**COMMISSION OF INQUIRY INTO THE DECLINE OF SOCKEYE SALMON IN THE FRASER RIVER**

In the matter of His Excellency the Governor General in Council, on the recommendation of the Prime Minister, directing that a Commission do issue under Part 1 of the Inquiries Act and under the Great Seal of Canada appointing the Honourable Bruce Cohen as Commissioner to conduct an inquiry into the decline of sockeye salmon in the Fraser River

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# SUBMISSIONS OF THE PARTICIPANT GOVERNMENT OF CANADA ON INFECTIOUS SALMON ANAEMIA VIRUS

**DECEMBER 29, 2011**

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## I. OVERVIEW

1. Since mid-October 2011 there have been samples taken from British Columbia (BC) salmon and tested for infectious salmon anaemia (ISA) with some presumptive positive results for ISAV or another orthomixovirus. Validation tests have been performed with no confirmed findings of ISA in BC salmon to date. The various tests have been performed by knowledgeable scientists. Those same scientists agree that further inquiry is warranted, and this is being undertaken.

2. There are several possible scientific explanations for the mixed results from testing and differing views whether any results could be false positives or negatives. Further inquiry and research is required before a definitive conclusion can be reached on whether ISAV, an ISAV like virus, or ISA is present in BC salmon. Even if positive findings are confirmed it will be necessary to then determine what it is that has been detected and whether it causes disease in Pacific and farmed Atlantic salmon. ISA is known to be lethal to Atlantic salmon, including those in most finfish farms in BC. It is not known to affect Pacific salmon, although they can be carriers. ISA has no impact on human health.

3. Further suggested research includes more sequencing information, challenge testing of assays used by some scientists, review of test methodology and lab protocols, and field surveillance to gather and test more samples. Dr. Kristi Miller opines that what she has detected has been in BC waters for decades.

4. By legislation, the Canadian Food Inspection Agency (CFIA) is the lead agency for the National Aquatic Animal Health Program (NAAHP). In response to the presumptive positive findings, CFIA is conducting lab assessments, reviewing test methodology and lab protocols, and formulating a surveillance plan (with input from DFO and others) with implementation to begin in the first quarter of 2012.

5. The Department of Fisheries and Oceans (DFO) supports CFIA in its work. The DFO lab in Moncton, New Brunswick has conducted validation tests to determine whether any presumptive positive findings of ISA can be confirmed. Under an Umbrella Memorandum of

Understanding (MOU), the CFIA uses DFO labs for diagnostic testing, since DFO has the scientific expertise and laboratory facilities to test and diagnose fish health and disease. DFO has developed this expertise over many decades. In developing the National Aquatic Animal Health Program (NAAHP) the Government of Canada (Canada) decided that it is most efficient and effective for CFIA and DFO to be partners in the testing and surveillance of aquatic animal health. The National Aquatic Animal Health Laboratory System (NAAHLS) is part of this.

6. Where suspect cases of a pathogen, such as ISAV, are reported to the CFIA, it is important to assess all the evidence, undertake validation tests, assess and analyse the test results in light of all available information, make inquiries and investigate. Then, where questions remain, to develop and implement a well thought-out surveillance and response plan. It would be unhelpful to simply rely and act upon non-validated and sometimes inconclusive and inconsistent test results without more being done. Sound science is based on observation and study. The objective is to protect against aquatic animal diseases and improve fish health and facilitate safe international trade of aquatic animals and animal products.

7. CFIA does not intend to report suspected or presumptive findings of ISAV or ISA to the World Organisation for Animal Health (OIE). Member countries are not required to report suspected cases. They are expected to investigate suspected cases and determine whether any report of a suspected case can be confirmed and, if so, to then report. With this, the OIE and other countries are aware of the presumptive positive findings from BC waters.

8. CFIA is proceeding in a cautious and prudent manner to gather all available information, including attempts to corroborate the presumptive positive findings. Regardless of the results of those tests, CFIA will also develop and implement a surveillance plan to obtain and test more samples from BC waters over several years.

9. As the above diagnostic work is being done, DFO Science continues to conduct ongoing multi-year research into ISAV and other pathogens in BC waters. This research is being done by Dr. Kyle Garver, DFO virologist, and by Dr. Miller, DFO molecular biologist, amongst others. This science research is important and, in time, may lead to findings that will inform whether to

change the assays or test methodology used in diagnostic testing and whether other new or emerging diseases should be regulated.

## II. REGULATORY REGIME

### A. International

#### i. World Organisation for Animal Health (formerly the *Office International des Épizooties*)

10. The need to fight animal diseases at the global level led to the creation of the Office International des Epizooties through an international agreement titled the International Agreement for the Creation of an *Office International des Epizooties* in Paris signed on January 25, 1924. In May 2003 the Office became the World Organisation for Animal Health but kept its historical acronym OIE. The OIE is dedicated to fighting animal diseases and improving animal health, worldwide, as more particularly set out below. Its objectives coincide and fit with the domestic need in Canada to protect aquatic animal health and maintain sustainable fisheries. The OIE is recognised as a reference organization by the World Trade Organization and in 2011 had a total of 178 member countries, including Canada.[1]

#### ii. International Standards

11. With modern globalisation, animal health measures have increasing importance to facilitate the safe international trade of animals and animal products while avoiding unnecessary impediments to trade. In light of this, the Agreement on the Application of Sanitary and Phytosanitary Measures encourages the members of the World Trade Organization to base their sanitary measures on international standards, guidelines and recommendations, where they exist.[2] Canada is a member of the World Trade Organization.

12. The OIE is the World Trade Organization reference organization for standards relating to animal health and infectious diseases. The OIE publishes two codes (Terrestrial and Aquatic) and

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[1] OIE World Organisation for Animal Health, *About us*, online: <http://www.oie.int/about-us/>.
[2] OIE World Organisation for Animal Health, *International Standards*, online: <http://www.oie.int/international-standard-setting/overview/>.

two manuals (Terrestrial and Aquatic) as the principle references for World Trade Organization members.[3]

### iii. *Aquatic Animal Health Code*

13. The OIE *Aquatic Animal Health Code* (the *Aquatic Code*) sets out standards for the protection and improvement of aquatic animal health and welfare and veterinary public health worldwide. These include standards for safe international trade in aquatic animals (amphibians, crustaceans, fish and molluscs) and their products. The health measures in the *Aquatic Code* should be used by the veterinary authorities of importing and exporting countries to provide for early detection, reporting and control of agents pathogenic to aquatic animals so as to prevent their transfer via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.[4]

14. The recommendations in the disease chapters (ss. 8 to 11) of the *Aquatic Code* are intended to prevent listed pathogens (including ISA) being introduced into the importing country, taking into account the nature of the traded commodity and the aquatic animal health status of the exporting country. Properly applied, the recommendations provide for trade with an optimal level of animal health security, incorporating the latest scientific findings and available techniques.[5]

### iv. *Aquatic Code:* Infectious Salmon Anaemia Reporting Requirements

15. Chapter 10.5 of the *Aquatic Code* specifically addresses ISA and provides a regulatory structure that addresses the identification and testing for ISA as well as the identification and

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[3] OIE World Organisation for Animal Health, *International Standards*, online: <http://www.oie.int/international-standard-setting/overview/>.
[4] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/en/international-standard-setting/aquatic-code/>.
[5] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/en/international-standard-setting/aquatic-code/>.

maintenance of ISA-free zones and regulates the importation of live aquatic animals, among other objectives.[6]

16. Article 1.1.3 of the *Aquatic Code* requires that the occurrence of "listed diseases" be reported to the OIE within 24 hours.[7] ISA is identified as a listed disease in Article 1.3 of the *Aquatic Code*.[8] However, the OIE does not require that suspected cases of ISA be reported - only confirmed cases must be reported.[9]

### v. *Aquatic Code:* Competent Authority

17. The *Aquatic Code* Glossary provides a definition of the competent authority responsible for the identification of reportable diseases within member countries:

> Competent authority: means the Veterinary Authority or other Governmental Authority of a Member having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health and welfare measures, international health certification and other standards and recommendations in the Aquatic Code in the whole territory.[10]

### vi. Canadian Food Inspection Agency Role and Responsibilities Supported by Department of Fisheries and Oceans Laboratories

18. CFIA is the lead agency in Canada for preventing the introduction or spread of aquatic animal disease of finfish molluscs and crustaceans. Included in its mandate is to protect the aquatic animal resource base and ensure the continued health and sustainability of aquatic animals in Canada. CFIA assumed its current role over aquatic animal health in December 2010 through amendments to the *Health of Animals Regulations*, along with the concurrent

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[6] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.10.5.htm>, ch 10.5.
[7] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.1.htm>, ch 1.1.
[8] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.3.htm>, ch 1.3.
[9] Exhibit 2120: E-mail dated 11/30/2011, from Brian Evans to Cornelius Riley, Subject: TR: ISA virus British Columbia, with attached copy of OIE letter to Alexandra Morton, Dr Kim Klotins, 19 December 2011, p 13:36 to 14:2.
[10] OIE World Organisation for Animal Health, *Aquatic Animal Health Code*, online: <http://www.oie.int/index.php?id=171&L=0&htmfile=glossaire.htm#sous-chapitre-2>, Glossary.

amendment to the *Reportable Diseases Regulations* in January 2011, which are detailed below. Prior to December 2010, there was no national aquatic animal health program in Canada.

19. The Chief Veterinary Officer of Canada is an office within CFIA and is designated as the competent authority for Canada within the meaning of the OIE *Aquatic Code*. As such, CFIA has the responsibility for the *Aquatic Code* and the identification of ISA within Canada.[11]

20. Under the federal National Aquatic Animal Health Program (NAAHP), described in further detail below, three DFO laboratories - the Gulf Fisheries Centre in Moncton, the Freshwater Institute in Winnipeg and the Pacific Biological Station in Nanaimo - plus the bio-containment laboratory in Charlottetown, comprise the National Aquatic Animal Health Laboratory System (NAAHLS).[12] NAAHLS laboratories have responsibility under the NAAHP for the diagnostic work required to confirm the presence of aquatic animal diseases in Canada. The Moncton laboratory is the NAAHLS reference lab for ISA.[13] These DFO laboratories provide diagnostic lab work under NAAHP as part of the CFIA mandate. While CFIA has regulatory responsibility for terrestrial and aquatic animal health in Canada by law, it uses DFO laboratory and resource facilities and expertise for aquatic animal health testing. CFIA does not have its own aquatic animal health laboratory resources. CFIA became responsible for aquatic animal health in Canada (in December 2010).[14]

### vii. OIE Reference Laboratories

21. An OIE reference laboratory is designated to:

> …pursue all the scientific and technical problems relating to a named disease or specific topic. The Expert, responsible to the OIE and its Members with regard to these issues, should be a leading and active researcher helping the Reference Laboratory to provide scientific and technical assistance and expert advice on topics linked to surveillance and control of the disease for which the Reference Laboratory is responsible. Reference Laboratories may also provide scientific and technical training

[11] Dr Kim Klotins, 16 December, 2011, p 132:26-32.
[12] Stephen Stephen, 16 December 2011, pp 86:36 to 87:3.
[13] Stephen Stephen, 16 December 2011, p 86:14-35.
[14] Stephen Stephen, 16 December 2011, p 86:14-35; Dr Kim Klotins, 16 December 2011, pp 85:28 to 86:1.

> for personnel from Members, and coordinate scientific and technical studies in collaboration with other laboratories or organisations, including through OIE Laboratory Twinning.[15]

22. There are two OIE reference labs for ISA: a) Dr. F. Kibenge, Atlantic Veterinary College (AVC), Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Prince Edward Island; and b) Dr. Birgit Dannevig, National Veterinary Institute, Oslo, Norway.[16]

23. Dr. F. Kibenge is a reference scientist for the OIE, but he clarified that the OIE is not an accrediting body. His lab is selected based upon their expertise. OIE reference labs are not accredited or audited by the OIE – rather, they are designated as reference labs to provide support to OIE member countries that do not have the veterinary or laboratory infrastructure to conduct investigations for the diseases for which the reference lab is responsible. Dr. F. Kibenge provides advice on ISA to Chile, for example.[17] Countries like Canada (with the NAAHLS), the United States and United Kingdom have their own system of national reference laboratories. In those countries, the competent authority is unlikely to use the OIE Reference Laboratory for confirmation of disease unless it is approved to do so.[18]

### viii. OIE Manual of Diagnostic Tests for Aquatic Animals (OIE Diagnostic Manual) – Definition of Suspect and Confirmed Cases of ISA and ISAV

24. Chapter 2.3.5 of the OIE Diagnostic Manual specifically addresses ISA.[19] The manual provides the international standard regarding the disease itself (including the various strains), life cycle, susceptibility of host species, vectors for transmission of the virus, and disease patterns, distribution and prevalence. It also sets out standard diagnostic methods for detection of ISA.

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[15] Exhibit 2111: CFIA Inspection Checklist – Animal Pathogen Containment Level 2 Laboratories.
[16] OIE World Organisation for Animal Health, *Reference Experts and Laboratories*, online: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>; Dr Frederick Kibenge, 15 December 2011, pp 74:45 to 75:3.
[17] Dr Frederick Kibenge, 16 December 2011, p 62:20-28.
[18] Exhibit 2011: OIE World Organisation for Animal Health: Reference Laboratories; Dr Frederick Kibenge, 15 December 2011, p 74:34-40; Dr Peter Wright, 16 December 2011, pp 99:46 to 100:25; 19 December 2011, pp 24:8 to 25:29, p 32:14-43; Dr Kim Klotins, 19 December 2011, p 46:14-32.
[19] Exhibit 1676: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).

25. The manual stipulates different definitions and requirements to establish suspected cases of the virus (ISAV) and the disease (ISA), and confirmed cases of ISAV and ISA:

> 7.1. Definition of suspect case
>
> ISA or infection with ISAV would be suspected if at least one of the following criteria is met:
>
> i) Clinical signs consistent with ISA or pathological changes consistent with ISA (Section 4.2) whether or not the pathological changes are associated with clinical signs of disease;
> ii) Isolation and identification of ISAV in cell culture from a single sample (targeted or routine) from any fish on the farm…;
> iii) Evidence for the presence of ISAV from two independent laboratory tests such as RT-PCR… and IFAT on tissue imprints…;
> iv) Detection of antibodies to ISAV.
>
> 7.2. Definition of confirmed case
>
> The following criteria in i) should be met for confirmation of ISA. The criteria given in ii) and iii) should be met for the confirmation of ISAV infection.
>
> i) Mortality, clinical signs and pathological changes consistent with ISA (Section 4.2), and detection of ISAV in tissue preparations by means of specific antibodies against ISAV (IFAT on tissue imprints… in addition to either:
>  A) isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm;
>  or
>  B) detection of ISAV by RT-PCR by the methods described in Section 4.3.1.2.3;
> ii) Isolation and identification of ISAV in cell culture from at least two independent samples (targeted or routine) from any fish on the farm tested on separate occasions…;
> iii) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm with corroborating evidence of ISAV in tissue preparations using either RT-PCR (Section 4.3.1.2.3) or IFAT…[20]

[20] Exhibit 1676 at pp 232-233: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).

26. The OIE Diagnostic Manual further states that, in suspected cases, an official investigation to confirm or rule out the presence of ISA and ISAV should be undertaken by the competent authority as soon as possible, including "inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in s. 4."[21] As elaborated later in these submissions, Canada is currently doing this.

27. The CFIA "case definitions" of suspected and confirmed ISAV and ISA mirror those found in the OIE Diagnostic Manual.[22]

## B. Federal

### i. National Aquatic Animal Health Program

28. There has been a uniform national program for terrestrial animal health for decades. Recent examples where it has been invoked in the disease context in high profile, successful prevention and response actions are with avian flu in birds and mad cow disease in cattle. As to aquatic animals, the federal and provincial governments and various stakeholders discussed for many years the need for a uniform national program to implement a regulatory framework which controls the spread of disease among aquatic animals.[23]

29. In 2005, the- NAAHP received initial funding from the federal government to develop a national aquatic animal health disease prevention and response program. The program is co-delivered by the CFIA and DFO under the legislative authority of the *Health of Animals Act*.[24] CFIA is the lead agency under the legislation. The NAAHP is a science-based regulatory program for aquatic animal diseases.[25] It was designed to be consistent with international OIE animal health management standards. This includes protecting Canadian aquatic resources (wild and farmed) from serious aquatic animal diseases and maintaining competitive international

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[21] Exhibit 1676 at p 232: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[22] Exhibit 2106: *Draft ISAV Hazard Specific Plan*, 21 April 2011, at p. 5-7; Dr Kim Klotins, 16 December 2011, pp 142:9 to 143:1.
[23] *Regulatory Impact Analysis Statement*, (2010) C Gaz II, Vol. 144, No. 26, online: <http://www.gazette.gc.ca/rp-pr/p2/2010/2010-12-22/html/sor-dors296-eng.html>.
[24] *Health of Animals Act*, SC 1990, c 21.
[25] Dr Kim Klotins, 16 December 2011, pp 85:28 to 86:13; Stephen Stephen, 16 December 2011, p 86:14-29.

market access.[26] To assist with the implementation and development of the NAAHP, CFIA has created an Aquatic Animal Health Committee, to ensure meaningful and ongoing input from all interested parties.[27]

### ii. Aquatic Animal Health Committee

30. The Aquatic Animal Health Committee includes provincial and territorial authorities for aquaculture and wild fisheries resource management, veterinary association representatives, Aboriginal groups and wild and farmed industry stakeholders.[28]

31. The NAAHP program development and its implementation is reported to the Canadian Council of Fisheries and Aquaculture Ministers, and to the Agriculture Federal/Provincial/Territorial Regulatory Assistant Deputy Minister Committees.[29]

32. The Aquatic Animal Health Committee is chaired by the CFIA and DFO actively participates.[30]

### iii. Canadian Food Inspection Agency

33. The CFIA regulates aquatic animal diseases of finfish, molluscs and crustaceans through the NAAHP. Using the *Health of Animals Act*, which brings Canada's aquatic and terrestrial animal health programs under the same legislative umbrella as of December 2010, the federal NAAHP responsibilities are co-delivered by the CFIA and DFO. The NAAHP consists of four main program elements:

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[26] Canadian Food Inspection Agency, *Aquatic Animal Health Committee*, online: <http://www.inspection.gc.ca/english/anima/aqua/comme.shtml>.
[27] Canadian Food Inspection Agency, *Aquatic Animal Health Committee*, online: <http://www.inspection.gc.ca/english/anima/aqua/comme.shtml>.
[28] Exhibit 2105 at p 40: *Draft Aquatic Animal Health Functional Plan*, 1 September 2010; Dr Kim Klotins, 19 December 2011, pp 88:41 to 89:31; Canadian Food Inspection Agency, *Aquatic Animal Health Committee*, online: <http://www.inspection.gc.ca/english/anima/aqua/comme.shtml>.
[29] Canadian Food Inspection Agency, *Aquatic Animal Health Committee*, online: <http://www.inspection.gc.ca/english/anima/aqua/comme.shtml>.
[30] Canadian Food Inspection Agency, *Aquatic Animal Health Committee*, online: <http://www.inspection.gc.ca/english/anima/aqua/comme.shtml>.

a. Program direction and regulation;

b. Field operations;

c. Diagnostic testing; and

d. Research and development.[31]

34. The CFIA, as the lead agency, has regulatory and enforcement responsibilities, provides overall program direction and field operations capability for the aquatic animal industries in Canada.[32] CFIA and DFO coordinate in performing field operations for surveillance and monitoring activities for the wild stock and farm fish. DFO provides laboratory support for diagnostic testing required by the NAAHP, and the delivery and supervision of diagnostic science research and development.[33] The program is consistent with international standards set by the OIE.[34]

35. The CFIA is responsible for the administration and enforcement of the following Acts that are relevant to ISA:[35]

***Health of Animals Act,* SC 1990, c 21**

a. For the purposes of the NAAHP, diseases that may affect aquatic animal species were added to the list set out in the *Reportable Diseases Regulations*. Under s. 5 of the Act, where the owner or person in possession of an aquatic animal becomes aware of the presence of ISA, that person must notify the nearest veterinary

[31] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program.
[32] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program; Dr Kim Klotins, 16 December 2011, p 132:26-32.
[33] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program; Dr Kim Klotins, 16 December 2011, p 132:26-32.
[34] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program.
[35] Canadian Food Inspection Agency, *Acts and Regulations*, online: <http://www.inspection.gc.ca/about-the-cfia/acts-and-regulations/eng/1299846777345/1299847442232>.

inspector. A veterinarian or analyst who suspects that an aquatic animal is affected must also notify a veterinary inspector.

b. The Act allows the CFIA to inspect, collect information and samples in order to confirm disease, including ISA, and take steps to prevent the introduction of and spread of disease, including ISA.

c. The Act also allows the CFIA to conduct various activities for disease response, including for ISA, such as declaration of Infected Places, Control Areas, issuance of licences and permits, disposal and treatment of affected animals and things and compensation.

***Health of Animals Regulations,* CRC, c 296**

a. Amendments came into effect in December 2010 which, for the first time, extend the regulations to apply to aquatic animals, including finfish such as salmonids.[36]

b. The Regulations allow the CFIA to conduct activities for disease response, including as to ISA, such as quarantine and disinfection.

c. The Regulations also allow the CFIA to take steps to prevent introduction of aquatic animal diseases, such as ISA, from other countries and from infected areas within Canada.

***Reportable Diseases Regulations,* SOR/91-2**

a. Amendments came into effect on January 5, 2011 to add diseases to the list or reportable diseases.[37] ISA is a reportable disease and there is now an obligation for certain categories of professionals and other persons in Canada to notify the CFIA if ISA is suspected or known to occur in one or more animals in Canada.

[36] Dr Kim Klotins, 16 December 2011, p 130:13-15.
[37] Dr Kim Klotins, 16 December 2011, p 130:16-19.

***Compensation for Destroyed Animals Regulations*, SOR/2000-233**

a. The Regulations allow the CFIA to pay compensation for certain activities carried out by the CFIA during disease response; the current maximum is $30 per animal.

36. For clarity, the *Health of Animals Act* and the *Health of Animals Regulations* were originally established to control and eradicate diseases of animals by providing the basis for meeting domestic and import disease control requirements for terrestrial animals. As noted, the *Health of Animals Regulations* and the *Reportable Diseases Regulations* were amended in December 2010 and January 2011 respectively, in order to provide a similar level of protection to aquatic animals.[38]

37. Specifically, the relevant regulatory amendments include the addition of aquatic animals to the *Health of Animals Regulations* (Part XVI (ss. 190-202)), including susceptible species of aquatic animals listed in Schedule III (effective December 22, 2010), and the addition of aquatic animal diseases of national and international significance, including ISA, to the list set out in the *Reportable Diseases Regulations* (on January 5, 2011).[39] In addition, the *Health of Animals Regulations* was amended so that international imports of any of the aquatic animals listed in Schedule III of the *Health of Animals Regulations* will require an import permit issued by the CFIA, effective December 10, 2011.[40]

38. These amendments were enacted to extend the application of the general provisions of the existing Regulations to aquatic animals for the purposes of the NAAHP and to add specific authorities to regulate and control the spread of disease in susceptible species of aquatic animals listed in Schedule III, thereby creating a national framework to address health risks to aquatic animals.

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[38] Dr Kim Klotins, 16 December 2011, pp 129:14 to 130:27; *Regulatory Impact Analysis Statement*, (2010) C Gaz II, Vol. 144, No. 26, online: <http://www.gazette.gc.ca/rp-pr/p2/2010/2010-12-22/html/sor-dors296-eng.html>.
[39] Dr. Kim Klotins, 16 December 2011, pp 129:14 to 130:27; *Regulatory Impact Analysis Statement*, (2010) C Gaz II, Vol. 144, No. 26, online: <http://www.gazette.gc.ca/rp-pr/p2/2010/2010-12-22/html/sor-dors296-eng.html>.
[40] Stephen Stephen, 16 December 2011, pp 130:28 to 132:3.

39. Currently, Canada is in a transition state with components of the NAAHP being phased in over time. CFIA has assumed authority for exports of aquatic animals, imports of aquatic animals and notification and disease response in cultured and wild aquatic animal species for those diseases listed as reportable under the *Reportable Diseases Regulations*, or immediately notifiable under Schedule VII of the *Health of Animals Regulation*.

### iv. CFIA – Reportable Diseases

40. CFIA is responsible for preventing the introduction or spread of aquatic animal diseases of finfish, including salmon, molluscs and crustaceans.[41]

41. Animal diseases fall into three categories:

a. Reportable Diseases are of significant importance to aquatic animal health or to the Canadian economy.[42] While these diseases are described as occurring in limited areas within Canada, CFIA online notes that "[a]nyone who owns or works with aquatic animals and knows of or suspects a reportable disease is required by law to notify the CFIA".[43] If a reportable disease is detected, then the CFIA begins an investigation.[44]

b. Immediately Notifiable Diseases are described as serious diseases of concern to animal health and to the Canadian economy (given the international trade consequences).[45] The *Health of Animals Regulations* enables controls to prevent the introduction and spread of these diseases under the import permit provisions (ss. 190-195).[46] However, they are diseases that are not known to exist in Canada and only regulated parties such as "laboratories are required to contact the CFIA

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[41] Canadian Food Inspection Agency, *Aquatic Animal Diseases*, online: <http://www.inspection.gc.ca/animals/aquatic-animals/diseases/eng/1299156296625/1320599059508>.
[42] Exhibit 1566: Fisheries and Oceans Canada, *Fish Health Protection Regulations - Manual of Compliance*, online: <http://www.dfo-mpo.gc.ca/science/enviro/aah-saa/regulation-reglements-eng.htm>.
[43] Exhibit 2128: *Changes to the Health of Animals Regulations – Aquatic Animal Diseases*, 10 December 2011.
[44] Exhibit 2128: *Changes to the Health of Animals Regulations – Aquatic Animal Diseases*, 10 December 2011.
[45] *Regulations Amending the Health of Animals Regulations, 2010*, SOR/2010-296.
[46] *Regulations Amending the Health of Animals Regulations, 2010*, SOR/2010-296.

regarding the suspicion or diagnosis of these diseases".[47] The Regulatory Impact Analysis Statement notes that if after an investigation the immediately notifiable disease is found to be established in Canada, then disease control measures can be established.[48]

c. Annually Notifiable Diseases are those "present in Canada and are a concern to some of Canada's trading partners. Only laboratories are required to contact the CFIA regarding the suspicion or diagnosis of these diseases".[49] The Regulatory Impact Analysis Statement explains that these diseases do not warrant a national program as they are found throughout Canada; do not have sufficient impact on fish stocks; or, in the case of captive aquatic animals, can be controlled by treatment or by biosecurity measures.[50]

### v. Mandatory Reporting Requirements

42. By two notices dated January19, 2011, CFIA notified veterinarians, aquatic animal health specialists and the public generally of their mandatory reporting obligations under the *Health of Animals Act*. The first notice, addressed to veterinarians and aquatic animal health specialists, describes their obligations pursuant to s. 5(2) of the *Health of Animals Act*. Sub-section 5(2) requires these persons to report any "suspicion" of a reportable disease to a veterinary inspector at a local CFIA Animal Health Office.[51] The second notice, addressed to all "Canadians who own or work with aquatic animals," describes the mandatory obligation of the public to report the presence of a reportable disease to the CFIA under s. 5(1) of the *Health of Animals Act*.[52]

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[47] Exhibit 2128: *Changes to the Health of Animals Regulations – Aquatic Animal Diseases*, 10 December 2011.
[48] *Regulatory Impact Analysis Statement*, (2010) C Gaz II, Vol. 144, No. 26, online: <http://www.gazette.gc.ca/rp-pr/p2/2010/2010-12-22/html/sor-dors296-eng.html>.
[49] Exhibit 2128: *Changes to the Health of Animals Regulations – Aquatic Animal Diseases*, 10 December 2011.
[50] *Regulatory Impact Analysis Statement*, (2010) C Gaz II, Vol. 144, No. 26, online: <http://www.gazette.gc.ca/rp-pr/p2/2010/2010-12-22/html/sor-dors296-eng.html>.
[51] Exhibit 2027: *Mandatory Notification of Reportable Aquatic Animal Diseases*, 19 January 2011; Dr Kim Klotins, 16 December 2011, p 89:20-22.
[52] Exhibit 2103: *Mandatory Notification of Reportable Aquatic Animal Diseases*, 19 January 2011; Dr Kim Klotins, 16 December 2011, pp 89:44 to 90:3.

43. DFO distributed these notices to all departmental management committee members including the Deputy Minister, Assistant Deputy Ministers and Regional Directors General with a request that this notice be provided to all DFO staff, including scientists, who are involved in rearing, holding and undertaking research in respect of aquatic organisms. This distribution occurred on or about February 7, 2011.[53] A reminder notice attaching the two January 19, 2011, notices was sent to the same recipients on November 28, 2011.[54]

44. The CFIA documents the receipt of notifications and information about the affected animals and other epidemiological information required to make decisions during a disease response on the Call Log/AquaPIQ.[55]

### vi. Situation Reports

45. CFIA prepares internal Situation Reports to summarize information about the disease notifications, status, steps taken, and planned operations.[56] Daily Situation Reports were prepared following the reports in mid-October of suspected ISA and provides a helpful chronology on the steps taken in response to notification of ISA by the CFIA from October 19, 2011 through December 8, 2011, and continuing.

### vii. Department of Fisheries and Oceans

46. DFO is responsible for three regulations relevant to diseases in fish: s. 56(b) of the *Fishery (General) Regulations*, the *Fish Health Protection Regulations* and the *Pacific Aquaculture Regulations*.[57] As of December 2011, the *Fish Health Protection Regulations* requirement for a permit to import salmonids into Canada has been removed to avoid duplication with the recently amended *Health of Animals Regulations*. Eventually, the *Fish Health Protection Regulations* will be rescinded as CFIA continues to take on a greater role in the area

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[53] Stephen Stephen, 16 December 2011, pp 134:36 to 135:7.
[54] Exhibit 2116: Email of Dr Siddika Mithani to various people, 28 November 2011; Stephen Stephen, 16 December 2011, p 134:20-28.
[55] Exhibit 2126: *CFIA Call Log by Ray J Fletcher*, 30 November 2011.
[56] Exhibit 2107: *Situation Report (Internal) Update #3*, 20 October 2011.
[57] *Fishery (General) Regulations*, SOR/93-53; *Fish Health Protection Regulations*, CRC, c 82; *Pacific Aquaculture Regulations*, SOR/2010-270.

of aquatic animal diseases. However, it should be noted that the schedules to the *Fish Health Protection Regulations* do not list ISA.[58] The *Pacific Aquaculture Regulations* specifically list ISA as a disease to be reported to DFO. Licence conditions for all marine finfish aquaculture operators in British Columbia, granted under the authority of the *Pacific Aquaculture Regulations*, specifically list ISA as a disease to be reported to DFO. The CFIA is working with DFO to remove regulatory duplication.

47. Today, DFO has responsibility under NAAHP for undertaking science research, diagnostic testing, and providing advice to CFIA on diagnostic testing pursuant to a MOU between the two departments.[59] DFO has undertaken science research for many decades. The NAAHP envisions a partnership between CFIA and DFO in respect of aquatic animal health, recognizing DFO's expertise and knowledge with respect to aquatic disease diagnostic testing and research paired with CFIA's regulatory authorities under the *Health of Animals Act* and expertise and knowledge in animal disease, disease diagnosis and disease control.[60]

### viii. CFIA/DFO Memorandum of Understanding (MOU)

48. Under the MOU between the CFIA and DFO, DFO established the NAAHLS in 2006.[61] NAAHLS includes designated scientists and laboratories in the Gulf Fisheries Centre (GFC) in Moncton, the Pacific Biological Station (PBS) in Nanaimo, the Freshwater Institute in Winnipeg and the Charlottetown Aquatic Animal Pathogen Bio-containment Laboratory in Charlottetown. These labs are linked into a national platform for diagnostic testing, with common procedures, quality management systems, and sample receipt and tracking procedures.[62]

49. Each laboratory is designated as the national reference laboratory for specific disease diagnostic testing procedures. The GFC in Moncton is the national reference laboratory for testing for ISA. Other laboratories, whether or not under NAAHLS can undertake screening and surveillance testing for ISAV, but validation of any positive tests results is required by the GFC before positive results can be considered confirmed. The analysts in GFC and Ms. Gagné's lab in

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[58] Stephen Stephen, 16 December 2011, pp 130:40 to 131:44.
[59] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program.
[60] Stephen Stephen, 16 December 2011, p 86:19-29.
[61] Exhibit 2127: Umbrella Memorandum of Understanding (MOU) On The Development And Implementation of A National Aquatic Animal Health Program.
[62] Stephen Stephen, 16 December 2011, pp 86:41 to 87:3; Dr Peter Wright, 16 December 2011, p 87:12-29.

particular, are designated by the CFIA under the *Health of Animals Act* to undertake testing for ISAV for the CFIA.[63]

50. CFIA is responsible for the diagnosis of ISA and infection with ISAV, including evaluation of test results, and administration and regulation of the *Health of Animals Act*. By legislation, CFIA makes the final decision on the status of aquatic animal health in Canada for purposes of reporting to the OIE. The CFIA conducts sampling, and performs investigations to confirm and report on the presence of diseases. DFO, for its part, conducts science research into ISA and ISAV, along with other viruses and diseases, and also performs laboratory testing for other DFO programs. However, as with others, a DFO scientist has a duty to report any suspect cases of ISA to CFIA, who then conducts a formal investigation and integrates information from DFO into the broader picture.[64]

### C. Provincial

51. The BC Ministry of Agriculture is interested to have the provincial laboratory in Abbotsford to be considered for future laboratory work in testing for disease. [65] Presently, the Province is not involved in the DFO NAAHP laboratory network testing for disease. However, like others, the provincial lab is required to notify the CFIA if they suspect ISA or presence of ISAV.

### D. Other Agencies' Roles

52. Other agencies or persons, such as universities, the aquaculture industry, or private individuals that may be involved in testing for virus or disease have an obligation to report any positive findings to CFIA. At that point, CFIA will conduct an investigation to confirm the report and consider whether this impacts their present diagnostic testing.[66] Some university or other third party laboratories may be considered for the NAAHLs network for future disease

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[63] Exhibit 2022: *Letter of Designation*, 28 October 2011; Stephen Stephen, 16 December 2011, p 86:30-35; Dr Peter Wright, 16 December 2011, pp 99:26 to 100:25; Dr Kim Klotins, 16 December 2011, p 88:8-30.
[64] Stephen Stephen, 16 December 2011, p 108:12-45; Dr Kim Klotins, 16 December 2011, p 132:26-32.
[65] Dr Kim Klotins, 16 December 2011, p 122:1-9.
[66] Stephen Stephen, 16 December 2011, pp 109:43 to 110:10; Dr Kim Klotins, p 110:16-32.

testing. Presently, no third party laboratories are involved in the DFO NAAHP laboratory network testing for disease.

## III. ISAV, ISA and Real-time RT-PCR Testing

### A. What are ISAV and ISA?

53. ISAV is an orthomyxovirus, and has a similar structure to a flu virus.[67] It is an RNA virus consisting of eight segments of RNA of varying lengths.[68] It is the only orthomyxovirus presently known to infect fish.[69] There are over 20 different isolates (or strains) of ISAV, which have been divided into two genotypes: North American and European. Each genotype consists of numerous strains, some of which are virulent, and some of which are avirulent.[70]

54. The virus ISAV can infect Atlantic salmon, and can cause a communicable disease known as infectious salmon anaemia, or ISA. The virus has also been found in various species of wild fish, which can be carriers of the virus without having the disease. The OIE Diagnostic Manual states that the only known natural outbreaks of ISA have been in farmed Atlantic salmon.[71] As stated by Dr. Are Nylund, there is a large difference between the presence of the virus (ISAV) and the presence of actual disease (ISA).[72]

55. The OIE Diagnostic Manual defines ISA as follows:

> Infectious salmon anaemia (ISA) is an orthomyxovirus infection of sea-farmed Atlantic salmon (*Salmon salar*) (28) inducing a systemic and lethal condition characterised by severe anaemia and variable haemorrhages and necrosis in several organs. The disease course is

[67] Dr Frederick Kibenge, 15 December 2011, pp 9:39 to 10:10.
[68] Dr Frederick Kibenge, 16 December 2011, p 1:25-40.
[69] Dr Frederick Kibenge, 15 December 2011, pp 9:39 to 10:10.
[70] Nellie Gagné, 16 December 2011, p 71:5-18.
[71] Exhibit 1676 at p 223: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[72] Dr Are Nylund, 15 December 2011, p 10:18-27.

> prolonged with low daily mortality (0.05–0.1%) typically only in a few cages, but cumulative mortality may become very high.[73]

56. The OIE Diagnostic Manual further describes the clinical signs of ISA to include pale gills, exophthalmia, distended abdomen, blood in the anterior eye chamber, skin haemorrhages especially of the abdomen, as well as scale pocket oedema. Naturally infected Atlantic salmon appear lethargic and may keep close to the wall of the net pen.[74]

57. ISA and ISAV have no impact on human health, but ISA can have a serious impact on the salmon aquaculture industry and commercial fishers if fish have to be destroyed and/or markets and trade are restricted or closed.[75]

### B. Geographic Distribution of ISA and ISAV

58. Initially reported in Norway in the mid-1980s, ISA in Atlantic salmon has since then been reported in Canada (New Brunswick in 1996 and Nova Scotia in 2000), the United Kingdom (Scotland in 1998), the Faroe Islands (2000), USA (Maine in 2001) and in Chile. The virus has been reported from rainbow trout in Ireland in 2002 and from coho salmon in Chile.[76]

### C. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Testing

59. RT-PCR is a method amplifying (or replicating) a particular RNA sequence in order to determine whether it is present in a sample, and if so, in what amount. The first step involves reverse transcription (RT) of RNA, which involves the creation of complementary DNA (or cDNA) out of the RNA in the sample.

60. Once that is complete, a PCR process is initiated in order to amplify (or make many copies of) the DNA. The process involves repeated cycles of heating and cooling the sample.

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[73] Exhibit 1676 at p 222: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[74] Exhibit 1676 at p 226: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[75] Exhibit 2029 at p 1: *News Release – No Confirmed Cases of Infectious Salmon Anaemia in British Columbia*; Dr Frederick Kibenge, 16 December 2011, p 63:10-21; Dr Frederick Kibenge and Nellie Gagné, 16 December 2011, p 64:18-43; Dr Kim Klotins, 16 December 2011, p 96:16-35.
[76] Exhibit 1676 at p 224: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).

First, the sample is heated to a particular temperature in order to 'melt' the DNA, or separate its double strands into single strands. Next, the temperature is lowered to a level that allows 'primers' to bind to the single strands. A primer is a small sequence of DNA complementary to the DNA being targeted. Once the primer binds to one point on a strand (known as annealing), a polymerase enzyme will bind to the strand and begin copying it. Sometimes, at this stage, the temperature is raised again to allow the polymerase enzyme to work faster.[77]

61. Upon completion of each cycle, if primers have annealed to a target sequence, the sequence will be copied and there will be twice as much DNA (including the cDNA of interest) of the target sequence than at the beginning of the cycle. The cycle is repeated numerous times – the number of cycles required, and the timing and temperature of each stage of a single cycle, will vary depending on the testing protocol involved.[78] In addition, in testing for a given virus, different labs may use different primers that target different segments – and different portions of segments – of the viral genome.

62. In conventional RT-PCR testing, the results of the DNA amplification are visualized by gel electrophoresis. The brighter the band on the gel, the more copies of the target DNA are present. In real-time RT-PCR testing, which is a newer method, a probe is used which creates an increasing level of fluorescence in each sample as the target sequence is multiplied. Real-time RT-PCR results are stated in "Ct values" with Ct being the number of cycles at which the fluorescence rises above the threshold a certain level of fluorescence is produced. The fewer cycles it takes to produce a certain level of fluorescence, the larger the amount of starting DNA of interest was present in the sample. In other words, in testing for sequences from an RNA virus, the lower the Ct value, the more of that sequence was originally present in the sample.[79]

63. PCR and RT-PCR are very sensitive techniques capable of identifying a single copy of DNA or RNA, respectively. Due to this high level of sensitivity these tests can be prone to

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[77] Exhibit 2058: *Beginner's guide to Real-time PCR.*
[78] Exhibit 2058: *Beginner's guide to Real-time PCR.*
[79] Exhibit 2058: *Beginner's guide to Real-time PCR*; Nellie Gagné, 15 December 2011, p 11:27-43.

contamination which can result in false positive results.[80] The risk of contamination is reduced through good laboratory practices and the use of controls within each assay. It is extremely important to have good laboratory procedures to avoid contamination and have highly trained and skilled scientists and technicians conducting tests and interpreting the results.[81]

64. Primers used in PCR and RT-PCR can bind non-specifically to similar sequences on non-target DNA/RNA. If both primers non-specifically bind a false positive result can result.[82] PCR and RT-PCR results thus require confirmation to ensure that the product which is being amplified is actually the target of interest. This is most often done by sequencing of the product and comparison of the sequence to known sequences from public databases. Another way to confirm the presence of ISAV is to test a portion of the original tissues for the presence of virus by cell culture.[83]

65. The success of PCR and RT-PCR analysis requires the target of interest to be present in good condition. Once samples are removed degradation of both the host and pathogen RNA occurs.[84] The NAAHP validated diagnostic test for ISAV tests each sample for the integrity of RNA.[85] If samples are found to have degraded RNA it is possible that any negative result obtained could be in error. In this case, the samples are reported as inconclusive negatives.[86] Samples that are degraded but produce a positive PCR or RT-PCR are reported as positives. However, the degradation of host and pathogen RNA does not prevent false positives resulting from contamination of the samples with positive material.[87]

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[80] Nellie Gagné, 16 December 2011, p 19:27-38.
[81] Exhibit 2046: QA/QC summary – Nellie Gagné, 14 November 2011; Dr Are Nylund and Nellie Gagné, 15 December 2011, pp 75:41 to 77:40; Dr Frederick Kibenge, Dr Are Nylund, Dr Kristi Miller and Nellie Gagné, 15 December 2011, p 87:6-20.
[82] Dr Are Nylund and Dr Kristi Miller, 15 December 2011, pp 57:45 to 59:7; Dr Are Nylund, 15 December 2011, p 100:11-39.
[83] Exhibit 1676 at pp 232-233: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[84] Nellie Gagné, 16 December 2011, p 16:8 to 17:2.
[85] Exhibit 2046 at p 4: QA/QC summary – Nellie Gagné, 14 November 2011.
[86] Nellie Gagné, 15 December 2011, p 16:9-24.
[87] Nellie Gagné, 16 December 2011, pp 15:45 to 18:23.

66. The NAAHP RT-PCR test for ISAV has been designed to identify all known strains of ISAV from both the North American and European genotypes. This test has undergone the process of validation to ensure its specificity and to determine its capacity (sensitivity) to detect ISAV.[88]

## IV. ISAV Chronology of 2011 Events (CFIA & DFO)

### A. Dr. Molly Kibenge's 2004 Results

67. Dr. M. Kibenge was a post-doctoral scientist in Dr. Simon Jones' laboratory at the PBS from approximately January 2003 to June 2004. In her work, Dr. M. Kibenge tested samples from Pacific salmon for ISA and other diseases, using RT-PCR and cell culture assays.[89] Dr. Jones testified that Dr. M. Kibenge obtained positive test results using a RT-PCR assay for segment 8, but the results were not repeatable on a consistent basis. She was not able to amplify genomes for segments 2, 6, and 7. Dr. Jones and Dr. M. Kibenge, therefore, decided in October 2003 to forward samples to Dr. F. Kibenge at the AVC for confirmatory testing.[90]

68. Twenty samples of chinook salmon were sent to Dr. F. Kibenge in a blind test. In fact, some of these were the samples that tested positive most frequently in segment 8. Dr. F. Kibenge was provided with ten salmon that had tested positive and ten fish that had tested negative with Dr. M. Kibenge's tests. Of the ten positive samples, Dr. F. Kibenge confirmed three positive test results and found seven to be negative. Of the ten negative samples, Dr. F. Kibenge also reported three positive test results, while confirming seven negative test results.[91]

69. In early 2004, Dr. M. Kibenge reported the results of her research in a draft paper titled, "Presence of Infectious Salmon Anaemia Virus nucleotide sequences in wild Pacific salmon."[92]

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[88] Exhibit 2000 at pp 10, 12-13: *Validation Pathway for NAAHLS Diagnostic Test Methods Molecular Analysis for Infectious Salmon Anaemia Virus*.
[89] Dr Simon Jones, 16 December 2011, p 126:2-3, p 126:20-22.
[90] Dr Simon Jones, 16 December 2011, p 126:26-45.
[91] Dr Simon Jones, 16 December 2011, p 127:13-24.
[92] Exhibit 2113: *Presence of Infectious Salmon Anaemia Virus nucleotide sequences in wild Pacific salmon*.

70. Dr. Jones testified to his concern at the continuing inconsistency in Dr. M. Kibenge's test results and inability to confirm the results, noting Dr. F. Kibenge's test results. Accordingly, Dr. M. Kibenge, after meeting with Dr. Jones, Dr. Garth Traxler and Dr. Dorothy Kieser of the Fish Health Program at PBS, sent approximately 90 samples of chinook salmon to Ms. Nellie Gagné's laboratory at DFO Moncton for further confirmatory testing. The Moncton lab could not reproduce Dr. M. Kibenge's results.[93] At the time, Dr. Traxler was the senior virologist at PBS and Dr. Kieser was a fish pathologist (both now retired). Dr. Keiser was in charge of the diagnostic laboratory at PBS, and at that time she was responsible for the *Fish Health Protection Regulations.*[94]

71. Ms. Gagné testified that she tried to replicate Dr. M. Kibenge's results several times, using the same tests and methodologies as Dr. M. Kibenge used, and was in frequent communication with Dr. M. Kibenge to try to understand and reconcile their inconsistent results. Ultimately, all of Ms. Gagné's tests of Dr. M. Kibenge's salmons were negative for ISAV.[95]

72. Upon consideration of all of the available data, Dr. Jones and other members of the Fish Health Program at PBS (Dr. Traxler and Dr. Kieser) concluded that Dr. M. Kibenge's results were not a positive finding of ISAV.[96] In particular, Dr. Jones notes that the results of Dr. M. Kibenge, when compared to the test results of Dr. F. Kibenge and Ms. Gagné were inconsistent and could not be replicated.[97]

73. Dr. Jones testified that the draft manuscript yields some puzzling and inconsistent results that were not verifiable, and the results of the tests were considered to be negative. For example, the manuscript asserts at Table 1 that all 64 Cultus Lake sockeye samples tested positive for ISAV. However, in an earlier email from Dr. M. Kibenge to Dr. Jones, she noted that in her view

[93] Dr Simon Jones, 16 December 2011, p 127:19-44.
[94] Dr Simon Jones, 16 December 2011, p 126:10-14.
[95] Nelle Gagné, 15 December 2011, pp 26:43 to 28:8.
[96] Dr Simon Jones, 16 December 2011, pp 127:47 to 128:7.
[97] Dr Simon Jones, 16 December 2011, p 127:13-44.

the sockeye clones did not resemble any ISAV isolate, but rather shows homology to other species.[98]

74. Dr. Jones recommended to Dr. M. Kibenge that further research and testing was required to explain these inconsistent results. Dr. M. Kibenge left DFO for the AVC at University of Prince Edward Island in 2004. She and Dr. Jones communicated periodically by email up to the end of 2005 in connection with subsequent test results.[99] Thereafter, Dr. Jones had no further communication with Dr. M. Kibenge in connection with this manuscript until November 4, 2011, when Dr. M. Kibenge asked Dr. Jones' permission to submit the draft manuscript for publication. The manuscript had not changed from the draft prepared in March 2004, and in particular did not explain, consider or even refer to Ms. Gagné's inconsistent tests results, or any of Dr. M. Kibenge's subsequent testing after she left the PBS. Dr. Jones, noting these deficiencies, testified that he was surprised and disappointed to receive this request to publish the paper in light of these deficiencies.[100] In his opinion, the paper is not up to a standard suitable for publication in a peer-reviewed journal. Dr. Jones testified that he has written and reviewed numerous papers for science journals and is familiar with the standards expected for publication.[101]

75. In November 2011Ms. Gagné retested samples retained from Dr. M. Kibenge's 2004 research, using DFO's current validated RT-PCR assay and the Snow 2006 segment 8 assay. These test results were also negative (see section E).[102] Further, Dr. Miller testified that she compared sequences from Dr. M. Kibenge's research with her own and found some similarities, but also some mismatches, with her own sequencing results (described in section D below). [103]

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[98] Exhibit 2140: Email from Molly Kibenge to Simon Jones, 5 March 2004; Dr Simon Jones, 19 December 2011, p 112:4-28.
[99] Exhibit 2114: Emails between Simon Jones to Molly Kibenge, February 2005; Exhibit 2115: Emails between Molly Kibenge and Simon Jones, January 2006.
[100] Dr Simon Jones, 19 December 2011, pp 4:38 to 5:8.
[101] Dr Simon Jones, 19 December 2011, p 5:8-18.
[102] Nellie Gagné, 15 December 2011, pp 27:45 to 28:8.
[103] Dr Kristi Miller, 15 December 2011, p 83:4-14, pp 109:37 to 110:10.

### B. Dr. Frederick Kibenge's Tests and Results in October-November 2011 – CFIA Notification #1 and #2

76. Dr. F. Kibenge conducted real-time RT-PCR tests on wild Pacific salmon on four separate occasions in late 2011. [104]

a. **Sample Set #1B Hearts** – On October 15, 2011, Dr. F. Kibenge notified Dr. Brian Evans, Chief Veterinary Officer for Canada, that he had obtained two positive PCR results for ISAV segment 8 and segment 6 in 48 Rivers Inlet sockeye smolt hearts he received from Dr. Rick Routledge and student Nicole Gerbrandt of Simon Fraser University.[105] Dr. F. Kibenge reported that the detection of segment 6 indicates that the strain detected is similar to the European genotype.[106] These 48 smolts (Sample Set #1B) were part of a larger set of samples which became known as Sample Set #1.[107] This notification became known as CFIA Notification #1.[108]

b. **Sample Set #2 –** On October 20, 2011, Dr. F. Kibenge notified the CFIA that he had obtained three positive PCR results for ISAV segment 8 (one in an adult coho heart, one in an adult chinook gill and one in an adult chum gill).[109] One of those positives also tested positive for segment 6, which Dr. F. Kibenge reported indicates the European genotype.[110] The other two positives could not be classified as either the European or North American genotypes.[111] These samples were

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[104] Dr Fredrick Kibenge, 15 December 2011, pp 11:44 to 13:25.
[105] Exhibit 2005: *Content of information to provide from an OIE Reference Laboratory to inform the OIE on positive results of samples on OIE listed diseases*.
[106] Exhibit 2005 at p 3: *Content of information to provide from an OIE Reference Laboratory to inform the OIE on positive results of samples on OIE listed diseases*.
[107] Exhibit 2143: Work flow timeline.
[108] Exhibit 2141: ISAV #1 timeline.
[109] Exhibit 2107 at p 1: *Situation Report (Internal) Update #3, 20 October 2011*; Exhibit 2007: Email from Fred Kibenge to Jonathan Coady, 23 November 2011 attaching report "Alexandra Morton Samples (Sockeye Chinook and Coho) VT10142001_October 12 2011".
[110] Exhibit 2007: Email from Fred Kibenge to Jonathan Coady, 23 November 2011 attaching report "Alexandra Morton Samples (Sockeye Chinook and Coho)_VT10142001_October 12 2011".
[111] Exhibit 2007: Email from Fred Kibenge to Jonathan Coady, 23 November 2011 attaching report "Alexandra Morton Samples (Sockeye Chinook and Coho)_VT10142001_October 12 2011".

collected by Alexandra Morton at Weaver Creek, and became known as Sample Set #2.[112] This notification became known as CFIA Notification #2.[113]

c. **Sample Set #3 –** November 3, 2011: Dr. F. Kibenge notified Ms. Morton that he had not obtained any positive results for ISAV segment 8 from a set of five sockeye smolt hearts, five sockeye smolt gills and five herring hearts.[114] These became known as Sample Set #3.[115]

d. **Sample Set #4 –** In a report by Dr. F. Kibenge dated November 7, 2011, he describes negative results for ISAV segment 8 in samples from several adult coho, sockeye and pink salmon. These samples were submitted by Ms. Morton to Dr. F. Kibenge, and were collected from the Harrison River.[116]

77. Dr. F. Kibenge attempted to culture the virus in Sample Set #1 and #2. However, he was not able to culture the virus.[117] He noted that when a fish is clinically sick, one is normally able to culture the virus, and that some ISA strains (in particular, the avirulent HPR0 strain) are not culturable.[118]

78. Dr. F. Kibenge did not test the quality of the RNA in any of the samples.[119]

### C. Dr. Are Nylund's Tests and Results – October-November 2011

79. Dr. Nylund conducted real-time RT-PCR tests on wild Pacific salmon on three separate occasions in October and November 2011.[120]

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[112] Exhibit 2143: Work flow timeline.
[113] Exhibit 2142: ISAV #2 timeline.
[114] Exhibit 2010: Email from Fred Kibenge to Jonathan Coady, 23 November 2011 attaching report "Alexandra Morton Samples (Herring and Sockeye)_VT10312011_October 31 2011".
[115] Exhibit 2143: Work flow timeline.
[116] Exhibit 2009: *Testing Records: Alexandra Morton samples (Sockeye, Coho, Pink) VT11072011_November 07 2011*, 25 November 2011.
[117] Dr Frederick Kibenge, 15 December 2011, p 45:3-7.
[118] Dr Frederick Kibenge, 15 December 2011, pp 45:35 to 46:12.
[119] Exhibit 2075 at p. 11.
[120] Dr Are Nyland, 15 December 2011, pp 13:26 to 15:20.

a. **Sample Set #1B Gills –** In a report dated October 22, 2011 (and later finalized on November 2, 2011), Dr. Nylund reported RT-PCR results on the gill tissues from the same 48 sockeye smolts that Dr. F. Kibenge had tested from Sample Set #1.[121] While the initial report only noted negative results, the final report includes positive results from several reruns of tests on sample #26 and sample #36, which Dr. Nylund knew had both tested positive for Dr. F. Kibenge.[122] Dr. Nylund obtained a weak positive result (near the detection limit for the test) in sample #36, but he was not able to reproduce this result.[123] Dr. Nylund suggests in the November 2nd report that the positive result may not have been reproducible as the amount of viral genome present was too low.[124] Sample #26 continued to test negative.[125] Dr. Nylund also noted that the quality of the RNA may have been poor in these samples.[126]

b. **November 23, 2011 Report –** Dr. Nylund tested gills and hearts from 24 "salmonids collected in British Columbia." One of the gills tested positive for ISAV segment 7 once (but was not positive for segment 8) and was repeated once, but this result was not repeated in the other four reruns, even though one "should be able to repeat all positive results when Ct values are below 37" as here.[127] The heart from that fish tested negative. Dr. Nylund also reported that another sample tested positive for ISAV segment 7 in one of five runs on the heart tissue, but tested negative in the gill tissue.[128] Dr. Nylund then suggests that "we are not detecting any of the known ISA viruses from Europe (or eastern North America) [but] a more exact answer requires that we are able to sequence the RNA that is targeted by the

[121] Exhibit 2014: *Report-I, 27th October 2011;* Exhibit 2015: *Report, 2nd November 2011.*
[122] Exhibit 2015 at p 1: *Report, 2nd November 2011.*
[123] Exhibit 2015 at p 3: *Report, 2nd November 2011.*
[124] Exhibit 2015 at p 1: *Report, 2nd November 2011.*
[125] Exhibit 2015 at pp 1-2: *Report, 2nd November 2011.*
[126] Exhibit 2015 at p 2: *Report, 2nd November 2011.*
[127] Exhibit 2016 at p 1: *Report, 23rd November 2011.*
[128] Exhibit 2016 at p 1: *Report, 23rd November 2011.*

ISAV 7 assay." Dr. Nylund noted that the quality of the RNA in these samples was reasonable.[129]

c. **December 12, 2011 Report –** Dr. Nylund tested gills from an unknown number of "salmonids collected in British Columbia." None of the gills tested positive for ISAV, but a number did test positive for infectious hematopoietic necrosis virus (IHNV).[130] Dr. Nylund noted that the amount and quality of RNA in the samples was reasonable, and should not have influenced the results.[131]

80. Dr. Nylund was unable to sequence any ISA virus from any of these samples, and so was not able to verify that he was actually detecting the ISA virus. He noted, however, that his real-time assay is very specific and should only be detecting the ISA virus, though it may not detect all strains of the virus.[132]

### D. Dr. Kristi Miller's Tests and Results – November-December 2011

#### i. Wild Fraser Sockeye Salmon from 2007-2010

81. Dr. Miller is a molecular biologist and research scientist at PBS. She previously testified in August 2011 that her laboratory, in conjunction with Dr. Garver at PBS had tested her samples for ISAV as part of her work on the mortality-related signature, and that the results were negative.[133] On hearing of the recent positive results for ISA, she decided to go back to her Fraser sockeye samples from 2007 to 2010 and test them again for ISAV, using five different assays to test for ISAV segments 7 and 8.[134] Dr. Miller obtained positive PCR results with four of the five primer sets, though not necessarily in the same fish. She was also able to produce a short sequence of a portion of segment 7 which is 95% similar to known ISAV strains.[135]

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[129] Exhibit 2016 at p 1: *Report, 23rd November 2011*.
[130] Exhibit 2033 at p 1: *Report, 12th November 2011*.
[131] Exhibit 2033 at p 1: *Report, 12th November 2011*.
[132] Dr Are Nylund, 15 December 2011, p 15:2-20.
[133] Dr Kristi Miller, 24 August 2011, pp 52:26 to 53:39; 15 December 2011, p 20:12-21.
[134] Exhibit 2041: Primers and probes for ISAV; Exhibit 2042: Prevalence of ISAV identified using five distinct TaqMan assays in gill tissue from 2007-2010; Dr. Kristi Miller, 15 December 2011, p 20:8-41.
[135] Dr Kristi Miller, 15 December 2011, p 21:9-17.

82. Dr. Miller provided some of these samples (five positive and five negative) blind to Dr. Garver to see if he could confirm her results. He used both the DFO validated assay used at the Moncton lab, as well as the Plarre ISAV 7 assay used by Dr. Miller, and obtained positive results with the latter but not with the former.[136] He obtained positive results for two fish, with one testing positive in only one replicate, and the other testing positive in all three replicates.[137]

83. Dr. Miller also provided liver tissue samples to Ms. Gagné to see if she could confirm her results. Using the DFO validated assay, the results obtained by Ms. Gagné were negative.[138] Dr. Miller also used the DFO validated primers in a conventional PCR test (ie, without the probes), and the results were negative.[139]

84. With these samples, Dr. Miller's lab performed a genomic analysis and found that there was a very strong genomic response in the fish that tested positive for the ISAV 7 sequence, and that that response was influenza-like.[140] She notes that this response is present despite the low Ct values in the PCR results, but that this does not mean that the virus is causing disease or mortality.[141] She stated that this only indicates that animals that have positive Ct values are also responding in some way that is similar to responses of mammals to influenza infection.[142]

85. These results have led her to conclude that there is a variant or divergent strain of ISAV present in Fraser sockeye that is not detectable with existing assays.[143] She notes that it is 95% similar to known strains, and that her sequence contains three fixed differences that have been present in Fraser sockeye (and pinks) since at least 1986. This suggests to her that this variant strain of ISAV has existed since at least 1986, but "probably quite considerably longer than

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[136] Dr Kristi Miller, 15 December 2011, pp 22:40 to 23:36.
[137] Exhibit 2043: Excel Spreedsheet of ISAV Results (Garver, comparing Genetics lab protocol with Gagné lab protocol).
[138] Nellie Gagné, 15 December 2011, p 24:9-20.
[139] Dr Kristi Miller, 15 December 2011, p 24:22-30.
[140] Dr Kristi Miller, 15 December 2011, pp 48:28 to 49:38.
[141] Dr Kristi Miller, 15 December 2011, p 50:26-34, pp 87:28 to 88:21.
[142] Dr Kristi Miller, 15 December 2011, p 87:28 to 88:16.
[143] Dr Kristi Miller, 15 December 2011, p 22:2-39.

that."[144] Dr. Miller testified that it has not been established that this ISAV variant causes disease.[145]

86. Dr. F. Kibenge was largely in agreement with Dr. Miller's conclusions.[146] Ms. Gagné, however, was more cautious in her interpretation of Dr. Miller's results. Ms. Gagné noted that the results show very weak positives using different primers for different segments of the virus, and that this was difficult to interpret and warranted further testing.[147]

87. Dr. Nylund was sceptical of Dr. Miller's methodology and conclusion. He noted that the methodology used by Dr. Miller was novel and not standard, and may lead to false positives through 'non-specific annealing', where the primers attach to random RNA or DNA, which are later amplified in the PCR process, give a false positive result.[148] In this regard, he noted that the sequences obtained by Dr. Miller were either identical to the primers she had used, or included a stop codon (which indicates the end of a sequence) in a portion of the sequence where no stop codon should exist.[149] Ms. Gagné noted that if the stop codon lies in a portion of the sequence that codes for a crucial protein, it is likely the virus cannot function.[150]

88. While Dr. Miller acknowledged the stop codon, she disagreed with Dr. Nylund that her results were due to non-specific annealing.[151]

### ii. Farmed Salmon from Creative Salmon from Winter 2010/2011

89. As part of a project looking into jaundice syndrome and winter mortality at Creative Salmon aquaculture facilities, Dr. Miller also tested farmed adult chinook samples from these facilities on the West Coast of Vancouver Island for ISAV, among other pathogens. She

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[144] Dr Kristi Miller, 15 December 2011, pp 51:45 to 52:33.
[145] Dr Kristi Miller, 15 December 2011, p 60:12-24.
[146] Dr Frederick Kibenge, 15 December 2011, p 9:1-14.
[147] Nellie Gagné, 16 December 2011, p 8:18-47.
[148] Dr Are Nylund and Dr Kristi Miller, 15 December 2011, pp 57:45 to 59:7; Dr Are Nylund, 15 December 2011, p 100:11-39.
[149] Dr Are Nylund and Dr Kristi Miller, 15 December 2011, pp 58:19 to 59:7; Dr Are Nylund, 15 December 2011, pp 104:21 to 105:6.
[150] Nellie Gagné, 16 December 2011, pp 28:15 to 29:11.
[151] Dr Kristi Miller, 15 December 2011, pp 94:45 to 95:25, p 142:6-23.

obtained positive results for ISAV, with similar Ct values and a similar prevalence as in wild Fraser sockeye. However, she noted that there is no evidence that ISAV is causing the jaundice syndrome, or other disease or mortality.[152]

### E. Ms. Nellie Gagné's Tests and Results – October-December 2011

90. Ms. Gagné of the DFO Moncton lab conducted real-time RT-PCR tests on wild Pacific salmon on six separate occasions in late 2011:

a. Sample Set #1A – whole smolts from Sample Set #1 not previously tested, Moncton testing completed on November 7, 2011;

b. Sample Set #1B – the previously necropsied carcases (hearts, kidneys and gills removed) of the 48 smolts from Sample Set #1, Moncton testing completed on November 16, 2011;

c. Sample Set #1B – kidney extracts from the 48 smolts from Sample Set #1, Moncton testing completed on November 3, 2011;

d. Sample Set #2 – adult salmon collected by Alexandra Morton at Weaver Creek, Moncton testing completed on November 15, 2011;

e. Reruns of Dr. M. Kibenge's samples (discussed above), Moncton testing completed on December 9, 2011; and

f. Dr. Miller's samples (discussed above), Moncton testing completed on December 8, 2011.[153]

91. Ms. Gagné's results were all negative, including in Sample Set #1B (the 48 smolts in which Dr. F. Kibenge obtained his first set of positive results), in Sample Set #2 (adult salmon of

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[152] Dr Kristi Miller, 15 December 2011, pp 52:34 to 53:16; pp 95:54 to 96:37.
[153] Exhibit 2038 at p 1: *Technical information for DFO Moncton, based on sample sets for lab assessment regarding ISA in BC salmon*; Exhibit 2125; Email to/from Nellie Gagné enclosing tests on 2004 Molly Kibenge's samples.

various species from Weaver Creek in which Dr. F. Kibenge obtained his second set of positive results), in the reruns of Dr. M. Kibenge's samples (some of which had tested positive), and in samples submitted by Dr. Miller (some of which had tested positive).[154] Ms. Gagné used her own segment 8 assay for these tests (which is consistent with OIE guidelines), but also retested Sample Set #1B (kidney extracts), Sample Set #2, and Dr. M. Kibenge's samples with the Snow 2006 segment 8 assay. The results were again negative.[155]

92. Ms. Gagné was also unable to culture ISAV from these samples, but noted that some strains are not culturable, including the non-virulent HPR0 strain.[156] Thus, the Moncton lab has not found any confirmed positive results for ISAV.

93. Ms. Gagné also tested the quality of the RNA from the Sample Set #1A, #1B and #2, and found that the RNA showed extensive to total degradation.[157] She also noted that the condition of the physical samples in Sample Set #1A and #1B was quite poor.[158] For these reasons, although her PCR results were negative, she qualified the results as "inconclusive."[159]

94. Ms. Gagné also explained that she obtained a very weak positive (a Ct of 38, which is close to the limit of detection for the test) in one well for one sample from Sample Set #2, though it was not a sample that had tested positive for Dr. F. Kibenge.[160] The practice of the Moncton lab is to re-extract the DNA from the sample to attempt to replicate the positive signal. After three attempts, Ms. Gagné was unable to repeat the positive result.[161] Thus, she also interpreted this result as "inconclusive."[162] She noted that she would not normally have reported such a

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[154] Exhibit 2038 at p. 1: *Technical information for DFO Moncton, based on sample sets for lab assessment regarding ISA in BC salmon*; Nellie Gagné, 15 December 2011, p 16:6-8.
[155] Nellie Gagné, 15 December 2011, p 27:36 to 28:8, p 29:13 to 30:3; pp 66:21 to 67:2.
[156] Nellie Gagné, 15 December 2011, p 45:23-28; Dr Kristi Miller, 15 December 2011, p 118:27-43.
[157] Nellie Gagné, 15 December 2011, p 16:9-24.
[158] Exhibit 2038 at p 1: *Technical information for DFO Moncton, based on sample sets for lab assessment regarding ISA in BC salmon.*
[159] Exhibit 2038 at p 1: *Technical information for DFO Moncton, based on sample sets for lab assessment regarding ISA in BC salmon*; Nellie Gagné, 15 December 2011, p 16:9-24.
[160] Nellie Gagné, 15 December 2011, pp 17:44 to 19:8.
[161] Nellie Gagné, 15 December 2011, p 17:7-31.
[162] Nellie Gagné, 16 December 2011, p 22:28-41.

result, but would have waited and attempted to reproduce the result first.[163] She explained the possibility that this was a false positive as follows:

> In our hands, this is -- this can be false positives, and the company employed [Applied Biosystems] can confirm this, they have document about that. You can occasionally see a signal in one well, close to the limit of the assays, which can be due to the reporter, the fluorescence being present due to priming between your primers and probes, and the probe gets degraded and that creates fluorescence, but it doesn't mean you have a specific result.[164]

### F. Test Results – Expert Witness Conclusions and Next Steps

95. In the absence of further inquiry, tests and research, the scientist do not have a clear answer as to why different labs are obtaining different PCR results from the same fish. Dr. F. Kibenge thought that it was possible that the differences were due to the fact that different labs were testing different tissues from the same fish. He noted that further study is required to determine which tissues may be best to sample in Pacific salmon.[165]

96. Dr. F. Kibenge also suggested that the different methodologies used in the various labs can have an outcome on the results of the test, even when using the same primers.[166] A paper co-authored by Ms. Gagné in 2005 explains the difficulty in repeating results in different laboratories.[167] The scientists agreed that diagnostic protocols and interpretation of test results should be standardized across all laboratories.[168]

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[163] Nellie Gagné, 15 December 2011, p 17:12-31.
[164] Nellie Gagné, 15 December 2011, p 17:32-43.
[165] Dr Frederick Kibenge, 15 December 2011, pp 31:26 to 32:35.
[166] Dr Frederick Kibenge, 15 December 2011, pp 32:36 to 33:8.
[167] Exhibit 2003: P Nérette et al, "Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus" Journal of Fish Disease (2005); Dr Frederick Kibenge, Dr Kristi Miller, Dr Are Nylund and Nellie Gagné, 15 December 2011, pp 86:20 to 87:5.
[168] Exhibit 2003: P Nérette et al, "Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus" Journal of Fish Disease (2005); Dr Frederick Kibenge, Dr Kristi Miller, Dr. Are Nylund and Nellie Gagné, 15 December 2011, pp 86:20 to 87:5.

97. Dr. Miller stated that it was likely that whatever is being detected in BC is simply too variant to be picked up consistently with existing assays, although she did note that Ms. Gagné's assay should detect what she has so far sequenced.[169]

98. Importantly, based on the positive results found by Dr. F. Kibenge and Dr. Miller, the scientists agree that further inquiry, research and in particular, sequencing information is needed to determine whether there is an ISAV-like virus or other orthomyxovirus present in BC. Although Dr. Miller reported 95-100% similarity to known ISA strains based on her sequencing of a portion of segment 7, Dr. Nylund identified the presence of a stop codon within these sequences (as agreed by Dr. Miller above), which he and Ms. Gagné suggest means that this gene would be non-functional.

99. And while Ms. Gagné agrees that more sequence information is needed, she also noted that known ISA viruses have existed in nature for thousands of years and have evolved with their hosts, and that the current level of presence of the virus is below even a 'carrier' level in a non-susceptible species.[170]

100. However, none of the above test results (either from individual labs or taken collectively) mean that ISAV has been detected in BC – the PCR positives, even assuming they are all true positives, only indicate that the virus is suspected to be present according to the OIE definitions.[171] The PCR positive results also do not meet CFIA's case definition for confirmed ISAV infection.[172]

101. There are PCR results for segments 7 and 8, and a partial sequence of segment 7, but it is unknown if the positive results are detecting a variant virus, and if so, whether it is virulent or avirulent. There is presently no evidence that whatever is being detected causes disease or mortality in wild or farmed fish. Again, further inquiry and research is needed.

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[169] Dr Kristi Miller and Nellie Gagné, 15 December 2011, p 33:14-39.
[170] Nellie Gagné, 15 November 2011, pp 60:25 to 61:25.
[171] Exhibit 1676 at pp 232-233: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[172] Exhibit 2106 at p 5: *Draft ISAV Hazard Specific Plan*, 21 April 2011.

102. If there is a previously undetected virus (or viruses) in wild and farmed Pacific salmon, more sequence information should be obtained or the new virus should be cultured so that its relationship to known viruses can be determined. Sequence information is also needed to develop and validate assays that are specific to any new virus.[173]

## V. CFIA Investigation

### A. CFIA Response to Confirm Report of an Infectious Disease

103. As noted above, CFIA was notified by Dr. F. Kibenge of presumptive positive ISAV test results on two separate occasions: October 15 and 20, 2011. In late November and early December, Dr. Miller notified CFIA (directly and indirectly via Mr. Stephen) of her test results.

104. In response to these notifications, CFIA has taken the following steps, each of which are discussed in more detail below:

a. CFIA sought samples from the Dr. F. Kibenge and Dr. Miller for corroborative testing at Ms. Gagné's NAAHLS reference lab in Moncton.

b. CFIA traced the origin of the samples tested by Dr. F. Kibenge, Dr. Nylund in Norway and Dr. Miller.

c. CFIA investigated the chain of custody for the various samples collected and tested by others.

d. CFIA placed quarantine orders pursuant to s. 6 of the *Health of Animals Act* on samples collected by Simon Fraser University and the University of BC identified in its chain of custody investigation.

e. CFIA conducted confirmatory testing on the samples that had been collected, through Ms. Gagné's ISAV reference lab in Moncton.

[173] Dr Frederick Kibenge, 16 December 2011, pp 46:17 to 47:31.

f. CFIA commissioned an assessment of Dr. F. Kibenge's and Ms. Gagné's labs as part of the investigation using an independent scientist from the University of Guelph.

g. CFIA interpreted the test results pursuant to a number of factors, including principles of diagnostic veterinary medicine, and concluded that the samples tested by Dr. F. Kibenge and Dr. Are Nylund were negative for ISAV. The testing and interpretation of Dr. Miller's findings is ongoing.

h. CFIA, in collaboration with DFO informed the public, international trade partners and provincial authorities of the results of the investigation on an ongoing basis.

i. A surveillance plan to confirm the presence or absence of ISA (and other diseases) has begun in collaboration with DFO, with the aim of implementing it in early 2012 after consultation with stakeholders.

105. Dr. Klotins testified about the steps taken by CFIA to investigate the test results reported by Dr. Routledge (who is a statistician, not a biologist[174]), Ms. Morton, Dr. F. Kibenge, and also the subsequent test results from Dr. Miller's lab at the PBS. In addition, the CFIA Situation Reports and DFO Issue Updates provide an overview of the progress of this investigation. In addition, the CFIA produced timelines of the investigation.[175]

## B. Background – Plans and Procedures

### i. Aquatic Animal Health Functional Plan (Draft, September 1, 2010)

106. The Aquatic Animal Health Function Plan represents an overarching view of how CFIA would conduct disease response internally, and how CFIA would work with any partners that CFI has agreements with. To support this plan with specific policies and procedures, CFIA has developed a policy for the receipt and processing of a mandatory notification and determination

[174] Exhibit 2070: Curriculum Vitae of Dr Rick Routledge, 20 October 2011.
[175] Exhibit 2107: CFIA Situation Reports; Exhibit 2109: Issue Updates – as per calls with CFIA, 7 December 2011; Exhibit 2141: ISAV #1 timeline; Exhibit 2142, ISAV #2 timeline; Exhibit 2143: Work flow timeline.

of an initial inspection. CFIA has developed procedures describing the steps to be taken for notification and undertaking investigations within CFIA. For example, to support the Aquatic Animal Health Function Plan, hazard specific plans and sampling procedure have been developed.[176]

### ii. ISAV Hazard Specific Plan

107. CFIA has developed a Hazard Specific Plan starting with four diseases, one of which is ISAV, that provides a specific disease response for each.[177] One purpose of the Hazard Specific Plan is to define what in the circumstances is a "positive" test for ISAV at it applies to an individual fish, or for a fish population.[178]

### iii. Sampling Procedures

108. CFIA has also developed sampling procedures for cultured finfish, cultured molluscs and crustaceans that help with the Aquatic Animal Health Functional Plan.[179]

## C. Collection and Quarantine of Samples

109. The CFIA initiated the investigation into the possible presence of ISAV in BC after CFIA Notification #1, CFIA Notification #2, and correspondence with Dr. Miller and Dr. Nylund, as described above.[180]

110. For each of the notifications CFIA had to determine where the samples and their various sub-sets were located, whether they should be quarantined, and the chain of custody (where they had come from, how they had been collected, stored, and transported to address concerns

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[176] Exhibit 2105: *Draft Aquatic Animal Health Functional Plan*, 1 September 2010; Exhibit 2106: *Draft ISAV Hazard Specific Plan*, 21 April 2011; Dr Kim Klotins, 16 December 2011, pp 93:43 to 44:14.
[177] Dr Kim Klotins, 16 December 2011, p 94:6-10; Exhibit 2106: *Draft ISAV Hazard Specific Plan*, 21 April 2011.
[178] Exhibit, 2106: *Draft ISAV Hazard Specific Plan*, 21 April 2011; Dr Kim Klotins, 16 December 2011, p 97:1-5.
[179] Dr Kim Klotins, 16 December 2011, p 94:11-16.
[180] Exhibit 2032: Transcription News Conference, 2 December 2011; Exhibit 2005: Content of information to provide from an OIE Reference Laboratory to inform the OIE on positive results of samples on OIE listed diseases, Dr. Fred Kibenge, October 15, 2011; Exhibit 2107, *Situation Report (Internal) Update* #2, 19 October 2011; Exhibit 2107 at p 1: *Situation Report (Internal) Update #3*, 20 October 2011; Exhibit 2007: Email from Fred Kibenge to Jonathan Coady, 23 November 2011 attaching report "Alexandra Morton Samples (Sockeye Chinook and Coho) VT10142001_October 12 2011"; Exhibit 2142: ISAV #2 timeline; Exhibit 2107: *Situation Report (Internal) Update #12*, 4 November 2011; Exhibit 2014: *Report-I, 27 October 2011*; Exhibit 2015: *Report, 2nd November 2011*.

regarding cross-contamination and cross-reaction, among other things).[181] Dr. Klotins testified to the significance of investigating the chain of custody of samples (from point of collection to point of reporting and every step in between), out of concerns of cross-contamination of the samples.[182]

### i. Sample Set # 1

111. Dr. F. Kibenge of the AVC notified the CFIA of his ISA test results on October 15, 2011 (CFIA Notification #1). These tests were done on the hearts (Sample Sub-set #1B Heart) taken from a sample of 48 smolts (Sample Sub-set #1B[183]), that were part of a large sample of smolts collected by researchers at the University of British Columbia and Simon Fraser University from Rivers Inlet in BC in May and June of 2011 (Sample Set #1). [184] These heart samples were collected by CFIA operations staff and sent to the GFC in Moncton for further testing.

112. CFIA operations staff quarantined and collected the 299 samples from Sample Set #1 at Simon Fraser University (Sample Set #1A) on October 18th, 2011. The next day CFIA sent these to the GFC lab in Moncton for further testing. Simon Fraser University also has other samples collected in 2009 and 2010. CFIA placed these in quarantine onsite at Simon Fraser University.[185]

113. DFO NAAHLS staff collected the kidney extracts from the 48 smolts (Sample Sub-set #1B Kidney) located in Dr. Miller's lab at PBS on October 20, 2011, and sent these samples to the GFC lab in Moncton for further testing.[186]

114. CFIA operations staff collected the 48 smolt carcasses (Sample Sub-set #1B Carcasses) on October 21, 2011 from a private residence in Nanaimo and transported them to PBS. On October 24, 2011, DFO sent these samples to the GFC lab in Moncton for further testing.

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[181] Exhibit 2107: *Situation Report (Internal) Update #2 to #18*; Exhibit 2030: Transcription, News Conference, 8 November 2011; Exhibit 2032: Transcription, News Conference, 2 December 2011.
[182] Dr Kim Klotins, 16 December 2011, pp 97:34 to 98:18.
[183] Exhibit 2143: Work flow timeline.
[184] Exhibit 2107: *Situation Report (Internal) Update #2*, 19 October 2011; Exhibit 2143: Work flow timeline.
[185] Exhibit 2107: *Situation Report (Internal) Update #2*, 19 October 2011.
[186] Exhibit 2107: *Situation Report (Internal) Update #3*, 19 October 2011.

115. CFIA determined that the gills from the 48 smolts (Sample Sub-set #1B Gills) had been sent to Norway by Ms. Morton. On November 2, 2011, CFIA received from Ms. Morton a report from Dr. Nylund regarding these samples (and subsequently received information directly from Dr. Nylund).[187]

116. An additional 61 samples remained at the University of British Columbia (Sample Sub-set #1C). CFIA placed these under quarantine on October 20, 2011, collected them on October 25, 2011, and sent them to the GFC lab in Moncton for further testing on October 26, 2011.

### ii. Sample Set #2

117. On October 20, 2011, in a verbal report, Dr. F. Kibenge notified the CFIA of another potential ISAV detection at his AVC lab (CFIA Notification #2).[188] Eleven heart and gill samples collected at Weaver Creek in October 2011 yielded positive results at the AVC lab (Sample Set #2). Fifteen samples (5 herring hearts, 5 sockeye heart and gills) yielded negative results at the AVC lab (Sample Set #3). On October 26, 2011, CFIA collected homogenates prepared from Sample Sets #2 by Dr. F. Kibenge from the AVC and sent them to the GFC in Moncton for further testing.

### iii. Dr. Miller's Samples

118. Shortly after Mr. Stephen learned of Dr. Miller's positive test results for ISA, he informed Dr. Kiley by phone.[189] CFIA officials have interviewed Dr. Miller in connection with these test results.[190] Dr. Miller provided samples to Ms. Gagné's lab for testing (see above). In the week prior to the ISA hearings, Dr. Miller obtained further test results on chinook salmon provided by the Creative Salmon fish farm located on the West Coast of Vancouver Island, and on archived sockeye and pink salmon samples retained by Dr. Miller's lab. These test results

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[187] Exhibit 2107: *Situation Report (Internal) Update #12*, 4 November 2011; Dr Kim Klotins, 16 December 2011, p 95:2-26.
[188] Dr Kim Klotins, 19 December 2011, p 39:14-18.
[189] Stephen Stephen, 16 December 2011, p 107:31-33.
[190] Dr Kim Klotins, 16 December 2011, p 110:21-29.

were communicated to Mr. Stephen and Mr. Mark Saunders of DFO.[191] CFIA has been notified and its investigation into Dr. Miller's test results is ongoing.[192]

### iv. Testing of Samples and Interpretation of Results

119. The samples collected by CFIA were sent to Ms. Gagné's lab at the GFC in Moncton for further testing and/or storage. The results of this testing are discussed above. Ms. Gagné reported her test results to DFO and CFIA in a series of laboratory reports.[193]

120. CFIA in the course of its investigation must consider not only the RT-PCR test results received from the GFC, the AVC and other labs, but also interpret these results in light of other information available. Analytical research in isolation is not sufficient; diagnostic analysis must be undertaken to interpret analytical research results. All of the witnesses agreed that the RT-PCR test, as a screening test for ISAV, is not a perfect test and ideally requires confirmation through other research, testing and investigation. The CFIA requires further evidence, such as relevant epidemiological information (ie, clinical observation, histopathology, diagnostic test characteristic, expected prevalence of disease, and other epidemiological considerations used in diagnostic veterinary medicine). CFIA also requires results from other tests (ie, cell culture, indirect fluorescent antibody test) for confirmation of ISAV. Also, CFIA requires that PCR tests that are not validated require further research to determine their reliability and test characteristics.[194]

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[191] Dr Kristi Miller, 15 December 2011, p 51:2-3, p 66:31-33; Exhibit 2055: E-mail from Kristi Miller-Saunders to Stephen Stephen, 1 January 2001 with attachments (note: the January-01-01 date is an error; the email was communicated on 13 December 2011, Dr Kristi Miller, 15 December 2011, p 51:30-31.
[192] Dr Kim Klotins, 16 December 2011, p 110:20-29; p 144:1-4; 19 December 2011, p 28:2-6.
[193] Exhibit 2002: *Laboratory Report*, 17 November 2011; Exhibit 2036: *Laboratory Report*, 6 December 2011; Exhibit 2037, *Laboratory Report*, 1 December 2011.
[194] Dr Kim Klotins, 19 December 2011, p 27:8-23, p 29:26-45, p 43:30-37.

### v. Decision on Release of Collected and Quarantined Samples

121. Dr. Routledge and Ms. Morton have requested that CFIA return the samples collected by CFIA. The decision whether or not to return samples is one to be made, in most cases, by the local CFIA veterinary inspector that imposed the quarantine. A decision has not yet been made with respect to the samples collected from Dr. Routledge. CFIA has responded to Ms. Morton requesting further information about the samples to which she is referring; Ms. Morton has not responded to this inquiry.[195] Section 49 of the *Health of Animals Act* addresses the collection of samples in the course of an inspection. CFIA, in making the determination as to whether to retain or return the samples, must among other things ensure that the samples were not contaminated when they were sent to the lab in Moncton, or that samples are available if needed for the ongoing investigation.[196]

### vi. AVC and GFC Lab Assessments

122. A component of the CFIA investigation into the reported findings of ISAV is an assessment of the methodologies and procedures used by the labs in Canada that tested for ISAV. Both Dr. F. Kibenge's lab at the AVC and Ms. Gagné's lab at the GFC are part of this assessment. CFIA is considering undertaking an assessment of Dr. Miller's lab in PBS.[197]

123. The purpose of the assessment was to reconcile the differences in results between the two labs when testing the same samples and using the same or similar RT-PCR assays. The assessment is intended to consider the functional laboratory capability of the two labs for the diagnosis of ISAV, and in particular to:

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

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[195] Dr Kim Klotins, 19 December 2011, pp 47:39 to 48:8.
[196] Dr Kim Klotins, 19 December 2011, p 47:22-23; 16 December 2011, p 99:9-25, pp 100:26-47 to 101:1-6, p 144:5-10.
[197] Dr Kim Klotins, 19 December 2011, p 7:9-23.

b. assess conformity of ISA testing with acceptable practices (ie, OIE standards).[198]

124. The assessment will assist CFIA in determining whether the test findings in the AVC lab were true or false positives.[199] The assessments reviewed the protocols and methodologies used in the respective labs, the bio-containment and quality control measures used, the training of the staff and other factors that could inform interpretation of the inconsistent test results.[200]

125. The AVC and Moncton labs were assessed by a panel of experts, including two scientists from CFIA and an outside expert, Dr. Davor Ojkic, an avian virologist and immunologist from the Animal Health Laboratory at the University of Guelph. The site visits for the lab assessments occurred on November 18, 2011 (for the AVC) and November 17, 2011 (for the Moncton lab).[201] The reviewers followed the same process and procedure in each lab.[202] The written assessment of the AVC was prepared first and is complete.[203] The written assessment of Ms. Gagné's lab at GFC Moncton is underway; an early draft of the assessment is in evidence.[204]

## D. Assessment of the AVC Lab

### i. Cross-Contamination of the Samples

126. The report notes that there were several issues in the AVC lab that created a risk of cross-contamination of the samples being tested, including the locations of the receipt of samples, cloning and inoculation in the lab, the location of RNA extraction in the lab, and the use of non-

[198] Exhibit 2121: CFIA - Aquatic Animal Health Laboratory Assessment Working Group National Emergency Response Team (NERT), 10 November 2011.
[199] Dr Kim Klotins, December 16, 2011, p 138:38-41
[200] Dr Peter Wright, 19 December 2011, p 34:9-20.
[201] Exhibit 2122: *Summary of Information from a Document Review and On-Site Visit (November 18, 2011) for the ISA OIE Reference Laboratory at Atlantic Veterinary College*; Dr Peter Wright, 16 December 2011, pp 114:34 to 115:2.
[202] Exhibit 2121: CFIA - Aquatic Animal Health Laboratory Assessment Working Group National Emergency Response Team (NERT), 10 November 2011; Dr Peter Wright, 16 December 2011, pp 114:34 to 115:2.
[203] Exhibit 2075: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[204] Exhibit 2074: Infectious Salmon Anaemia (ISA) Laboratory Assessment: NAAHLS Laboratory Global Fisheries Center, Department of Fisheries and Ocean.

dedicated equipment (pipettes).[205] The report further noted that the positive controls used in the AVC lab had low Ct values, and were, thus a possible source of cross-contamination. It also noted that the positive controls were genomic RNA, which makes distinguishing between true positives and cross-contamination difficult.[206]

127. By contrast, Ms. Gagné and Dr. Nylund both described some of the practices normally used in labs to avoid cross-contamination, including taking in samples in an area separate from where the work is done, separate rooms for each stage of the RT-PCR process, the use of different pipettes at different stages of the test, and the use of an artificial insert into positive controls to distinguish them from true positives.[207]

128. Ms. Gagné also noted that when she tested the quality of the RNA in a reference gene in the samples, she obtained a no Ct, which she has never seen before. She stated that she would normally have rejected such degraded samples. She further noted that one would not expect a positive result with such extensive degradation, as the RNA in the virus degrades along with the RNA in the fish. She suggested that one possible explanation for the positive results despite the RNA degradation was the contamination of Dr. F. Kibenge's samples.[208]

129. Dr. F. Kibenge stated several times in his testimony that he had ruled out cross-contamination as a possible cause of his positive results, but did not elaborate.[209] He also disagreed with the statement in the report that his positive controls were a potential source of cross-contamination, although Ms. Gagné testified that they were.[210] Ms. Gagné also stated that she would have taken additional precautions, including the introduction of blanks alongside the

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[205] Exhibit 2075 at p 11: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[206] Exhibit 2075 at Appendix 2, p 5: Exhibit 2075, *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[207] Dr Are Nylund and Nellie Gagné, 15 December 2011, pp 75:41 to 77:40.
[208] Nellie Gagné, 16 December 2011, pp 15:45 to 18:23.
[209] Dr Frederick Kibenge, 16 December 2011, p 18:29-32, p 39:6-12.
[210] Dr Frederick Kibenge and Nellie Gagné, 16 December 2011, pp 13:13 to 14:8.

samples.[211] She noted that the report shows deviation from what should be done to avoid cross-contamination.[212]

### ii. Test Procedures

130. The report notes a number of issues with the test procedures used in the AVC lab, including: the lack of testing of the quality of the RNA in the samples, inconsistencies in the concentration of the RT-PCR master mix, and aberrant results in the negative and positive controls were not reported or explained.[213] On the first point, the report notes that the failure to test the quality of the RNA means that the extent to which RNA degradation may have affected the test results cannot be known.[214] On the third point, the report states that when a negative or positive control is reactive when it should not be, the proper procedure is to retest.[215]

### iii. Interpretation of Test Results

131. Numerous issues with the AVC lab interpretation of RT-PCR results are noted in the report, including:

a. Three of five positives were reported on the basis of only one well (of two) having a positive result;

b. A lack of consideration for the shape of the curve in the interpretation of the results, including a reporting of 'flat' curves as positive results; and

c. A reliance on Ct values alone in the interpretation of results without any visual inspection of the curves.[216]

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[211] Nellie Gagné, 16 December 2011, p 18:25-34, pp 19:39 to 20:10.
[212]Nellie Gagné, 16 December 2011, p 20:4-10.
[213] Exhibit 2075 at p 11: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[214] Exhibit 2075 at p 11: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[215] Exhibit 2075 at p 7: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[216] Exhibit 2075 at p 7: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011. Exhibit 2123: LC480 Data Analysis of ISAV Testing at AVC,

132. On the first point, Ms. Gagné explained the practice of running a PCR test on multiple wells from the same sample. If a sample is positive, one should see the same Ct values in both wells, except where the Ct value in one well is very high (ie, a weak signal). She stated that a positive signal in one well indicates that the sample should be retested.[217] The report also states that Dr. F. Kibenge ought to have retested samples that were only positive in one well, rather than reporting them as positives.[218]

## VI. DFO Aquatic Animal Health Science

133. Pursuant to its MOU with CFIA, DFO supports CFIA by providing scientific and laboratory support for diagnostic testing for the NAAHP. Under the MOU, DFO also supports CFIA by conducting research utilizing NAAHP funding, DFO also conducts research into ISA outside the auspices of the NAAHP for its own fish health and fish management purposes, including through the work of Dr. Miller on genomic signatures and Dr. Garver on the monitoring of ISAV and other pathogens.[219] There is an important distinction to be drawn between the diagnostic research and testing to support the requirements of CFIA to determine whether there is confirmation of a disease, and the analytical and academic research undertaken by DFO scientists on ISA and other diseases and pathogens.

134. In DFO, there are two streams of work related to aquatic disease issues:

a. The bulk of the work takes place under the aquatic animal health program activity element. This is in turn divided into NAAHP and non-NAAHP diagnostic and research activities. The NAAHP activities are focussed on testing for the various NAAHP programs, including export certification, surveillance, response to a notification, and on targeted research for the development and validation of

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29 November 2011.
[217] Nellie Gagné, 16 December 2011, pp 72:17 to 73:12.
[218] Exhibit 2075 at p 7: *Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory, Atlantic Veterinary College*, 14 December 2011.
[219] Stephen Stephen, 16 December 2011, p 108:12-45; Dr Kim Klotins, 16 December 2011, p 132:26-32.

improved diagnostic procedures for regulatory work. This stream of work has a very strong emphasis on standardization, test validation and consistency and these labs are targeting International Organization for Standardization 17025 certification.[220] The non-NAAHP activities include diagnostic and vet support for enhancement facilities and research into the identification and characterization of pathogens and disease and impacts on many aquatic animals, including salmon.

b. The second stream takes place under departmental funding such as the Biotechnology and Genomics program and is aimed at broad-based improvements to DFO's broad regulatory activities, through the adoption of leading-edge genomics research and biotechnology tools and techniques. This includes research into pathogens and their effects on host gene response. This is aimed at developing methods to predict salmon performance and survival. This is the area in which Dr. Miller and the PBS Molecular Genetics laboratory operates.[221]

135. Mr. Stephen testified to the support that DFO is giving to the funding of analytical research into ISAV and other diseases and pathogens that may affect Pacific salmon. In particular, he noted that Dr. Miller's research as part of the Genomics Research and Development Initiative has just been granted $462,000 over the next three years for further genomics research into parvovirus and related research. This research represents 20% of the Genomics Research and Development Initiative budget administered by Mr. Stephen in his role as Director of the Biotechnology and Aquatic Animal Health Science Branch of DFO. Since 1999, Dr. Miller has received $2.8 million in funding from this source.[222] Ms. Gagné's lab at GFC in Moncton will receive $171,600 dollars over the same period for research into non-pathogenic ISA. [223]

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[220] Nellie Gagné, 15 December 2011, p 65**:**4-19; Dr Peter Wright, 19 December 2011, pp 32:46 to 33:18.
[221] Dr Kristi Miller, 15 December 2011, pp 48:34 to p 49:21. Dr Miller also received research funding from the DFO Aquaculture Collaborative Research and Development Program among other program funding.
[222] Stephen Stephen, 16 December 2011, p 109:2-28.
[223] Nellie Gagné, 15 December 2011, p 80:15-36.

136. Dr. Wright testified to the somewhat different requirements and standards required to confirm the presence of ISA or ISAV in accordance with requirement of the *Health of Animals Act* and standards set by the OIE. Dr. Wright testified about the distinction between analytical and diagnostic testing in the field of regulatory veterinary medicine. For example, diagnostic laboratories have to meet particularly stringent quality control and bio-containment standards, and for that reason in many labs the diagnostic and analytical research labs are physically separated. DFO laboratories in particular are operating under and working towards the high quality standards required under an International Organization for Standardization 17025 designation.[224]

137. DFO diagnostic testing on behalf of CFIA and under NAAHP requires the use of validated tests. The standards for a validated test are set out in the OIE Diagnostic Manual. The OIE does not require that any particular test be used, but rather that the test used meets certain standards and criteria. The NAAHP requires that tests meet these validation criteria with all the enumerated performance requisites.[225] The assay for ISA used by Ms. Gagné at DFO Moncton is considered comparable to other segment 8 assays published by Snow and Plarre. All of these assays meet this validation requirement.[226]

138. Dr. Wright noted that the steps in this testing are governed by the OIE validation pathway. The OIE has developed a "validation template" that specifically requires a test to be fit or suited for its intended purposes (ie, as a screening or confirmatory test). This is considered a key criterion for validation. The OIE template incorporates four distinct phases for validation. Moreover, the OIE has created a registry for diagnostic tests that fulfil these validation requirements.[227]

139. Stage 1 of the validation pathway is an analytical validation of the assay used, including the repeatability, sensitivity, inclusivity (and exclusivity) of the assay. This testing of the

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[224] Dr Peter Wright, 19 December 2011, pp 17:36 to 18:18.
[225] Dr Peter Wright, 16 December 2011, p 102:6-13, p 103:1-21, p 140:5-46; Exhibit 1676: "Chapter 2.3.5. Infectious Salmon Anaemia" *Manual of Diagnostic Tests for Aquatic Animals* (2009).
[226] Dr Peter Wright, 16 December 2011, p 102, 37-43.
[227] Exhibit 2117, Peter Wright et al "Development of a Framework for International Certification by OIE of Diagnostic Tests Validated as Fit for Purpose"; Dr Peter Wright, 16 December 2011, p 141:21-47.

"analytical sensitivity" of the assay used is looking at the limit of detection of that assay.[228] At this stage, the validation is the "scientific proof that the tests that you're using actually works and that it's repeatable and it's reliable."[229]

140. Stage 2 of the validation pathway is a diagnostic evaluation of the assay used. This stage looks at the performance of the assay in the context of its ability to detect disease or exposure in animals. At this stage, results are expressed in terms of probabilities. That is, "what's the probability that if [the sample] tests positive or that if you have an infected animal that it will test positive or, on the other hand, the specificity if you have a non-infected, non-diseased animal it tests negative."[230]

141. Finally, the competent authority interprets the test results in accordance with a number of diagnostic considerations, including clinical indicators and other contextual factors, in accordance with epidemiological principles.[231] Dr. Klotins testified that the test results are but one factor in the veterinary diagnosis: "[s]o even in veterinary medicine, when we get test results it is not the laboratory that makes the determination of the disease or not. They tell us under their protocols they believe the tests are positive or inconclusive and then the clinician makes the decision, the interpretation on what those test results actually mean to the patient."[232]

142. This rigorous validation exercise is required to satisfy strict regulatory animal health requirements and to satisfy trade partners and external stakeholders. As Dr. Wright put it, "[s]o basically what it's [the validation process] doing is it is providing a tool for the program to use to either detect and/or manage disease and to qualify animals for movement. It supports the import/export. It supports all kinds of things. But you need all of those credentials in place in order to be able to withstand any type of scrutiny of the testing you're doing and the reliability of the results that you're generating."[233]

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[228] Dr Peter Wright, 16 December 2011, p 101:7-22.
[229] Dr Peter Wright, 16 December 2011, pp 138:38 to 139:4.
[230] Dr Peter Wright, 16 December 2011, p 101:23-34, p 139:5-27.
[231] Dr Peter Wright, 16 December 2011, p 101:35-42.
[232] Dr Kim Klotins, 19 December 2011, p 43:30-37.
[233] Dr Peter Wright, 16 December 2011, p 139:29-37.

143. Some witnesses testified to the process by which analytical (or "pure") research is incorporated on an ongoing basis into diagnostic testing methods and validation approaches.[234] Dr. Wright emphasized that this process is a multi-disciplinary one involving scientists who conduct research, scientists who develop diagnostic tests and epidemiologists.[235]

144. The implications of this distinction between testing for research purposes, and for diagnostic testing of reportable diseases for regulatory purposes, is clearly illustrated by the testing and results generated by Dr. Miller, an expert in molecular genetics, using assays for ISA that were not validated in accordance with OIE standards for diagnostic veterinary medicine. However, if the research of Dr. Miller and others demonstrates that a strain of ISAV exists in Pacific waters, and it is not picked up by existing validated ISAV pathways, a procedure exists for updating the assays and methodologies to incorporate the results of research into the validation process.[236] As Dr. Wright testified, there is a rigorous process by which a research tool is validated for use as a diagnostic tool.[237]

## VII. Reporting and Communications

145. Canada has an obligation for reporting on disease issues to a variety of audiences, including trade partners and international organizations (OIE) responsible for protecting animal health worldwide. In addition, Canada communicates regularly on these matters with academic and other institutions involved in disease research and diagnostics and the general public.

146. With respect to the reported findings of ISAV in Pacific salmon, the CFIA communicated with and briefed key trading partners, and authorities in other provinces, about the results of the

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[234] Stephen Stephen, 16 December 2011, p 110:4-14; 19 December 2011, p 9:37-47; Dr Kim Klotins, 19 December 2011, p 9:28-35.
[235] Dr Peter Wright, 19 December 2011, p 10:2-15.
[236] Dr Peter Wright, 16 December 2011, p 103:38-44.
[237] Dr Peter Wright, 16 December 2011, pp 110:34 to 111:5; 19 December 2011, p 30:39-45, p 31:17-27; Exhibit 2117: Peter Wright et al, "Development of a Framework for International Certification by OIE of Diagnostic Tests Validated as Fit for Purpose".

investigation.[238] CFIA Policy and Programs Branch is providing information to embassies and consulates in other counties, and with CFIA veterinarians posted abroad so that trade partners can be briefed on the investigation and have an opportunity to ask questions.[239]

147. Each audience has different requirements with respect to the type and format of the information provided. For example, when communicating with scientists and scientific international organizations, the information is usually conveyed in technical and prescribed formats.

148. Communicating with the general public requires conveying the essence of the message in terms understandable by laypersons without the use of technical language. Consequently, the precision of language is lessened relative to that used in communicating with scientific audiences and public information documents will not always have the same strict standards of accuracy used in academic or international forums. Added to this, public information documents are not always written by scientists or technicians.

149. There was some attention given in the evidence to Ministerial communication statements and transcripts of technical briefings of October 24, November 8 and December 2, 2011.[240] The Ministerial statements state that there has never been any confirmed finding of ISA in BC, which is correct. As Ms. Gagné stated in response to questions regarding the Ministerial statements, there is not yet any evidence of the disease ISA in BC salmon.[241]

150. There was criticism in the questioning of there being no reference to science researchers' findings of presumptive positive results. As Mr. Stephen explained presumptive positive results

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[238] Dr Kim Klotins, 19 December 2011, p 36:12-23, p 37:24-29; Exhibit 2138: Aquatic Animal Health's Technical Briefing Regarding the Reported Suspect Finding of Infectious Salmon Anaemia Virus (ISAV) in BC, 10 November 2011.
[239] Exhibit 2107, *Situation Report (Internal) Update #4*, 21 October 2011.
[240] Exhibit 2028: Federal Investigation into Infectious Salmon Anaemia Virus in British Columbia Salmon, 24 October 2011; Exhibit 2089, Statement from the Federal Minister of Fisheries and Oceans Canada, Keith Ashfield and British Columbia Minister of Agriculture, Don McRae on new test results indicating that there are no confirmed cases of ISA in British Columbia Salmon, 9 November 2011; Exhibit 2004, Statement from the Federal Minister of Fisheries and Oceans Canada, Keith Ashfield, on Negative Infectious Salmon Anaemia Test Results in British Columbia Salmon, 2 December 2011.
[241] Nellie Gagné, 16 December 2011, pp 27:3 to 28:5.

are not normally reported until validation tests have been done. This is because when a new, unexpected presumptive result is found it is prudent and sound science to investigate whether something has truly been detected and, if so, what, before pronouncing on a presumptive finding.

151. At the time of the Ministerial statements validation testing was either incomplete or had found that the presumptive positive results could not be repeated or confirmed. On cross-examination by Mr. McDade, Mr. Stephen confirmed the accuracy of the October 24, 2011 communication statement;[242] Mr. Stephen and Dr. Klotins confirmed the accuracy of the November 8, 2011 communication statement;[243] and Ms. Gagné confirmed the accuracy of the December 2, 2011 communication statement.[244] On cross by Mr. Rosenbloom, Mr. Stephen also confirmed the accuracy of the December 2, 2011 communication statement.[245]

152. With this, the transcripts of the technical briefings were made public to provide further particulars of ongoing validation testing to determine whether ISA is present in Pacific salmon in BC. Those interested can read the technical briefings.[246]

## VIII. Future Activities in relation to Surveillance and Testing (CFIA and DFO)

### i. CFIA Surveillance Plan

153. CFIA and DFO are presently working on a draft Surveillance Plan for ISAV, IPNv and IHNv in Anadromous Salmonids in BC that is presently in its second or third internal draft.[247] Once CFIA and DFO are satisfied with this internal draft, the CFIA will circulate it more broadly to external partners and stakeholders. CFIA anticipates having this surveillance plan in place by the late spring of 2012.[248] The present draft suggested a proposed sampling of 3,850 wild and

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[242] Stephen Stephen, 19 December 2011, pp 39:28 to 40:7.
[243] Stephen Stephen and Dr Kim Klotins, 19 December 2011, pp 41:22 to 43:37.
[244] Nellie Gagné, 16 December 2011, pp 26:34 to 29:27.
[245] Stephen Stephen, 19 December 2011, pp 70:34 to 72:43.
[246] Exhibit 2030: Transcription, News Conference, 8 November 2011; Exhibit 2032: Transcription, News Conference, 2 December 2011.
[247] Exhibit 2112, *Surveillance Plan for ISAV, IPNV, and IHNV in Anadromous Salmonids in British Columbia*, November 2011.
[248] Dr Kim Klotins, 16 December 2011, p 120:37-42 and p 121:1-14.

farmed fish per year, including six Pacific salmon species, 350 animals per species and per population.[249]

154. Surveillance results are public and transparent. CFIA will provide their surveillance reports to the public.[250] There will consultation in developing the surveillance plan, likely in January.[251]

155. CFIA selected ISAV, IPNv and IHNv to be part of the draft surveillance plan as other countries are asking Canada to demonstrate freedom from these diseases. Without the testing, CFIA will be unable to establish. As further information becomes available this list of viruses/disease may be lengthened. CFIA keeps an eye on what is happening globally in terms of infectious diseases in salmon.[252]

156. CFIA has implemented a similar surveillance plan for molluscs on the west coast that is used to demonstrate the health status of oysters and manila clams to other importing countries. The CFIA is designing the salmonid surveillance plan to be consistent with OIE standards so that it will be accepted by importing countries[253] Mr. Stephen noted that the NAAHP has not yet been audited by a foreign country but expects the EU to do an assessment next year.[254]

157. The CFIA is designing the surveillance plan in a manner that will take into account any new scientific information regarding test methodologies and epidemiology of the viruses and the diseases, such as some of the concerns found in Dr. Miller's results. CFIA will continually review their methodologies to ensure they are using the appropriate tests in terms of sensitivity and specificity to overcome any limitations of the ISA test.[255]

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[249] Exhibit 2112 at p 13, *Surveillance Plan for ISAV, IPNV, and IHNV in Anadromous Salmonids in British Columbia*, November 2011.
[250] Dr Kim Klotins, 19 December 2011, p 67:1-5.
[251] Dr Kim Klotins, 19 December 2011, pp 59:33 to 60:3.
[252] Dr Kim Klotins, Dr Peter Wright and Dr Simon Jones, 19 December 2011, pp 60:22 to 61:19.
[253] Dr Kim Klotins, 19 December 2011, p 26:16-41.
[254] Stephen Stephen, 19 December 2011, p 26:42-47.
[255] Dr Kim Klotins, 19 December 2011, p 29:1-19, pp 63:39 to 64:7.

158. CFIA does not publicly report preliminary results, they have to confirm the results first.[256]

159. CFIA's duty is to prove facts and verify the presence, or absence, of any disease that has impacts for all Canadians, First Nations, fishers, aquaculture, and their international partners.[257]

### ii. DFO Testing

160. DFO has previously conducted tests of wild salmon for ISA.[258] In 2010/2011 DFO conducted test of 637 and 232 Georgia Strait sockeye, respectively, and all tests for ISAV were negative. [259] These tests were conducted by the National Aquatic Animal Health Program laboratory in Nanaimo by Dr. Garver. Further, Dr. M. Kibenge conducted her 2004 post-doctoral research report on ISA.[260] Dr. Jones testified:

> 1But to determine whether there is a virus, don't
> 2 we go through this sequence, if I can call it
> 3 that, of determining by lab of a positive result,
> 4 then sequencing, culturing, and then determining
> 5 whether there's a pathogenic event going on that
> 6 may be killing fish. Do you agree with this?
> 7 Absolutely, and we have obtained samples
> 8 from Fraser River sockeye, both in the virology
> 9 and the parasitology program over many, many
> 10 years. We have never seen any evidence of
> 11 clinical disease that would be typically
> 12 associated with ISAV. We've never seen pathology,
> 13 or we've never isolated the virus. There's no
> 14 information that would lead us to believe that
> 15 that finding was a real finding.[261]

161. Once DFO became the primary regulator for aquaculture in Dec 2010, DFO established a Fish Health Audit and Surveillance program modelled after the program that the Provincial

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[256] Dr Kim Klotins, 19 December 2011, p 38:3-6.
[257] Stephen Stephen, 16 December 2011, p 113:1-19.
[258] Stephen Stephen, 19 December 2011, p 49:39-47.
[259] Exhibit 2145: Email from Laura Hawley to Kyle Garver, 9 November 2011.
[260] Stephen Stephen, 19 December 2011, p 50:1-45.
[261] Dr Simon Jones, 19 December 2011, p 75:1-15.

Government had been operating. (A description of this program is found at exhibit 1662, and Dr. Mark Sheppard testified on August 31, 2011, that DFO operates that program.)[262]

162. The purpose of this activity is the diagnostics/detection of an exotic disease (to the province) and/or emerging infectious diseases. Sites are selected by random sampling and targeted sampling. Sampling was aimed at achieving a 95% confidence of detection of 2% disease prevalence among farmed fish during a quarter.[263]

163. DFO improved on the Provincial Audit program by granting the DFO fish health staff both guardian and inspector powers under the *Fisheries Act* which allows them to enter sites and obtain samples without the permission of the farm operator.[264]

## IX. CONCLUSION

164. Based on the testing and analysis to date there is no confirmed finding ISA is present in salmon in BC. While there are indications that a virus of some form is circulating in the wild population in BC, it remains to be determined whether the presumptive positive findings are true positives. Further inquiry is needed and this is being done with dispatch.

165. Moreover, if further tests or surveillance result in positive tests for ISAV, IHNV, or IPNV, the CFIA will determine if its case definitions have been met. If they have, then the OIE and/or Canada's trade partners, and Canadians will be informed of the changed health status of wild and cultured salmonids in BC. It is important to proceed in a planned, focused way in accordance with sound science.

166. The scientists testified that even if ISAV is present there is no evidence that it is virulent for wild sockeye salmon. On the contrary, the OIE Diagnostic Manual states that the only known natural outbreaks of ISA have been in farmed Atlantic salmon. In Chile which has proven cases

[262] Dr Mark Sheppard, 31 August 2011, p 23:5-19.
[263] Dr Mark Sheppard, 31 August 2011, pp 57:37 to 58:26.
[264] Andrew Thomson, 1 September 2011, pp 25:26 to 26:25, p 75:19-43.

of ISA, wild fish populations have not been affected, nor have farmed coho populations.[265] There has been no mass die-off in farmed fish in BC.

167. With respect to the mandate of this Commission, after hearing the testimony and reviewing the evidence of the last three days of hearings, Canada confirms its statement in paragraph 287 of its Final Submission regarding the causes of the decline in Fraser sockeye stocks. The new evidence given in the December 2011 hearings does not indicate that any different weight should be attributed to disease being a factor in the decline experienced in 2009 than is the case before hearing this new evidence. Most certainly, further inquiry into the recent reports of ISAV is called for before reaching definitive conclusions, as noted and as supported and recommended by the scientists who testified in December.

168. Since the Final Submissions were completed, the focus of the hearings has shifted from the parvovirus to the ISA virus. The rapidity of the shift is demonstration of how quickly science in this area can evolve. Despite this change in focus, Canada reiterates points made in paragraphs 313 to 316 of its final submission with respect to the topic of disease which stress that more research is required and speculation should be avoided pending conclusive findings. No scientific information to date has indicated that either the parvovirus or ISA virus is negatively impacting Pacific sockeye salmon.

169. Regarding future sustainability, Canada has indicated that it is continuing to fund research into virus issues including parvovirus and ISA. Moreover, CFIA in partnership with DFO has begun work on a more intensive and extensive surveillance program to monitor this situation.

[265] Dr Kim Klotins, 19 December 2011, pp 25:30 to 26:1.

170. Given the significance of the wild salmon fishery to First Nations, Canada also recognizes the need to consider appropriate Aboriginal involvement in these monitoring and reporting programs.

All of which is respectfully submitted,

Dated this 29th day of December, 2011.

for G. McNeill

Mitchell Taylor, Q.C.
Counsel for the Participant Government of Canada

# INDEX OF AUTHORITIES

## Legislation



## Secondary Sources