Affidavit #2 of Dr. Gary D. Marty
Affirmed April 26, 2011

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

In the matter of Her Excellency the Governor General in Council, on the recommendation of the Prime Minister, directing that a commission do issue under Part I 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.

AFFIDAVIT

I, Dr. Gary D. Marty, in the City of Abbotsford in the Province of British Columbia, AFFIRM THAT:

1. I am a Veterinary Pathologist and am employed as the Fish Pathologist with the Animal Health Centre, of the Ministry of Agriculture and as such have personal knowledge of the matters hereinafter deposed to, save and except where stated to be based upon information and belief, and where so stated, I verily believe the same to be true.

2. Veterinary pathology is the science that studies disease in animals. Veterinary pathologists examine animal tissue and body fluids to diagnose disease and predict outcomes. By determining causes of disease, veterinary pathologists help maintain herd health and establish if there is a risk to humans

handling or consuming the meat or milk of food animals. With experience in diseases of multiple species, veterinary pathologists are uniquely qualified to perform studies to advance our understanding of the cause and methods to prevent disease in animals and humans. Attached to this my affidavit as Exhibit "A" is a true copy of how the American College of Veterinary Pathologists describes my profession on its website, which is located at the following URL: http://www.acvp.org/public/WhatIsVetPath.cfm.

3. All members of the American College of Veterinary Pathologists (Diplomate ACVP) have completed a doctorate in veterinary medicine degree (DVM) and at least three years of post-veterinary school training in veterinary pathology. In addition to these minimum requirements, I have a bachelor's of science degree (BSc) in Fisheries and Wildlife Biology, a master's of science degree (MSc) in Fisheries Biology, and a Ph.D. in Comparative Pathology. Attached to this my affidavit as Exhibit "B" is a true copy of my *curriculum vitae.*

4. The Animal Health Centre, of the Ministry of Agriculture operates an accredited Veterinary Medical Diagnostic Laboratory. To be accredited there are a number of stringent requirements that must complied with. Attached to this my affidavit as Exhibit "C" is a true copy of the Requirements for an Accredited Veterinary Medical Diagnostic Laboratory from the American Association of Veterinary Laboratory Diagnosticians (AAVLD), Inc.

5. The AAVLD has training and experience requirements for veterinary pathologists in an Accredited Veterinary Medical Diagnostic Laboratory (p. 22 of Exhibit "C"). The minimal qualifications are a DVM and MSc or 2 years residency in pathology; the preferred qualifications are a DVM, PhD, Diplomate ACVP, + 5 years of experience. By 1996 I had completed all of the preferred education and certification qualifications, and I now have 15 years of relevant experience.

6. The Animal Health Centre receives two types of cases. The first type involves analysis of samples from the science-based Fish Health Auditing and

Surveillance Program. The second type involves analysis of samples submitted directly by the aquaculture industry.

7. Mr. McDade's April 13, 2011 letter on behalf of the Aquaculture Coalition identifies 35 cases of concern from 2007 - 2010 that were based on tissues submitted directly by the aquaculture industry to the Animal Health Centre. All 35 cases include a diagnosis of sinusoidal congestion in the liver. The comments section of 34 of these case reports includes a standard sentence, "Sinusoidal congestion is one of the classic lesions associated with [infectious salmon anaemia virus (ISAV)] infection[s], but ISAV has never been identified in British Columbia." The relevant comment for case 2007-1859 is the same except that is does not include the word "sinusoidal".

8. All of my pathology reports are written with the understanding that they will be interpreted by a veterinarian with training and experience in fish medicine. I expect fish health veterinarians to understand that "one of the classic lesions associated with ISAV infection" is very different from more specific language that was not used in my reports. For example, I did not report that I had found "a lesion pathognomonic for ISAV", and I did not report that I had found "a lesion diagnostic for ISAV".

9. In my view, results from the Auditing and Surveillance Program provide a high degree of confidence that the finding of sinusoidal congestion recorded in these 35 case reports is not a result of ISAV infection.

Fish Auditing and Surveillance Records

10. The Fish Health Auditing and Surveillance Program was operated entirely by the Province from 2003 - March 2010 for both Atlantic salmon and Pacific salmon species. From July 2010 - March 2011 samples were collected and results posted as part of a similar program (Atlantic salmon only) operated by the BC Centre for Aquatic Health Sciences in Campbell River. As part of

these voluntary programs, all farm companies rearing the target species participated. For both programs, all diagnostic samples were analyzed at the Animal Health Centre in Abbotsford. Each quarter, farms for audit were selected at random from the list of all stocked farms in the Province, with selection to ensure that sampling within zones and subzones was representative of the number of stocked farms in each area. Diagnostic analysis of each fish included histopathology and bacteriology; also, fish tissues were pooled in lots of up to 5 fish each for Polymerase Chain Reaction "PCR" analysis of 5 pathogens of concern.

11. From the first quarter of 2006 though the first quarter of 2011, samples from 2,880 fish from this program were analyzed by the Animal Health Centre. All fish tested negative by PCR for ISAV.

12. Analyzed samples included livers from 2,434 Atlantic salmon examined by histopathology; of these, 188 (7.7%) had moderate or severe sinusoidal congestion in the liver. Also, livers from 446 Pacific salmon (Chinook salmon and coho salmon) were examined by histopathology; of these, 13 (2.9%) had moderate or severe sinusoidal congestion in the liver.

13. Because sinusoidal congestion in the liver is a nonspecific change that can result from anything that causes blood vessel walls to become distended, diagnostic information other than histopathology is needed to determine possible causes. In contrast, PCR tests are highly sensitive and specific for identifying the presence of infectious organisms in tissue samples.

14. Every one of the 201 cases of sinusoidal congestion in the liver tested negative by PCR for ISAV. In my view, this clearly rules out ISAV as a potential cause of sinusoidal congestion in these cases. Based on these results, I do not view the presence of sinusoidal congestion in a BC farm salmon to be worthy of reportable suspicion of ISAV. Animal Health Centre veterinary virologist Dr. John Robinson reviewed the PCR results from nearly all of these cases, and I

believe he did not view the negative PCR results for ISAV to be worthy of reportable suspicion of ISAV.

15. Additionally between 2006 and 2011 Program histopathology and PCR results were reviewed by at least one of 4 Provincial fish health veterinarians (Drs. Joanne Constantine, Ian Keith, Mark Sheppard, or Andrea Osborn) or one private veterinarian (Dr. Sonja Saksida). I believe they did not view the presence of sinusoidal congestion along with negative PCR results for ISAV to be worthy of reportable suspicion of ISAV.

16. Mr. McDade's April 13, 2011 letter mentions case 20072_P.3-8 from the Auditing program, which includes a farm diagnosis of "haemolytic anaemia"; the letter does not note, however, that all tissues from this case were negative by PCR for ISAV.

17. My confidence that ISAV is not in BC is further strengthened by 100% negative test results from every farm fish tested by PCR for ISAV as part of the BC Fish Health Auditing and Surveillance Program. Results from this program from 2003 through the first quarter of 2010 are freely available to the public at the Ministry web site which is located at the following URL: http://www.agf.gov.bc.ca/ahc/fish_health/index.htm.

18. Results from this program from the third quarter and fourth quarter of 2010 are freely available to the public at the BC Centre for Aquatic Health Sciences web site which is located at the following URL: http://www.cahs-bc.ca/fish-health-audits.php. Results for the first quarter of 2011 are not yet posted.

Records from Samples Submitted Directly by the Aquaculture Industry

19. The Animal Health Centre receives tissue samples for analysis directly from farm companies or their veterinarians. This is an important part of the

Animal Health Centre's mission as a government supported laboratory. By providing subsidized diagnostic services for the animal agriculture industry, the Animal Health Centre's goal is to increase the chance for early detection of new or foreign diseases, and to support the health of farm animals in the Province. Reasons for submission of diagnostic samples from the aquaculture industry vary. Common reasons include looking for the cause of disease in sick fish, ruling out disease in fish before they are moved, or monitoring causes of "background mortality" in fish that die on the farms but are not part of an epidemic.

20. Tissue samples are harvested in the field, preserved for shipment, and sent to the Animal Health Centre with a submission form that includes client-selected specific requests for analysis (e.g., PCR or histopathology). The aquaculture industry has options for diagnostic services, and I am usually not told whether the client also submitted samples to other diagnostic laboratories.

21. The Animal Health Centre conducts the diagnostic tests requested by the client. However, if something in the requested results warrants additional diagnostic tests, I will contact the client and recommend additional tests. My requests for additional diagnostic tests have never been refused. This helps to ensure that we detect new diseases if they occur.

22. All 35 case reports listed in Mr. McDade's April 13, 2011 letter were submitted directly by the client for analysis. AHC Case 09-3272 provides a good example of the scenario in which additional tests were warranted. The client requested PCR analysis of Infectious Hematopoietic Necrosis Virus "IHNV", Viral Hemorrhagic Septicemia Virus "VHSV", and ISAV, all which were negative. However, the heart of one fish submitted for histopathology had nonspecific lesions similar to what has been described with the disease "heart and skeletal muscle inflammation". Some scientists think that this disease might be a result of infection with salmon alphaviruses, which are a significant problem in Europe but are not known to occur in British Columbia. With the

client's approval, I ordered a PCR test for salmon alphavirus. The results were negative, and this is evidence that salmon alphaviruses are not in BC.

23. In my view, analysis of samples from both the science-based auditing program and direct submissions from the farms provides a high degree of confidence about the diseases present and not present in the BC aquaculture industry. Specifically, I have a high degree of confidence that BC is free from ISAV.

Reporting to the Canadian Food Inspection Agency

24. In December, 2010 the *Reportable Disease Regulations*, SOR/91-92 enacted pursuant to the *Health of Animals Act*, S.C. 1990 c. 21 was amended to include Infectious Salmon Anaemia ("ISA") for the first time. ISAV is the virus that causes the disease ISA.

25. I have contacted Dr. Kim Klontins, who is Acting National Manager, Disease Control Contingency Planning, Aquatic Animal Health Division, Canadian Food Inspection Agency "CFIA". Dr. Klontins informed me that the system for Federally Reportable Diseases was not in place until January 5, 2011. Therefore, any cases occurring before January 5, 2011, are NOT reportable to Federal Authorities. Attached to this my Affidavit as Exhibit "D" is a true copy of an email dated Fri 2011-04-15 5:59 a.m. with attachments from Dr. Klontins to myself confirming this information.

26. Even though reporting for the 35 cases in question was not officially required, based on what happened with an outbreak of another OIE reportable disease in August 2001, I believe that any detection of ISAV in these cases would have been reported to Federal authorities and other fish health veterinarians in BC.

27. I was advised by Dr. Joanne Constantine via e-mail on April 26, 2011, that the Province did report an outbreak of Infectious Hematopoietic Necrosis Virus ("IHNV") in farmed salmon to federal authorities in August, 2001. At the time Dr. Constantine was the fish health veterinarian for the Province; she now serves as the National Manager of the Import/Export Section of the Aquatic Animal Health Division of CFIA. An epidemiological study of this IHNV outbreak included participation by 100% of the affected farms, and the results have been published in the peer-reviewed scientific literature (Saksida, S.M. 2006. Infectious haematopoietic necrosis epidemic (2001 to 2003) in farmed Atlantic salmon *Salmo salar* in British Columbia. Diseases of Aquatic Organisms 72:213-223.).

28. All submissions to the Animal Health Centre are treated as confidential medical records, but the Ethics Bylaws of the College of Veterinarians of British Columbia (formerly the British Columbia Veterinary Medical Association) states under "Disclosure of Information" (Item 91) that "...records, a copy thereof or the information therein must be released forthwith to: (a) any party that has an urgent and compelling need for the information in order to ensure the well-being of animal(s)..." In my view, an outbreak of ISAV constitutes an urgent and compelling need to report to all fish health veterinarians, provincial, and federal authorities; therefore, I would have reported an outbreak if had occurred before January 5, 2011, even though it was not explicitly required.

29. For all 35 cases of concern, the submitting veterinarian reviewed diagnostic results from the Animal Health Centre as part of their complete analysis of the health of the fish under their care. I believe none of the veterinarians who reviewed the diagnostic results believed that there was a credible suspicion of ISAV. Also, diagnostic PCR or virology results from 22 of the cases were verified by Animal Health Centre veterinary virologist Dr. John Robinson and I am advised by him that he did not find a credible suspicion of ISAV in any of the cases he verified.

30. While I disagree with Ms. Morton's characterization of the 35 individual fish necropsies she identified, I did advise Dr. Klontins on April 14, 2011 of the existence of the 35 cases, and that based on my medical examination I do not suspect ISA in any of the cases identified.

Interpretation of Sinusoidal Congestion

31. Mr. McDade's April 13, 2011 letter on behalf of the Aquaculture Coalition identifies 35 cases of concern from 2007 - 2010. I have reviewed each of these reports, and disagree with Ms. Morton's conclusions based on these reports. Generally speaking Ms. Morton's comments are based on an incomplete reading and incorrect interpretation of the "comments" section of these reports.

32. The World Organization for Animal Health (OIE) publishes online a Manual of Diagnostic Tests for Aquatic Animals 2010 (the "Manual") which is located at the following URL: (http://www.oie.int/international-standard-setting/aquatic-manual/access-online/). Chapter 2.3.5 of the Manual is entitled "Infectious Salmon Anaemia" and is attached to this my Affidavit as Exhibit "E"; the salient provisions are referred to below.

33. Within Chapter 2.3.5 of the Manual, section 4.2.3 describes "Microscopic pathology associated with ISA: Histological changes in clinically diseased Atlantic salmon are variable, but can include the following:

- Numerous erythrocytes in the central venous sinus and lamellar capillaries where erythrocyte thrombi also form in the gills.
- Multifocal to confluent haemorrhages and/or hepatocyte necrosis at some distance from larger vessels in the liver. Focal accumulations of erythrocytes in dilated hepatic sinusoids.

- Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and eventually haemorrhage into the lamina propria.
- Spleen stroma distended by erythrocyte accumulation.
- Slight multifocal to extensive diffuse interstitial haemorrhage with tubular necrosis in the haemorrhagic areas, erythrocyte accumulation in the glomeruli in the kidney.
- Erythrophagocytosis in the spleen and secondary haemorrhages in liver and kidney.

34. Also within this chapter of the Manual, section 7.1 gives the "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, as described in Section 4.3.1.2.1;

iii. Evidence for the presence of ISAV from two independent laboratory tests such as (Reverse Transcriptase Polymerase Chain Reaction Tests) RT-PCR (Section 4.3.1.2.3) and an indirect fluorescent antibody test "IFAT" on tissue imprints (Section 4.3.1.1.2); and

iv. Detection of antibodies to ISAV.

35. My primary area of expertise at the Animal Health Centre is histopathology, the study of the microscopic anatomical changes in diseased

tissue. Histopathology is a powerful method for identifying unknown diseases, but it is not very specific for diagnosis of salmon viruses, including ISAV. Therefore, I do not rely on histopathology alone as the basis for reporting the suspicion of ISA to federal authorities.

36. The role of the veterinarian is to put all the clinical and laboratory findings together to compose the best diagnosis possible. A single nonspecific lesion (e.g., sinusoidal congestion) is **not** sufficient grounds to report suspicion of ISAV to federal authorities. Such nonspecific clinical signs probably occur among a few of the several fish that die daily in BC fish farms. It would not be helpful to report such nonspecific findings to federal authorities.

37. I expect that fish health veterinarians will also read my entire comment to put any specific clause in the proper context. The text I have used in my comment relating to the diagnosis of "sinusoidal congestion of the liver" has varied over the years, but the first two sentences of my standard comment are usually, "Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV." I expect that a fish health veterinarian will read this sentence and be reminded that "sinusoidal congestion" is a nonspecific change that can result from anything that causes blood vessel walls to become distended.[1] By reading my comment, fish health veterinarians are also reminded that sinusoidal congestion can also occur after fish die (i.e., a postmortem artifact) if the liver is lower than other organs. Over time the blood drains into and distends sinusoids and sometimes other blood vessels in the liver.

38. Therefore, "sinusoidal congestion" is not a very specific change because it can occur in any fish that dies from any cause. In BC, based on the comment

[1] Substances that can cause sinusoidal congestion are called circulating vasodilators. Sinusoids are the name of blood vessel capillaries in the liver.

in my report, I expect that a fish health veterinarians reading a diagnosis of "sinusoidal congestion" will proceed to consider looking for bacteria, VHSV (a somewhat common virus among wild and farm fish in BC during the cool seasons), or any other cause of inflammation. I include a sentence that states that sinusoidal congestion is also associated with ISAV to ensure that fish health veterinarians remain vigilant (i.e., they need to look out for other clinical signs and mortality patterns that might be associated with ISAV); however, based on the wording I use, they should not think that I actually suspect ISAV in their fish.

Individual Reports

39. Attached to this my affidavit as Exhibit "F" is a document that I prepared which summarizes all 35 case reports in question.

40. Attached to this my affidavit as Exhibit "G" is a true copy of the Glossary and Chapter 1.1 of the Aquatic Animal Health Code.

41. I affirm this affidavit in support of the Province's position that Ms. Morton not be relieved from her undertaking and for no other purpose.

SWORN BEFORE ME at the City of Abbotsford, in the Province of British Columbia, this 26 day of April 2011.

A Commissioner for taking Affidavits for British Columbia

Howard Wiens
Barrister & Solicitor
#305 - 2692 Clearbrook Road
Abbotsford, B.C. V2T 2Y8

Print Commissioner's name

Title

604 - 850 - 6640
Phone Number

Dr. Gary Marty

Affidavit #2 of Dr. Gary D. Marty
Affirmed April 26, 2011

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

In the matter of Her Excellency the Governor General in Council, on the recommendation of the Prime Minister, directing that a commission do issue under Part I 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.

AFFIDAVIT #2 OF DR. GARY MARTY

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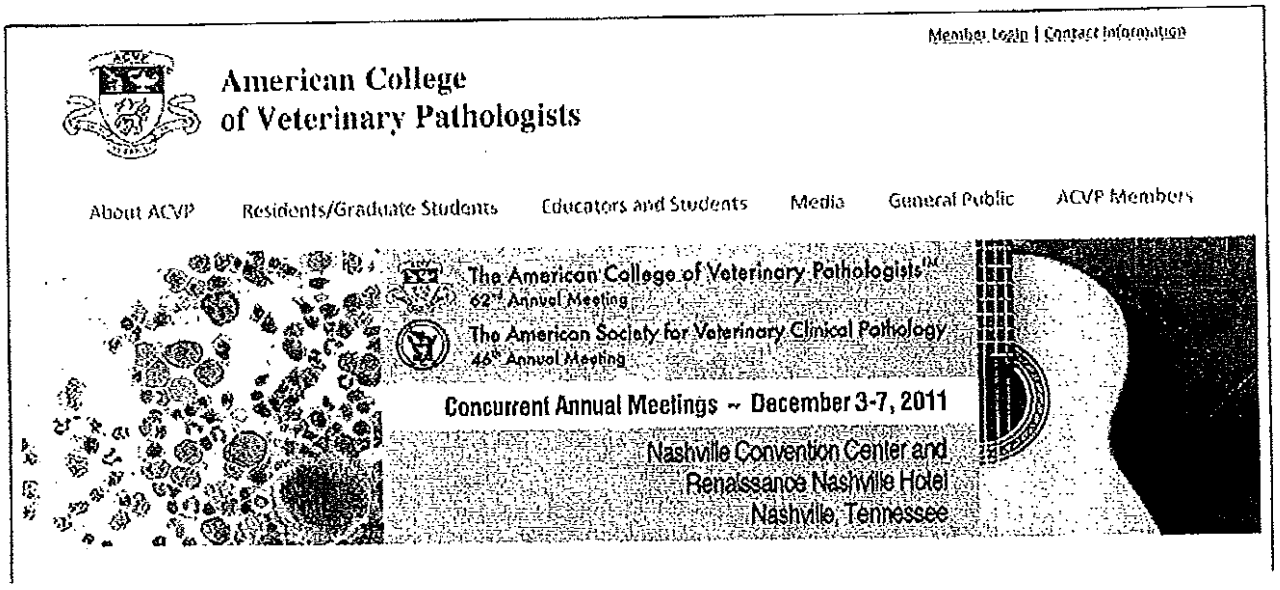

This is Exhibit "A" referred to in the affidavit of DR. GARY MARTY sworn before me at Abbotsford in the Province of British Columbia this 26 day of April, 2011

A Commissioner for taking Affidavits within the Province of British Columbia

FOR THE PUBLIC

Home

About ACVP

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Fundraising/ Development

Glossary of Terms

WHAT IS VETERINARY PATHOLOGY

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WHAT IS VETERINARY PATHOLOGY

Veterinary pathology is the science that studies disease in animals.

Why Are Veterinary Pathologists Important?

Veterinary pathologists improve and protect human and animal health by:

- Diagnosing disease in companion and zoo animals, and wildlife. Veterinary pathologists examine animal tissue and body fluids to diagnose disease and predict outcomes.
- Diagnosing disease in food-producing animals. By determining causes of disease, veterinary pathologists help maintain herd health and establish if there is a risk to humans handling or consuming the meat or milk of food animals.
- Contributing to drug discovery and safety. Because of their broad-based biomedical training, veterinary pathologists serve as key members of pharmaceutical research and development teams.
- Conducting research. With experience in diseases of multiple species, veterinary pathologists are uniquely qualified to perform studies to advance our understanding of the cause and methods to prevent disease in animals and humans.

What are Examples of Contributions of Veterinary Pathologists?

Veterinary pathologists are often among the first to recognize a new disease or health hazard. For example, veterinary pathologists were the first to recognize that a new disease agent, West Nile Virus, had invaded North America. Other ACVP diplomates have performed pioneering research on the potential applications of stem cells, conducted scientific experiments on the Space Shuttle, contributed to conservation efforts of African cheetahs, helped restore Prince William Sound after the Exxon Valdez oil spill, and assisted investigations of the nuclear power plant accidents at Three Mile Island and Chernobyl. Veterinary pathologists play critical roles on research teams to alleviate AIDS, SARS, cancer, chronic wasting disease, monkeypox and bioterrorism.

Where Do Veterinary Pathologists Work?

- Diagnostic Laboratories. These include private and state diagnostic laboratories, contract laboratories, academic institutions, zoos, and wildlife agencies.
- Academia. Institutions include veterinary or medical schools and research universities.
- Industry. This includes pharmaceutical, biotechnological, chemical and agrochemical industries, and supporting contract research organizations.
- Government. Examples of these agencies include the U.S. Army Veterinary Corps, Armed Forces Institute of Pathology, Centers for Disease Control, Environmental Protection Agency, Food and Drug Administration, United States Department of Agriculture, and the National Institutes of Health.

This is Exhibit "B" referred to in the
affidavit of DR. GARY MARTY
sworn before me at Abbotsford
in the Province of British Columbia this
26 day of APRIL, 20 11

A Commissioner for taking Affidavits
within the Province of British Columbia

GARY D. MARTY

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EDUCATION:

Ph.D. – Comparative Pathology, June 1996, University of California, Davis. Major professor - Dr. David Hinton.

D.V.M. – Iowa State University, Ames, Iowa. May 1987.

M.S. – Fisheries Biology, December 1986, Iowa State University, Ames, Iowa; Major professor - Dr. Robert Summerfelt

Graduate (no degree) – Texas A&M Univ., Summer 1983, Veterinary Anatomy (Aquatic Animal Medicine Program); Major Prof. - Dr. Raymond Sis

B.S. – Fisheries and Wildlife Biology, May 1983, Iowa State University, Ames, Iowa.

POSITIONS AND EMPLOYMENT:

2004 – now Fish Pathologist, Animal Health Centre, British Columbia Provincial Government
2004 – 2006 Assistant Research Pathologist (25-50% time), University of California, Davis
2001 – 2004 Lecturer in Gastrointestinal and Renal Anatomy, University of California, Davis
1997 – 2004 Assistant Research Pathologist, University of California, Davis
1996 – 1997 Postdoctoral researcher, Fish Pathology and Toxicology, Univ. of California, Davis
1987 – 1996 Postgraduate researcher, Comparative Pathology, University of California, Davis

ACADEMIC APPOINTMENTS (nonsalary):

Oct. 2007 – June 2011 Affiliate Faculty, University of Alaska, Fairbanks, School of Fisheries and Ocean Sciences
July 2006 – now Research Associate, University of California, Davis

SPECIALTY CERTIFICATION: Diplomate, American College of Veterinary Pathologists

VETERINARY PRACTICE LICENSES: State of Iowa (1987 - present); Province of British Columbia (public practice, 2005 - 2009); state of Alaska (1995 - 2002)

SPECIAL SKILLS: Fish health analysis (larvae, juveniles, and adults), fish necropsy, fish histopathology (tissues from 262 fish species examined), CYP1A immunohistochemistry, digital photomicroscopy

PUBLICATIONS: (from most recent to earliest)

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2010. Failure of population recovery in relation to disease in Pacific herring. Dis. Aquat. Org. 90:1-14. doi:10.3354/dao02210

3. **Marty, G.D.**, and R.A. Heintz. 2010. Ruptured yolk sacs and visceral fungi in emergent pink salmon alevins: histopathology and relation to marine survival. Dis. Aquat. Org. 88:115-126. doi:10.3354/dao02150

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6. **Marty, G.D.** 2008. Anisakid larva in the viscera of a farmed Atlantic salmon (*Salmo salar*). Aquaculture. 279:209-210.

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9. **Marty, G D.**, S.M. Bower, K.R. Clarke, G. Meyer, G. Lowe, A.L. Osborn, E.P. Chow, H. Hannah, S. Byrne, K. Sojonky, and J.H. Robinson. 2006. Histopathology and a real-time PCR assay for detection of *Bonamia ostreae* in *Ostrea edulis* cultured in western Canada. Aquaculture. 261:33-42.

10. Chen, M.F., J.A. Apperson, **G.D. Marty**, and Y.W. Cheng. 2006. Copper sulfate treatment decreases hatchery mortality of larval white seabass *Atractoscion nobilis*. Aquaculture. 254:102-114.

11. Farag, A.M., T. May, **G.D. Marty**, M. Easton, D.D. Harper, E.E. Little, and L. Cleveland. 2006. The effect of chronic chromium exposure on the health of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Aquat. Toxicol. 76:246-257.

12. Carls, M.G., R.A. Heintz, **G.D. Marty**, and S.D. Rice. 2005. Cytochrome P4501A induction in oil-exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. Mar. Ecol. Prog. Ser. 301:253-265.

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14. Carls, M.G., S.D. Rice, **G.D. Marty**, and D.K. Naydan. 2004. Pink salmon spawning habitat is recovering a decade after the Exxon Valdez oil spill. Trans. Am. Fish. Soc. 133:834-844.

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16. **Marty, G.D.**, T.J. Quinn, G. Carpenter, T.R. Meyers, and N.H. Willits. 2003. Role of disease in abundance of a Pacific herring (*Clupea pallasi*) population. Can. J. Fish. Aquat. Sci. 60:1258-1265.

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19. Arkush, K.D., A.R. Giese, H.L. Mendonca, A.M. McBride, **G.D. Marty**, and P.W. Hedrick. 2002. Resistance to three parasites in the endangered winter-run Chinook salmon: effects of inbreeding and major histocompatibility complex genotypes. Can. J. Fish. Aquat. Sci. 59: 966-975.

20. Carls, M. G., **G. D. Marty**, and J. E. Hose. 2002. Synthesis of the toxicological impacts of the *Exxon Valdez* oil spill on Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska U.S.A.. Can. J. Fish. Aquat. Sci. 59:153-172.

21. Quinn, T.J. II, **G.D. Marty**, J. Wilcock, and M. Willette. 2001. Disease and population assessment of Pacific herring in Prince William Sound, Alaska. In: Herring: Expectations for a new millennium, edited by F. Funk, J. Blackburn, D. Hay, A.J. Paul, R. Stephensen, R. Toreson and D. Witherell, University of Alaska Sea Grant, AK-SG-01-04, Fairbanks, pp. 363-379.

22. Hershberger, P.K., R.M. Kocan, N.E. Elder, **G.D. Marty**, and J. Johnson. 2001. Management of Pacific herring spawn-on-kelp fisheries to optimize fish health and product quality. N. Am. J. Fish. Manag. 21:976-981.

23. Chen, M.F., S. Yun, **G.D. Marty**, T.S. McDowell, M. House, K.D. Arkush, and R.P. Hedrick. 2000. A *Piscirickettsia salmonis*-like bacterium associated with mortality of white seabass (*Atractocion nobilis*). Dis. Aquat. Org. 43:117-126.

24. Hedrick, R.P., T.S. McDowell, **G.D. Marty**, K. Mukkatira, D.B. Antonio, K.B. Andree,

Z. Bukhari, and T. Clancy. 2000. Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies. Dis. Aquat. Org. 42:53-59.

25. Hedrick, R.P., O. Gilad, S. Yun, J.V. Spangenberg, **G.D. Marty**, R.W. Nordhausen, M.J. Kebus, H. Bercovier, A. Eldar. 2000. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. J. Aquat. Anim. Health 12:44-57.

26. Davis, C.R., **G.D. Marty**, M.A. Adkison, E.F. Freiberg, and R.P. Hedrick. 1999. Association of plasma IgM with body size, histopathologic changes, and plasma chemistries in adult Pacific herring *Clupea pallasi*. Dis. Aquat. Org. 38:125-133.

27. Hedrick, R.P., T.S. McDowell, M. Gay, **G.D. Marty**, M.P. Georgiadis, and E. MacConnell. 1999. Comparative susceptibility of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) to *Myxobolus cerebralis* the cause of salmonid whirling disease. Dis. Aquat. Org. 37:173-183.

28. **Marty, G.D.**, M.S. Okihiro, E.D. Brown, D. Hanes, and D.E. Hinton. 1999. Histopathology of adult Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 56:419-426.

29. Carls, M.G., **G.D. Marty**, T.R. Meyers, R.E. Thomas, and S.D. Rice. 1998. Expression of viral hemorrhagic septicemia virus in pre-spawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. Can. J. Fish. Aquat. Sci. 55:2300-2309.

30. **Marty, G.D.**, E.F. Freiberg, T.R. Meyers, J. Wilcock, T.B. Farver, and D.E. Hinton. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32:15-40.

31. **Marty, G.D.**, J.E. Hose, M.D. McGurk, E.D. Brown, and D.E. Hinton. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 54:1846-1857.

32. **Marty, G.D.**, R. Heintz, and D.E. Hinton. 1997. Histology and teratology of pink salmon larvae near the time of emergence from gravel substrate in the laboratory. Can. J. Zool. 75:978-988.

33. **Marty, G.D.**, J.W. Short, D.M. Dambach, R. Heintz, N.H. Willits, S.D. Rice, J.J. Stegeman, and D.E. Hinton. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome-P4501a induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Can. J. Zool. 75:989-1007.

34. Kocan, R.M., **G.D. Marty**, M.S. Okihiro, E.D. Brown, and T.T. Baker. 1996. Reproductive success and histopathology of individual Prince William Sound Pacific herring 3 years after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 53:2388-2393.

35. Hose, J.E., M.D. McGurk, **G.D. Marty**, D.E. Hinton, E.D. Brown, and T.T. Baker. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989-1991. Can. J. Fish. Aquat. Sci. 53:2355-2365.

36. Weidmer, M., M.J. Fink, J.J. Stegeman, R. Smolowitz, **G.D. Marty**, and D.E. Hinton. 1996. Cytochrome P450 induction and histopathology in pre-emergent pink salmon from oiled streams in Prince William Sound, Alaska. Am. Fish. Soc. Symp. 18:509-517.

37. Brown, E.D., T.T. Baker, J.E. Hose, R.M. Kocan, **G.D. Marty**, M.D. McGurk, B.L. Norcross, and J. Short. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the *Exxon Valdez* Oil Spill. Am. Fish. Soc. Symp. 18:448-462.

38. **Marty, G.D.**, R.C. Summerfelt, and D.E. Hinton. 1995. Histopathology of swimbladder noninflation in walleye (*Stizostedion vitreum*) larvae: role of development and inflammation. Aquaculture 138:35-48.

39. **Marty, G.D.**, D.E. Hinton, and J.J. Cech, Jr. 1995. Oxygen consumption by larval Japanese medaka with inflated or uninflated swim bladders. Trans. Am. Fish. Soc. 124:623-627.

40. DeKoven, D.L., J.M. Núñez, S.M. Lester, D.E. Conklin, **G.D. Marty**, L.M. Parker, and D.E. Hinton. 1992. A purified diet for medaka (*Oryzias latipes*): refining a fish model for toxicological research. Lab. An. Sci. 42:180-189.

41. **Marty, G.D.**, S. Wetzlich, J.M. Núñez, A. Craigmill, and D.E. Hinton. 1991. Fish-based biomonitoring to determine toxic characteristics of complex chemical mixtures: documentation of bioremediation at a pesticide disposal site. Aquat. Toxicol. 19:329-340.

42. **Marty, G.D.**, J.M. Núñez, D.J. Lauren, and D.E. Hinton. 1990. Age-dependent changes in toxicity of N-nitroso compounds to Japanese Medaka (*Oryzias latipes*) embryos. Aquat. Toxicol. 17:45-62.

43. **Marty, G.D.**, J.J. Cech, Jr., and D.E. Hinton. 1990. Effect of incubation temperature on oxygen consumption and ammonia production by Japanese medaka, *Oryzias latipes*, eggs and newly hatched larvae. Environ. Toxicol. Chem. 9:1397-1403.

44. **Marty, G.D.**, and R.C. Summerfelt. 1990. Wound healing in channel catfish by epithelialization and contraction of granulation tissue. Trans. Am. Fish. Soc. 119:145-150.

45. Zicker, S.C., **G.D. Marty**, G.P. Carlson, J.E. Madigan, J.M. Smith, and B.W. Goetzman. 1990. Bilateral renal dysplasia with nephron hypoplasia in a foal. J. Am. Vet. Med. Assoc. 196:2001-2005.

46. **Marty, G.D.**, and R.C. Summerfelt. 1988. Inflammatory response of channel catfish to abdominal implants: a histological and ultrastructure study. Trans. Am. Fish. Soc. 117:401-416.

47. **Marty, G.D.**, and R.C. Summerfelt. 1986. Pathways and mechanisms for expulsion of surgically implanted dummy transmitters from channel catfish. Trans. Am. Fish. Soc. 115:577-589.

MANUSCRIPT IN PRESS: none

MANUSCRIPTS IN REVIEW:

1. Waltzek, T.B., **G.D. Marty**, M.E. Alfaro, W.R. Bennett, M. Haulena, E.S. Weber III, and R.P. Hedrick. [Acceptable for publication after revision, 7 April 2011]. A systemic iridovirus from threespine stickleback (*Gasterosteus aculeatus*) represents a new megalocytivirus species in the family *Iridoviridae*. Dis. Aquat. Org.

2. Saksida, S.M., **G.D. Marty**, S. St-Hilaire, S.R.M. Jones, H.A. Manchester, C.L. Diamond, and J. Bidulka. Health assessments of juvenile pink salmon (*Oncorhynchus gorbuscha*) in the Broughton Archipelago of western Canada. J. Fish Dis.

BOOK CHAPTER:

Marty, G.D. 2008. Effects of the *Exxon Valdez* oil spill on Pacific herring in Prince William Sound, Alaska. pp. 925-932 *in* The Toxicology of Fishes, edited by R.T. Di Giulio and D.E. Hinton. CRC press, Boca Raton.

EXPERT PANEL:

1. Viral hemorrhagic septicemia virus (VHSV) expert panel. Sponsors: Canadian Food Inspection Agency (CFIA) and United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS). Duties: provide input for generating a weighted list of risk factors predictive of VHSV occurrence in North America; review resultant manuscript for publication. Dates of service: April - September, 2007. Publication:

Expert, VHSV. 2010. Viral hemorrhagic septicemia virus (VHSV IVb) risk factors and association measures derived by expert panel. Prev. Vet. Med. 94:128-139. doi:10.1016/j.prevetmed.2009.11.020.

EXTRAMURAL FUNDING: (from most recent to earliest)

1. North Pacific Research Board; RO319; Retrospective analysis of pigmented macrophage aggregates as markers of Pacific herring population health: $68,198; July 1, 2003 - June 30, 2007. Role, principal investigator.
2. United States Dept. of Commerce, National Oceanic and Atmospheric Administration; Field necropsy and Immunohistochemical localization of CYP1a in intertidal fish in Prince William Sound, Alaska; $3,000 and $13,278; May 1, – Oct. 31, 2004, and Nov. 1,

2004 – March 31, 2005. Role, principal investigator.

3. United States Dept. of Commerce, National Oceanic and Atmospheric Administration; Field necropsy and Immunohistochemical localization of CYP1a in intertidal fish in Prince William Sound, Alaska; $3,000 and $13,278; May 1, – Oct. 31, 2004, and Nov. 1, 2004 – March 31, 2005. Role, principal investigator.
4. National Institutes of Health, Institutional National Research Service Award (5T35RR007067) Veterinary Student Research Training Program: $218,540; December 1, 1999 - Nov. 30, 2004. ROLE: co-investigator.
5. Alaska Dept. of Fish and Game; contract # IHP-95-054, to determine the role of disease in population decline of Pacific herring in Prince William Sound, Alaska: $827,400; March 1995 - Oct. 2004. Role, principal investigator.
6. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 40HCNF300044; Immunohistochemical localization of CYP1a in intertidal fish exposed to weathered crude oil in the laboratory; $3,065; May 1, 2003 - Dec. 31, 2003. Role, principal investigator.
7. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # NFFS740-2-00013; Effect of weathered crude oil on crescent gunnels in Prince William Sound, Alaska, 12 years after the Exxon Valdez Oil Spill; $29,716; May 1, 2002 - Oct. 31, 2002. Role, principal investigator.
8. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 40HCNF000040; CYP1a Immunohistochemistry in larval pink salmon collected from Prince William Sound, Alaska, 10 years after the Exxon Valdez Oil Spill; $5,648; May 1, 2002 - Oct. 31, 2002. Role, principal investigator.
9. National Science Foundation, grant #9871962; Role of parasites and disease in health and population abundance of adult Pacific herring: $286,414; Feb. 1, 1999 - Jan. 30, 2002. Role, principal investigator.
10. U.S. Geological Survey, Biological Resources Division, ECRC Field Research Station, Jackson, WY, order # 01CRPR00238; Provide training in necropsy and preservation of juvenile and adult trout, and adult common carp for histopathology. Process tissues for histopathological analysis. $2,065.20; November 19, 2001 - April 5, 2002. Role, principal investigator.
11. U.S. Geological Survey, Biological Resources Division, ECRC Field Research Station, Jackson, WY, order # 01CRSA0346; Provide training in necropsy and preservation of juvenile and adult trout for histopathology. Process tissues for histopathological analysis: $5,692; March 20, 2001 - Sept. 1, 2001. Role, principal investigator.
12. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 40HCNF000040; processing of larval fish tissues for histopathology and immunohistochemistry; $36,644; March 1, 2000 - June 15, 2001. Role, principal investigator.
13. United States Geological Survey, Biological Resources Division, ECRC Field Research Station, Jackson, WY, contract # 99CR-R00652; processing for histopathology of larval and juvenile chinook salmon exposed to chromium; $5,691.60, Sept. 28, 1999 - February 28, 2000. Role, principal investigator.
14. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 40HCNF900116; processing of histologic gonad tissues and larval fish immunohistochemistry; $7,268.54; May 10 - Sept. 30, 1999. Role, principal investigator.
15. Alaska Dept. of Fish and Game; contract # IHP-98-050, to write a final report on damage

assessment fish histopathology studies after the *Exxon Valdez* Oil Spill: $6,532.25; August 19, 1998 - June 30, 2000. Role, principal investigator.

16. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 40HCNF800114, provide training in identifying coho salmon gonad tissues, and histologic processing of tissues $5,634; May 1 - Sept. 30, 1998. Role, principal investigator.
17. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 43ABNF501457, to determine the extent of histopathological lesions in Pacific herring adults exposed to petroleum hydrocarbons: $12,475; March 1995 - March 1996. Role, coinvestigator.
18. Prince William Sound Aquaculture Corporation, Cordova, Alaska. Contract for histopathological analysis to determine causes of abnormal pink salmon mortality; April 1994 - Sept. 1994; $8,280.00. Role, principal investigator.
19. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, contract # 43ABNF301765, to determine the extent of histopathological effects in pink salmon: $20,870; July 1993 - Nov. 1994. Role, coinvestigator.
20. Sigma Xi Grant-in-Aid of Research: $250, June 1984. Role, coinvestigator.

GRADUATE STUDENTS:

1. M.S., Ph.D. - Student: Peter-John Hulson; Major Professor, Terrance J. Quinn, III; Affiliation: University of Alaska, Fairbanks, Juneau Campus, Juneau, Alaska, USA; Role: committee member; Year of completion: M.S., 2007; Ph.D., ongoing.
2. Ph.D. - Student: Catherine Thomson; Major Professors, Ben F. Koop and Simon R. M. Jones; Affiliation: Centre for Biomedical Research, University of Victoria, Victoria, BC, Canada. Thesis title: "*Loma salmonae* in Chinook salmon (*Oncorhynchus tshawytscha*): improving detection, preventing infection, and increasing our understanding of the host response to a microsporidian parasite." Role: outside reviewer for dissertation. Year of completion: 2010.
3. Ph.D. - Student: Tayybah Shaheen; Major Professor, Tanveer Akhtar; Affiliation: Department of Zoology, University of Punjab, Lahore, Pakistan. Thesis title: "Studies on Chromium Toxicity in *Cyprinus carpio*: Brood Stock Progeny Relationship." Role: outside reviewer for dissertation; Year of completion: 2009.

TEACHING:

University of California, Davis -

1. **Course:** VME 416 (Fish Diseases, elective course for veterinary students); **Lecture title:** Functional anatomy of fish (50-minute lectures); **Dates:** April 7, 1998; April 4/6, 2000; April 3/5, 2001; April 4/9, 2002; April 1/3, 2003; April 12/14, 2005; April 3/5, 2007; Mar 31/April 2, 2009; April 5/7, 2011.
2. **Course:** VMD 432 (Gastrointestinal anatomy for 1st year veterinary students); prepared and presented 7 lectures and 7 laboratories on the microscopic and gross features of the gastrointestinal system of common domestic animals; **Dates:** Winter quarter, 2001, 2002, 2003, 2004.
3. **Course:** VMD 402D (Urinary System anatomy for 1st year veterinary students); prepared and presented 3 lectures and 3 laboratories on the microscopic and gross features of the urinary system of common domestic animals; **Dates:** Spring quarter, 2004.

4. **Course:** PMI 298 (graduate seminar in Special Pathology); **Lecture titles:** Normal gross and microscopic anatomy of fishes (50-minute lecture), AND Gross and microscopic pathology of fishes (50-minute lecture); **Dates:** May 10 and 17, 1999; May 9, 2001; April 24, 2002;
5. **Course:** PTX 230 and ECL 298 (Aquatic Toxicology/Ecotoxicology, graduate level); **Lecture title:** *Exxon Valdez* Oil Spill Damage Assessment (50-minute lecture); **Dates:** May 7, 1998; May 10, 2000.
6. **Course:** VMD452 (General Pathology, sophomore veterinary students); presented gross lesions and interpreted microscopic lesions; **Dates:** fall quarters, 1987-1989.
7. **Seminar:** Comparative Pathology (for veterinary resident training); presented four 50-minute Comparative Pathology seminars on spontaneous lesions in medaka (1989, 1991).

Iowa State University - Laboratory Animal Pathology graduate course; presented necropsy techniques, gross and microscopic anatomy, and histopathology of fish diseases: 1984, 1986, and 1988. Directed two 2-h laboratories and taught two 50-minute lectures: 1990 1994, 1998, 2000, and 2002.

Fish pathology study aides developed:

1. Fish Pathology Study Set (1990, 1998) - classic examples of fish lesions were compiled into a set that includes 50 microscope slides and a 35-page accompanying index and description.

ORAL PAPERS and SEMINARS PRESENTED: (from most recent to earliest)

1. **Marty, G.D.**, N.I. de With, M.P. Coombs, C.L. Diamond, H.A. Manchester, I.R. Keith, and M.E. Sheppard. Effect of postmortem change on the histopathologic diagnosis of lesions and pathogens in farmed Atlantic salmon. Annual meeting of the Fish Health Section of the American Fisheries Society; June 8-10, 2009 (Park City, Utah).

2. **Marty, G.D.**, M.E. Sheppard, M.P. Coombs, and H.A. Manchester. Histopathology of brain significantly increases the ability to determine cause of death in pen-reared Atlantic salmon. Annual meeting of the Fish Health Section of the American Fisheries Society; June 4-6, 2007 (Jackson Hole, Wyoming).

3. **Marty, G.D.** VHSV IVa in Alaska Herring. Canadian Food Inspection Agency VHS Biosecurity Workshop: Science and Epidemiology; May 29-30, 2007 (Guelph, Ontario).

4. **Marty, G.D.** Determining the cause of Pacific herring population decline in Prince William Sound, Alaska. Infectious Hematopoietic Necrosis Virus Research Workshop; January 18, 2007 (Campbell River, British Columbia).

5. **Marty, G.D.**, J.S. Odani, and A.M. O'Connor. Long-term effects of the 1989 *Exxon Valdez* oil spill versus a 1993 disease outbreak on hepatic pigmented macrophage aggregates in pacific herring from Prince William Sound, Alaska, USA. International Symposium on Aquatic Animal Health; Sept. 3-6, 2006 (San Francisco, California).

6. **Marty, G.D.**, G.A. Karreman, and Simon Jones. Prevalence and seasonality of new

pathogens in juvenile salmonids from inner coastal British Columbia, Canada. Western Fish Disease Workshop; June 26-28, 2006 (Victoria, British Columbia).

7. **Marty, G.D.**, T.J. Quinn II, T.R. Meyers, and S. Moffitt. Role of disease in limiting recovery of the Pacific herring population in Prince William Sound. Marine Science in the Northeast Pacific: Science for Resource Dependent Communities; Jan. 13-17, 2003 (Anchorage, Alaska).

8. **Marty, G.D.**, and T.R. Meyers. Long-term monitoring is necessary for understanding disease-related mortality of Pacific herring at the population level. Western Fish Disease Workshop; June 28, 2000 (Gig Harbor, Washington).

9. **Marty, G.D.**, and T.J. Quinn. Impact of two diseases on health and population abundance of adult Pacific herring; February 24, 2000. International Herring 2000 Symposium (Anchorage, Alaska).

10. **Marty, G.D.**, and T.R. Meyers. The Role of disease in limiting recovery of Pacific herring in Prince William Sound, Alaska; March 26, 1999. Symposium: Legacy of an oil spillC10 years after *Exxon Valdez* (Anchorage, Alaska).

11. **Marty, G.D.**, R.P. Hedrick, T.S. McDowell, M. Gay, M.P. Georgiadis, and E. MacConnell; Feb. 18, 1999. Comparative susceptibility of rainbow trout and brown trout to experimental infections with *Myxobolus cerebralis*; 5th Annual Whirling Disease Symposium (Missoula, Montana).

12. **Marty, G.D.**, and T.R. Meyers. Histopathology and Epizootiology of the North American Strain of Viral Hemorrhagic Septicemia Virus in Pacific herring in Prince William Sound, Alaska; Sept. 1, 1998; International Symposium on Aquatic Animal Health (Baltimore, Maryland).

13. **Marty, G.D.**, P.K. Hershberger, R.M. Kocan, and T.R. Meyers The Role of spawn-on-kelp pound fisheries in the expression of viral hemorrhagic septicemia virus (VHSV) in Pacific herring from Alaska; Sept. 5, 1997; annual meeting Fish Health Section, American Fisheries Society (Juneau, Alaska).

14. **Marty, G.D.**, R.M. Kocan, and T.R. Meyers. The role of disease in the abundance of two Pacific herring populations and implications for salmonids. Invited paper. June 3, 1997; Pathogens and Diseases of Fish in Aquatic Ecosystems (Portland, Oregon).

15. **Marty, G.D.** Epidemiology of *Ichthyophonus hoferi* in two Pacific herring populations in Alaska. August 7, 1996; Annual meeting Fish Health Section, American Fisheries Society (Madison, Wisconsin).

16. **Marty, G.D.** Role of Disease in the Abundance of Two Pacific Herring Populations in Alaska. Invited paper. July 16, 1996; Annual Meeting of the Western Division of the American Fisheries Society (Eugene, Oregon).

17. **Marty, G.D.**, E.F. Freiberg, T.R. Meyers, J. Wilcock, C.R. Davis, T.B. Farver, and D.E. Hinton. Role of *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus, and other causes of morbidity in declining Pacific herring populations in Prince William Sound, Alaska. Invited seminars. Jan. 17, 1996; *Exxon Valdez* Oil Spill Restoration Science Workshop (Anchorage, Alaska). Also, Oct. 19, 1995; for the Washington State University Department of Veterinary Microbiology and Pathology (Pullman, Washington).

18. **Marty, G.D.**, E.F. Freiberg, T.R. Meyers, J. Wilcock, C.R. Davis, T.B. Farver, and D.E. Hinton. *Ichthyophonus hoferi*, viral hemorrhagic septicemia virus, and other causes of morbidity in Pacific herring spawning in Prince William Sound in 1994. July 21, 1995. Annual meeting Fish Health Section, American Fisheries Society (Syracuse, New York).

19. **Marty, G.D.**, R.C. Summerfelt, and D.E. Hinton. Histopathology of swimbladder noninflation in walleye larvae: role of development and inflation. September 8, 1994. International Symposium on Aquatic Animal Health (Seattle, Washington).

20. ***Marty, G.D.**, M.S. Okihiro, and D.E. Hinton. Histopathologic analysis of chronic effects of the *Exxon Valdez* oil spill on Alaska fisheries. Nov. 19, 1992; annual meeting of the Alaska Chapter of the American Fisheries Society (Valdez, Alaska). Also, Feb. 4, 1993, *Exxon Valdez* Oil Spill Symposium (Anchorage, AK).

21. **Marty, G.D.**, M.S. Okihiro, J.M. Núñez, and D.E. Hinton. Morphology and management of *Mycobacterium avium* infection in laboratory-reared medaka *Oryzias latipes*. August 2, 1991. Annual meeting of the Fish Health Section of the American Fisheries Society (Newport, Oregon).

22. **Marty, G.D.**, J.M. Núñez, D.J. Lauren, and D.E. Hinton. Age-dependent changes in toxicity of N-nitroso compounds to Japanese Medaka (*Oryzias latipes*) embryos. July 20, 1989. Annual meeting of the Fish Health Section of the American Fisheries Society (Annapolis, Maryland).

23. **Marty, G.D.**, and R.C. Summerfelt. Tissue response of channel catfish to dummy transmitters surgically implanted in the peritoneal cavity. July 22, 1986. Annual meeting of the Fish Health Section of the American Fisheries Society (West Virginia).

*Best Paper Award.

POSTER PRESENTATION

1. Marty, G.D., T.J. Quinn II, and T.R. Meyers. Dec. 2-5, 2001. Relation of ulcers, viral hemorrhagic septicemia virus, and *Ichthyophonus hoferi* to Pacific herring population biomass in Prince William Sound, Alaska, 1994-2001. Annual meeting of the American College of Veterinary Pathologist. December 2001. Salt Lake City, Utah.

CONSULTING EXPERIENCE:

Expert Witness Testimony:

1. Shenandoah Fisheries, Ltd. v. Southern States Cooperative, Inc.; Circuit Court of Virginia

for the City of Richmond. On behalf of Shenandoah Fisheries; deposition (Dec. 15, 2000) and testimony at trial (April 24, 2001). Duties – Histopathological analysis of rainbow trout livers. Michie, Hamlett, Lowry, Rasmussen & Tweel, P.C., Charlottesville, Virginia, and Halver Corporation, Seattle, Washington. 1/4/00 - 4/25/01.

2. United States of America, State of West Virginia, and State of Ohio v. Elkem Metals Co. L.P., Ferro Invest III Inc., Ferro Invest II Inc., and Eramet Marietta Inc. Civil Action 2:03cv528 (S.D. Ohio). Deposition on behalf of Elkem Metals Co. L.P., Ferro Invest III Inc., Ferro Invest II Inc., and Eramet Marietta Inc. (June 20, 2005). Duties – Histopathologic analysis of tissue sections from freshwater drum, sauger, and gizzard shad produced by the United States of America; review of expert reports related to two fish kills.

Litigation Sensitive Research:

1. Fish health assessment of 250 spotted sand bass from San Diego Harbor (gross necropsy; histopathological analysis of liver, gill, kidney, and gonad). California Regional Water Quality Control Board, San Diego Region (through Exponent Corporation). August - December 2002.

2. Histopathological analysis of fathead minnow after acute toxicity testing, and review of the draft (March 8, 2001) report, "Formaldehyde Treatment Technologies, Re-certification Evaluation Report," generated by Cal/EPA, Department of Toxic Substances Control. For S & S Company of Georgia. 7/19/01- 12/31/01.

3. Histopathological analysis of larval and juvenile chinook salmon exposed to chromium. United States Geological Survey, Biological Resources Division, ECRC Field Research Station, Jackson, WY; 9/28/99 - 2/28/00.

4. Review studies on histopathology, growth, and disease challenge reported by the National Oceanic and Atmospheric Administration, U.S. Dept. of Commerce, as part of damage assessment of the Hylebos waterway in Commencement Bay, Tacoma, Washington. Hylebos Cleanup Committee (through contract with Striplin Environmental Associates, Olympia, WA); 12/20/97 - 1/30/99. Also, review the microscopic slides from rock sole (228 livers) and English sole (307 livers, 30 ovaries) that were used to generate the histopathology report. Litigation sensitive project.

5. Damage assessment on injured kelp bass populations in Southern California. United States Dept. of Commerce, National Oceanic and Atmospheric Administration (through contract with Applied Marine Sciences, Livermore, CA); 5/1/92 - 8/31/93. Necropsy and histopathology (liver, kidney, and ovary) and report writing. Litigation sensitive project.

Other Research:

1. Histopathology of koi carp exposed to experimental vaccine against koi herpesvirus (Cyprinid herpesvirus-3); February 2011; 524 organs examined from 89 fish. Department of Medicine & Epidemiology, University of California, Davis, California.

2. Necropsy and histopathology of steelhead trout exposed to steel pile driving in the Mad River, Arcata, California; sampling, June 29 – July 10, 2009; 315 fish examined. ICF

International for The California Department of Transportation (CalTrans).

3. Immunohistochemical analysis of cytochrome P4501A (CYP1A) expression in livers of masked greenling sampled from Prince William Sound, Alaska, summer 2004. 80 examined. United States Geological Survey, Biological Resources Division, Alaska Science Center, Anchorage, Alaska.

4. Necropsy and histopathology of Chinook salmon, shiner perch, and northern anchovy exposed to concrete pile driving in the Port of Oakland, August 2004. 204 fish examined. Strategic Environmental and the Port of Oakland Authority.

5. Histopathology of brood stock pallid sturgeon mortality in the endangered sturgeon recovery program operated by the U.S. Fish & Wildlife Service. Garrison National Fish Hatchery, Riverdale, North Dakota. July, 2003.

6. Gross examination and histopathological analysis of juvenile rainbow trout after acute toxicity testing. Phillips Petroleum Company (TOSCO). Nov. and Dec. 2001.

7. Histopathological analysis of brook trout and cutthroat trout sampled from Idaho streams contaminated with heavy metals; United States Geological Survey, Biological Resources Division, ECRC Field Research Station, Jackson, WY; 5/1/01 - 9/1/01; 11/1/01 - 5/1/02.

8. Gross necropsy and histopathological examination of white sturgeon tissues to determine causes of morbidity. Stolt Sea Farm, Elverta, CA. 1/26/00 - 4/26/00.

9. Effects of chronic crude oil exposure on early development of pink salmon; histopathology and immunohistochemistry interpretation of cytochrome P4501A expression. United States Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, AK; two contracts - 7/1/99 - 12/31/99, and 8/1/00-6/15/01.

10. Histology of gonads from coho salmon parr to determine potential for early sexual maturity in males ("jacks"). United States Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, AK; two contracts - 5/19/98 - 12/31/98, and 9/15/99 - 12/15/99.

11. Histopathology of larval white sea bass with bacterial infections after treatment with copper. California Dept. of Fish and Game. 9/25/99-9/28/99.

12. Histopathology of juvenile white sea bass to determine causes of mortality. Hubbs Sea World Research Institute, San Diego, CA; 8/26/99.

13. Determine causes of fathead minnow mortality in ambient water tests; Sacramento River Watershed Project, Central Valley Regional Water Quality Control Board; 12/26/97 - 12/31/98; subgross examination, histopathology, and report writing.

Diagnostic Pathology:

1. Pathologic diagnoses of veterinary pathology submissions (relief pathologist).
   a. Med Veterinary Laboratory, Concord, CA, July 1993 -Feb. 1995 (1085 cases);
   b. Phoenix Central Laboratories, Everett, WA, July 6 - October 18, 1997 (~1000 cases);
   c. IDEXX Laboratory, West Sacramento, CA, Sept. 15, 1998 - Jan. 1999 (~150 cases).

Training and Scientific Publication:

1. Provide expert advice and coauthor a manuscript that reports the most recent information on the effects of disease and the *Exxon Valdez* oil spill on the Pacific herring population of Prince William Sound, Alaska. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, Oct. 2005 – Sept. 30, 2006.

2. Train biologist and technicians to assess the prevalence of disease in Pacific herring. Write and publish a manual that describes the methods for assessing disease, including full-page color images showing significant diseases. Alaska Department of Fish and Game; March - May 2003.

3. Train technical personnel at a sewage treatment facility to preserve small fish for histopathological analysis. Histopathological analysis to determine causes of morbidity. South Bayside System Authority, Redwood City, CA; 10/1/99 - 12/30/00.

4. Provide expert advice and coauthor a synthesis manuscript entitled, "Synthesis of the toxicological and epidemiological impacts of the *Exxon Valdez* oil spill on Pacific herring." For the U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, Jan. - June 1999.

NATIONAL HONORS: National Institutes of Health, Environmental Pathology Training Fellowship (1987 - 1990, approximately $71,000);
Phi Kappa Phi Graduate Fellowship (1983 - 1984, $4500);
Soil Conservation Society of America Scholarship (1980, $750);

OTHER HONORS: University Fellowship, University of California, Davis, 1993, 1991.
Regent's Fellowship in Comparative Pathology, Univ. CA, Davis, 1990.
Graduate Research Excellence Award, Iowa St. Univ., 1986.
Honor Graduate, College of Agriculture, Iowa St. Univ., 1983.
Honor Societies: Gamma Sigma Delta, Phi Kappa Phi; Research Society: Sigma Xi.

WORK EXPERIENCE:

Aug. 2004 – present Fish Pathology: Fish Pathologist for the British Columbia Ministry of Agriculture 1767 Angus Campbell Road, Abbotsford, BC, V3G 2M3, Canada. Duties (100% time): provide fish pathology diagnostic services (necropsy and histopathology) for the province of British Columbia. Most work is in support of the large salmonid aquaculture industry in the Province, but duties also include shellfish pathology, aquarium fish pathology, and diagnostic services for investigation of fish mortality events (toxic spills) in natural waters.

Feb. 1997 - June 2006 Fish Toxicology/Pathology: Assistant Research Pathologist, Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of

California, Davis. Direct sampling and histopathologic analysis of adult Pacific herring from Alaska, with emphasis on determining causes of morbidity and mortality on a population scale; provided histopathologic analysis as a collaborator on toxicology projects with a variety of investigators. Served as the morphologic pathologist for the Fish Health Service and Fish Disease Laboratory of the College of Veterinary Medicine (1997-1998).

Oct. 1988 - Jan. 1997 Fish Toxicology/Pathology: Postgraduate researcher, Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis (Supervisor - Dr. David Hinton). Directed sampling, laboratory study, and histopathologic analysis of adult and larval fish tissues from Alaska, with emphasis on determining effects of the 1989 *Exxon Valdez* Oil Spill. Reviewed pathologic diagnoses in fish tissues from which other members of the laboratory had primary responsibility.

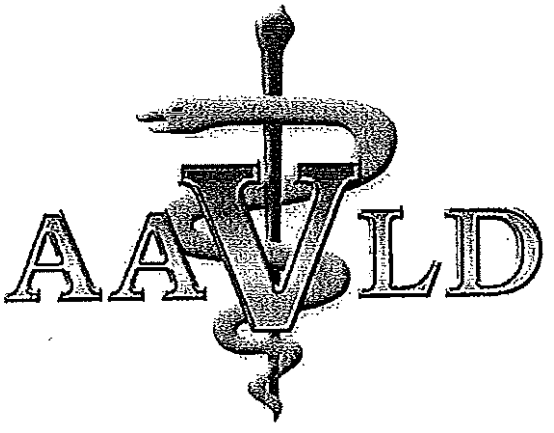
Sept. 1987- Feb. 1993 Veterinary Diagnostic Pathology: Graduate Student/Resident in Veterinary Medical Teaching Hospital (supervisor - Dr. Harvey Olander) and California Primate Research Center (1987-1988, supervisor- Dr. Judit Markovitz). Performed necropsies on a variety of vertebrate species (250 cases, 1987-1989), examined histologic slides, reported on significant gross and microscopic lesions. Taught senior veterinary students proper necropsy procedures, tissue preservation, and methods of describing lesions. Reported lesions in surgical biopsy specimens (three or four weeks per year, 40 cases per week, 1990-1993).

ORGANIZATIONS:

1. American College of Veterinary Pathologists
2. American Fisheries Society, Fish Health Section
3. Association of Aquaculture Veterinarians of British Columbia

REFERENCES: available upon request

REQUIREMENTS FOR AN ACCREDITED VETERINARY MEDICAL DIAGNOSTIC LABORATORY



AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS, INC.

Version 5.0

This is Exhibit "C" referred to in the affidavit of DR. GARY MARTY
sworn before me at Abbotsford
in the Province of British Columbia this
26 day of April, 20 11

A Commissioner for taking Affidavits within the Province of British Columbia

Table of Contents

Page

MISSION STATEMENT

The purpose of AAVLD accreditation program is to accredit public veterinary diagnostic laboratories in North America relative to technical and operational competence compatible with appropriate standards, and to provide an administrative assessment.

OBJECTIVES OF THE ACCREDITATION PROGRAM

- To provide a mechanism for objectively accrediting veterinary diagnostic laboratories
- To continuously emphasize the importance of excellence in veterinary diagnostic service
- To periodically evaluate and modify the accreditation process
- To keep laboratories cognizant of current technological advances in diagnostic veterinary medicine
- To keep laboratories informed of the impact of legislative mandates and other regulatory actions
- To promote adequate training of specialists in diagnostic veterinary medicine
- To encourage hiring of dedicated and innovative diagnosticians with appropriate training and experience
- To encourage acquisition and maintenance of facilities suitable and adequate to provide quality services
- To promote appropriate quality system programs
- To assist laboratories to meet or exceed the standards of the World Organization for Animal Health (Office International des Epizooties) (OIE) described in the OIE **Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008**

CLASSIFICATION

1.1 There are two types of accreditation: accredited and provisionally accredited.

1.2 An accredited laboratory is one that is capable of providing a full range of diagnostic services year-round in a majority of the following essential disciplines: necropsy, histopathology, clinical pathology, bacteriology, virology, mycology, parasitology, serology and toxicology. It is mandatory that a full service laboratory offers necropsy, histopathology, bacteriology, and virology on site. Mechanisms must exist for referral of those services not directly offered by the laboratory. The Accreditation Committee will evaluate the appropriateness of essential services referred to other laboratories.

1.3 A provisionally accredited laboratory is one that does not meet the requirements and guidelines but shows intent to do so. A provisionally accredited laboratory is given a period of time to correct the deficiencies noted. Provisionally accredited laboratories are required to document progress through periodic reports.

ADMINISTRATIVE REQUIREMENTS

2.1 Organization, Management and Personnel

2.1.1 Diagnostic laboratories reviewed for accreditation may be administered by a State/Provincial Department of Agriculture, a University, an Agricultural Experiment Station, a State/Provincial Department of Health, or by various combinations of such public institutions. The committee does not review commercial laboratories, or laboratory animal diagnostic laboratories supported by the National Institutes of Health.

2.1.2 The director/chief administrative officer shall be a veterinarian. The laboratory personnel shall be able to provide competence in all testing groups evaluated for accreditation. Minimum training levels are listed in the section on personnel qualifications in Appendix I.

2.2 Finance and Budget

2.2.1 The overall budget will be evaluated on the basis of salaries for personnel, operations, equipment, maintenance, travel, library resources and continuing education. The laboratory shall have sufficient resources to meet the requirements for accreditation as indicated in the support for the various disciplines and the overall administrative function of the laboratory.

2.2.2 As diagnostic laboratories are a vital part of disease surveillance and monitoring, finances must be available to sustain these assignments. Since these laboratories serve the public good, surveillance resources are not intended to be self-sufficient financially and require public financial support commensurate with the public good derived.

ACCREDITATION PROCESS

3.1 The Accreditation Committee will consider all written applications for an accreditation site visit from qualifying public laboratories, as described in the administrative requirements.

3.2 The Accreditation Committee of the American Association of Veterinary Laboratory Diagnosticians will evaluate each laboratory in terms of the degree to which it meets its own statement of objectives and the established criteria set by the Accreditation Committee.

3.3 Each accredited laboratory will be requested to provide an annual update at the time of dues payment that outlines changes in Chief Administrator or administrative structure, or any major changes in personnel, physical facilities, equipment, or budget that could affect accreditation status.

3.4 Steps of the Accreditation process:

3.4.1 Application. Applications are considered confidential by the committee.

3.4.2 Accreditation Committee review of the application. If the status and application of the laboratory is satisfactory, a site visit will be performed.

3.4.3 A site visit will be conducted according to the AAVLD Site Visit and Accreditation Audit Report Guidelines, SOP 102.1.

3.4.4 The site visit team provides written and oral reports and recommendations to the Accreditation Committee.

3.4.5 The committee may make changes to the written report and recommendations, and determines the classification status of tbe laboratory.

3.4.6 Reports, which are considered as confidential information, are sent to the laboratory director.

3.5 Accreditation is time limited. Laboratories are reaccredited periodically through reapplication. Accreditation may be withheld or withdrawn if the laboratory fails to meet the Requirements of the AAVLD Accreditation Committee.

SPECIFIC REQUIREMENTS

AAVLD incorporates by reference the World Organization for Animal Health (Office International des Epizooties) (OIE) document "OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008" from the Standards Commission of the Office International des Epizooties as a guide to specific requirements for accreditation. The entire OIE document is available to member laboratories through OIE. It is acknowledged that not all sections of laboratories conduct testing for infectious diseases. The good laboratory practice principles inherent in the OIE document still apply to those laboratory sections conducting work in areas other than infectious diseases.

4. Management requirements

4.1 Organization and Management

4.1.1 The laboratory or the organization of which it is part shall be an entity that can be held legally responsible.

4.1.2 The laboratory shall be organized and shall operate in such a way that it meets the requirements of this Standard whether carrying out work in its permanent facilities, at sites away from its permanent facilities, or in associated temporary or mobile facilities.

4.1.3 The laboratory shall have a clearly defined organizational system and structure. This shall be supported with organizational charts and job descriptions. Organizational charts shall indicate key personnel and the laboratory's place within the larger organization. Relationships between management, technical operations, support services, and quality activities shall be specified.

4.1.4 The laboratory shall:

a) have managerial and technical personnel with the authority and resources needed to carry out their duties and to identify the occurrence of departures from the quality system or from the procedures for performing tests, and to initiate actions to prevent or minimize such departures;
b) have arrangements to ensure that its management and personnel are free from any undue internal or external commercial, financial and other pressures and influences that may adversely affect the quality of their work;
c) have policies and procedures to ensure the protection of its clients' confidential information and proprietary rights, including procedures for protecting the electronic storage and transmission of results;
d) have policies and procedures to avoid involvement in any activities that would diminish confidence in its competence, impartiality, judgment or operational integrity;
e) specify the responsibility, authority and inter-relationships of all personnel who manage, perform or verify work affecting the quality of the tests;
f) provide adequate supervision of testing staff, including trainees, by persons familiar with the tests, their purpose, and the analysis of test results;
g) have technical management which has overall responsibility for the technical operations and the provision of the resources needed to ensure the required quality of laboratory operations;
h) appoint a member of staff as quality manager (however named) who, irrespective of other duties and responsibilities, shall have defined responsibility and authority for ensuring that the quality system is implemented and followed at all times; the quality manager shall have direct access to the highest level of management at which decisions are made on laboratory policy or resources;
i) appoint backups or deputies for key managerial personnel such as the quality manager.

> **NOTE:** In laboratories with a small number of personnel, individuals may have more than one function and it may be impractical to appoint deputies for every function.

4.2 Quality system

4.2.1 The laboratory shall establish, implement and maintain a quality system appropriate to the scope of its activities, including the type, range and volume of testing it undertakes.

The laboratory management shall document its policies, systems, programs, procedures and instructions to enable the laboratory to ensure to the extent possible, the quality of the test and diagnostic interpretations it generates. Documentation used in this quality system shall be communicated to, understood by, available to, and implemented by the appropriate personnel.

4.2.2 The laboratory management shall define and document the policies and objectives to be achieved by implementing the quality system. The laboratory management shall ensure that these policies and objectives are documented in a quality manual. The overall objectives shall be set out in a quality policy statement in the quality manual, stating the standard of performance to be achieved and maintained. The quality policy statement shall be issued under the authority of the chief executive. It shall include at least the following:

a) a statement of the laboratory management's intentions with respect to the standard of service it will provide;
b) the purpose of the quality system;
c) a requirement that all personnel concerned with testing activities within the laboratory familiarize themselves with the quality documentation and implement the policies and procedures in their work;
d) the laboratory management's commitment to good professional practice and quality of its diagnostic services to its client; and
e) the laboratory management's commitment to compliance with the AAVLD Standard.

NOTE: The quality policy statement and manual should be concise.

4.2.3 The quality manual shall include or make reference to the supporting procedures including technical procedures. It shall outline the structure of the documentation used in the quality system. The quality manual shall be maintained up to date.

4.2.4 The quality manual shall define the roles and responsibilities of technical management and the quality manager, including their responsibility for ensuring compliance with the AAVLD Standard.

4.3 Document Control

4.3.1 The document control system shall ensure that only the current version of the correct document is in use in the laboratory, and that documents needed for staff to perform their work are available at the work location.

4.3.2 The laboratory shall have documented policy, procedures, and/or work instructions that describe how laboratory documents affecting the quality of tests, including test methods, are reviewed, approved, issued, updated, revised, amended, retained or archived, and discarded. Procedures shall be reviewed and approved by authorized, qualified staff.

4.3.3 Amendments to documents shall be identified clearly in the text and reviewed and approved by an authorized, qualified officer, administrator or supervisor having access to pertinent background information concerning the change.

4.3.4 Documents shall be uniquely identified and accurately cross-referenced.

NOTE: In this context "document" means any information or instructions, in any format or medium, that have direct bearing on or affect the quality of test results, and includes not only the quality manual, policy, procedures, and instructions, but also test methods, worksheets, forms, international standards, and regulations.

4.4 Review of request or contract

4.4.1 The laboratory shall have documented policy and procedures that describe how the laboratory ensures that it is capable of and has the capacity for doing particular testing. The procedures shall ensure adequate review of the proposed work with laboratory staff and the client. The laboratory shall keep a record of the review and of client agreement.

4.4.2 The review shall also cover any work that is subcontracted by the laboratory.

4.5 Subcontracting of test services

The client shall be informed of and agree to any subcontracting of work.

4.6 Purchasing services and supplies

The laboratory shall have a policy and procedures to ensure that services and supplies meet pre-established specifications and will not adversely affect the quality of test results. These procedures shall include a description of the criteria for selection, evaluation, use, handling, and storage of materials and reagents having an effect or potential effect on test results.

4.7 Complaints

The laboratory shall have a policy and procedure for the resolution of complaints received from clients or other parties. Records shall be maintained of all complaints and of the investigations and corrective actions taken by the laboratory.

4.8 Control of nonconforming testing and test results

4.8.1 The laboratory shall have a policy and procedures that ensure that nonconforming testing (conditions that exist which have or could adversely affect the reliability of test results) is detected and promptly corrected. The laboratory shall have procedures for informing clients if test results are questionable or incorrect, particularly if this possibility is identified after test results have been reported to the client. These procedures shall describe who has the authority to withhold test results, implement corrective action, and authorize resumption of work.

4.8.2 When a serious issue or a risk to the quality of test results is identified, the laboratory shall ensure that appropriate corrective action procedures given in 4.9 shall be promptly implemented.

4.9 Corrective and preventive action

4.9.1 The laboratory shall have a policy and procedures for implementing corrective action when nonconforming work or departures from the policies and procedures in the quality system have been identified. The policy and procedures shall ensure:

a) designation of appropriate authorities responsible for implementation of corrective action(s);
b) investigative procedures are implemented to determine the root cause(s) of the problem;
c) upon identification, appropriate corrective action(s) are implemented;
d) documentation of any required changes to operational procedures;
e) once implemented, corrective action(s) are monitored to ensure effectiveness in overcoming the problem; and
f) when appropriate, areas of activity subject to corrective action are audited in accordance with 4.11.

> **NOTE:** Special internal audits need only be initiated when a serious issue or risk to the quality of test results or integrity of the quality system has been the subject of corrective action.

4.9.2 The laboratory shall identify potential sources of nonconformance and potential needs for improvement, either technical or with the quality system. Preventive action procedures shall include:

a) identification and evaluation of potential nonconformance or improvement;
b) development and implementation of an action plan, including appropriate controls; and
c) monitoring of effectiveness in reducing likelihood of nonconformance or in addressing specific needs for improvement.

> **NOTE:** Preventive action is a pro-active process. Identification of specific technical areas requiring preventive action often involves the ongoing monitoring and review of the validity of the test methods and the competence of the laboratory.

4.10 Records

All laboratory records must be maintained in an effective retrieval system and must be accurate, contemporaneous, attributable and legible. This retrieval system should include a system of classification of diseases. Records should be preserved in accordance with requirements for individual jurisdictions.

4.10.1 General

The laboratory shall have a records management system.

4.10.1.1 The laboratory shall establish and maintain procedures for identification, collection, indexing, access, storage, maintenance and disposal of quality and technical records. Quality records shall include reports from internal audits and management reviews, as well as, corrective and preventive action records.

4.10.1.2 All records shall be legible and shall be stored and retained in such a way that they are readily retrievable in facilities that provide a suitable environment to prevent damage or deterioration and to prevent loss. Retention times of records shall be established.

> **NOTE**: Records may be in the form of various types of media, such as hard copy or electronic media.

4.10.1.3 All records shall be held secure and in confidence.

4.10.1.4 The laboratory shall have procedures to protect and back up data and records held on computers at all times, and to prevent unauthorized access to or amendment of data or records on computers.

4.10.2 Technical records

4.10.2.1 The laboratory shall retain for a defined period of time, original observations, derived data, calibration records, staff records, a copy of each test report issued, and any other information necessary to recreate the activity. The records for each test shall contain sufficient information to facilitate identification of factors affecting the quality of test results and to enable the test to be repeated under conditions as close as possible to the original. The records shall include the identity of personnel.

4.10.2.2 Observations, data and calculation shall be clearly and permanently recorded and identifiable to the specific test at the time they are made.

4.10.2.3 When mistakes occur in records, each mistake shall be crossed out (not erased, made illegible nor deleted), and the correct value entered alongside. All such alterations to records shall be dated, signed or initialed by the person making the correction. In the case of computer—collected data, similar measures shall be taken to avoid loss or change of original data.

4.11 Internal audits

4.11.1 The laboratory shall periodically and in accordance with a predetermined schedule and procedure conduct internal audits of its activities to verify that its operations continue to comply with the requirements of the quality system and the AAVLD Standard. The internal audit program shall address all elements of the quality system, including testing activities. It is the responsibility of the quality manager to plan and organize audits as required by the schedule and requested by management. Such audits shall be carried out by trained and qualified personnel who are, wherever resources permit, independent of the activity to be audited. Personnel shall not audit their own activities except when it can be demonstrated that an effective audit can be carried out.

NOTE: In laboratories with a small number of personnel, effective internal audits may not be feasible. In such cases, it may be appropriate for two or more laboratories to cooperate in auditing each other.

4.11.2 When audit findings cast doubt on the effectiveness of the operations or on the quality of the laboratory's test results, the laboratory shall take timely and effective corrective and where appropriate preventive action, and shall notify clients in writing if investigations show that the laboratory results may have been affected (see 4.8).

4.11.3 The area of activity audited, the audit findings and corrective actions that arise from them shall be recorded. The laboratory management shall ensure that these corrective actions are discharged within an appropriate and agreed-upon time-frame.

4.12 Management reviews

4.12.1 The quality system and test related activities shall be reviewed by management at least once per year.

4.12.2 The laboratory shall have a procedure for performing a Management Review. The review shall take into consideration:

a) suitability of policies and procedures;
b) reports from managerial and supervisory personnel;
c) reports of recent internal audits;
d) corrective and preventive actions;
e) assessments by external bodies;
f) results of inter-laboratory comparisons or proficiency tests;
g) changes in the volume and type of work;
h) client feedback;
i) complaints;
j) other relevant factors, such as quality control activities, resources and staff training.

4.12.3 Findings from management reviews and the actions that arise from them shall be recorded. The management shall ensure that those actions are discharged within an appropriate and agreed-upon time frame.

4.12.4 This review and subsequent activities shall ensure the continuing suitability and effectiveness of the quality management system and shall ensure the introduction of necessary changes and improvements.

5. Technical requirements

5.1 General

5.1.1 Many factors can affect the reliability of test results. The extent to which these factors contribute to the reliability of test results differs between tests. The laboratory shall take account of these factors in developing or adopting test methods and related procedures for routine use, in the training and qualification of personnel, in the selection and calibration of equipment, and in the assessment of materials and reagents to he used in testing.

5.2 Personnel

5.2.1 The laboratory shall ensure the initial and ongoing competence of all laboratory personnel to do their assigned work.

5.2.2 The laboratory shall maintain current job descriptions for managerial, technical and key support personnel involved in testing and diagnostic interpretation, and the management shall authorize only staff who are documented as qualified and competent to do testing and related work.

5.2.3 The laboratory shall have a system which ensures the establishment and maintenance of a training program relevant to the present and anticipated needs of the laboratory.

5.3 Accommodation and environmental conditions

All aspects of the physical facilities must provide an appropriate environment for the conduct of the activities of all disciplines required for laboratory accreditation. Laboratories, offices, storage space and animal holding rooms shall be clean, maintained in good repair and be adequate in number and size for intended function of the laboratory. Adequate lighting and ventilation shall be provided. Safety, biosafety, biocontainment, and biosecurity features shall be incorporated as a part of the physical facility.

5.3.1 Laboratory facilities for testing, including but not limited to energy sources, lighting and environmental conditions, shall be such as to facilitate correct performance of tests. The laboratory shall ensure that the environment does not invalidate the results or adversely affect the required quality of any testing activity.

5.3.2 The laboratory shall monitor, control and record environmental conditions as required by relevant specifications or where they may influence the reliability of the results. Due attention shall be paid, for example, to biological sterility, dust, electromagnetic interference, radiation, humidity, airflow, electrical supply, temperature, and sound and vibration levels, as appropriate to the technical activities concerned. Test activities shall be stopped when the environmental conditions jeopardize the test results.

5.3.3 There shall be effective separation between neighboring areas in which there are incompatible activities. Measures shall be taken to prevent cross-contamination.

5.3.4 Access to and use of areas affecting test results shall be controlled.

5.3.5 The laboratory shall ensure the establishment and maintenance of safety, biosafety, biocontainment and biosecurity programs relevant to present and anticipated needs. The programs will provide staff training and address all necessary elements to ensure a safe work environment.

5.4 Test methods

5.4.1 General

5.4.1.1 The laboratory shall use appropriate test methods and related procedures for all animal disease diagnostic testing activities. Consideration shall be given to all factors that impact on the relevance of the test method and test results to a specific diagnostic interpretation or application. These factors include the suitability of the test method, its

acceptability by the scientific and regulatory communities, its acceptability to the client, and its feasibility given available laboratory resources. See 5.4.3.1 note.

5.4.1.2 Test methods shall be approved for use by qualified, authorized personnel, according to established procedures.

5.4.1.3 Tests shall be appropriately controlled.

5.4.1.4 The laboratory shall have written instructions for all tests and related procedures used in its routine activities, the calibration and operation of all relevant equipment, and the collection, handling, transport and storage of specimens and preparation of samples for testing.

5.4.1.5 Laboratories using test methods prepared by national and international standards-setting bodies and other external technical organizations shall have a system to receive updates of these methods in a timely manner.

> **NOTE:** International, regional or national standards or other recognized specifications that contain sufficient and concise information on any of the above subjects do not need to be rewritten as internal procedures if these standards are published in a way that they can be used as published by the operating staff in a laboratory. Consideration may need to be given to providing additional documentation for optional steps in the assay or additional details. As with all test methods, they shall be subject to document control (see 4.3).

5.4.2 Selection of methods

5.4.2.1 The client shall be informed of the test method chosen and if required, the laboratory shall provide the client with the rationale used in making this choice (see 5.4.1.1).

5.4.2.2 Analysts shall have a record of documented proficiency in the performance of the test. Proficiency shall be documented on an ongoing basis, at appropriate intervals. Assessment of proficiency sball be based on objective data, using blind samples of appropriate number and composition. These samples should be well characterized.

5.4.2.3 Test methods shall contain enough critical and descriptive information such that experienced personnel can properly perform the test within pre-established control limits without reference to other information sources. In addition, it shall include as appropriate:

a) evidence of document control;
b) relevant references;
c) a description of intended analyte(s)(e.g., antibody) and any quantities or ranges to be determined (e.g., titer);
d) any reference standards or reference materials required (e.g., reference strains, reference standards for antibody);
e) a description of the appropriate matrix or specimen for testing, including species (e.g., bovine serum);
f) safety considerations, including biocontainment level needed;
g) a list of and specifications for equipment, materials, and reagents, including software;
h) conditions for acceptance of specimens as fit for testing;

i) conditions for specimen identification, collection, handling, transportation and storage;
j) conditions for sample preparation;
k) a description of the controls used and their acceptance limits;
l) checks to be made prior to beginning the test procedure (e.g., equipment checks and calibrations);
m) acceptance criteria for results;
n) data to be recorded, and the method of analysis/transformation, presentation, and/or interpretation (e.g., how an absorbance reading is transformed and interpreted as a positive or negative result relative to a cut-off), and recording; and
o) most current description of the test procedure.

5.4.2.4 The test method shall be validated before it is incorporated into the routine diagnostic activities of the laboratory. The same prerequisite applies to an existing assay that has been modified if the modification affects the performance characteristics of the assay (see 5.4.3).

5.4.3 Validation of test methods

5.4.3.1 A test method, whether an international or national standard method, a harmonized method, or developed in-house shall be considered appropriate for routine diagnostic purposes if it has been validated, where possible according to the principles outlined in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* or other related OIE references. While it is preferred that all methods, developed in-house or drawn from reputable collections of standard methods, undergo an in-house validation using an appropriate number of samples from the population of interest, the user is not required to re-validate international or national standard methods, but shall be able to define, at least through reference to public or private documentation, the analytical sensitivity and specificity, accuracy and precision, diagnostic sensitivity and specificity and other parameters relevant to the use of the test method in the user's laboratory. The user shall provide documented evidence of data on and statistically valid assessment of comparative performance for those assays that are harmonized by interlaboratory comparison to an accepted and validated standard method.

NOTE: Test methods may be classified as "validated for use" by meeting the following criteria.

1) Ongoing documentation of internal or inter-laboratory performance using known reference standard(s) for the species and/or diagnostic specimen(s) of interest,

AND one or more of the following:

2) Endorsed or published by reputable technical organization (*e.g.: OIE Manual of Standards for Diagnostic Tests and Vaccines, US Food and Drug Administration's Bacteriologic Analytic Methods, Bergey's Manual of Determinative Bacteriology, American Society*

of Microbiology Manual of Clinical Laboratory Immunology, American Association of Avian Pathologists Isolation and Identification of Avian Pathogens, EPA protocols, American Fisheries Society Bluebook, AOAC, NAHLN);

3) Published in a peer-reviewed journal with sufficient documentation to establish diagnostic performance and interpretation of results;
4) Documentation of internal or inter-laboratory comparison to an accepted methodology or protocol.

5.4.3.2 Validation data, including all original observations, calculations, equipment monitoring and calibration records, and archived procedures used to formulate performance characteristics, shall be retained by the laboratory for at least as long as the assay is used for diagnostic purposes and for at least seven years after the assay has been retired from use.

NOTE: Depending on client needs, the laboratory may be required to define other diagnostic performance indicators such as positive and negative predictive values of the test. Such indicators may be particularly relevant to certain diagnostic applications or test populations.

5.4.4 Control of data

5.4.4.1 The laboratory shall ensure, using appropriate procedures, that all data resulting from test validation and all data relating to test results is secure, retrievable, and approved for use by specified, qualified personnel.

5.4.4.2 Manual calculations and data transfers shall be subject to appropriate checks in a systematic manner.

5.4.4.3 When computers or automated equipment are used for the acquisition, processing, recording, reporting, storage or retrieval of test data, the laboratory shall ensure that:

a) computer software, modified or developed by the user, is documented in sufficient detail and suitably validated or otherwise checked as being adequate for use, i.e., the laboratory shall implement and document changes to control procedures such that these activities can be recreated and an audit trail is established;
b) procedures are established and implemented for protecting the security, integrity, and retrievability of data; such procedures shall include, but not be limited to, integrity and confidentiality of data entry or collection, data storage, data transmission and data processing;
c) computers and automated equipment are maintained to ensure proper functioning and are provided with the environmental and operating conditions necessary to maintain the integrity of test data.

NOTE: Commercial software in general use within its designed application range may be considered sufficiently validated.

5.5 Equipment

The laboratory shall possess or have access to all equipment necessary for the correct performance of all services. All equipment shall be identified, properly maintained and calibrated with maintenance and calibration procedures documented.

5.5.1 The laboratory shall be furnished with all items of test and related equipment required for the correct performance of the tests. In those cases where the laboratory needs to use equipment outside its permanent control, it shall ensure that the requirements of this AAVLD standard are met.

5.5.2 Equipment and its software used for diagnostic activities shall be capable of achieving the accuracy required and shall comply with specifications relevant to the procedures concerned. Calibration programs shall be established for key equipment where these properties have a significant effect on the results.

5.5.3 Equipment shall be operated by authorized, qualified personnel. Up-to-date instructions on the use and maintenance of equipment (including any relevant manuals provided by the manufacturer of the equipment) shall be readily available for use by the appropriate laboratory personnel.

5.5.4 Each item of equipment used for test activities significant to a test result shall be uniquely identified.

5.5.5 Records shall be maintained of each item of equipment significant to the tests performed. The records shall include at least the following:

a) identity of the item of equipment;
b) manufacturer's name, type identification, and serial number or other unique identification;
c) verification that equipment complies with the specification;
d) the current location, where appropriate;
e) the manufacturer's instructions, if available, or reference to their location;
f) dates, results and copies of reports and certificates of all calibrations, adjustments, acceptance criteria, and the due date of next calibration;
g) maintenance carried out to date, and the maintenance plan;
h) damage, malfunction, modification or repair to the equipment.

5.5.6 Maintenance procedures shall be established.

5.5.7 Equipment calibrations shall be performed by qualified personnel using procedures appropriate to intended use, accuracy and precision required, and at appropriate intervals as historical data indicate.

5.5.8 Equipment that has been subjected to overloading or mishandling, or gives suspect results, or has been shown to be defective or outside specified limits, shall be taken out of service, clearly labeled or marked, and appropriately stored until it has been repaired and shown to perform correctly. The laboratory shall examine the effect of the defect or departure from specified limits on previous tests and shall institute the "Control of nonconforming work" procedure (4.8).

5.5.9 Whenever practical, all equipment under the control of the laboratory and requiring calibration shall be labeled, coded or otherwise identified to indicate the status of calibration or verification and the date when the next calibration or verification is due.

5.5.10 When, for whatever reason, equipment goes outside the direct control of the laboratory, the laboratory shall ensure that the function and calibration status of the equipment are checked and shown to be satisfactory before the equipment is returned to service.

5.5.11 Test equipment, including both hardware and software, shall be safeguarded from adjustments that would invalidate the test results.

5.6 Measurement traceability

5.6.1 Where indicated and when possible, the laboratory shall have traceability of all measurements, including the calibration of equipment to Standard International (SI) units.

5.6.2 Where traceability to SI units of measurement is not possible, the best available means for providing confidence in the results shall be applied, such as:

a) the use of suitable reference standards or materials certified to give a reliable characterization of the material;
b) mutual-consent standards or methods that are clearly speeified and agreed upon by all parties concerned;
c) participation in a suitable program of interlaboratory comparisons or proficiency testing.

5.6.3 Reference equipment, standards or materials used in conjunction with testing activities shall be handled, maintained, and stored in a manner that ensures proper performance and/or accuracy.

5.6.4 Biological reference material shall, where possible, be traceable to accepted international standards or to OIE reference materials (e.g., International Standard Sera).

5.6.5 Checks needed to maintain confidence in the status of working standards and reference materials shall be carried out according to defined procedures and schedules.

5.6.6 The laboratory shall have procedures for safe handling, transport, storage and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity.

5.7 Specimens

5.7.1 General

The laboratory shall have procedures for the collection of specimens to ensure that they are both appropriate to the test being undertaken and suitable for testing.

> **NOTE:** This applies to veterinary diagnostic laboratories only when the laboratory is directly responsible for specimen collection.

5.7.1.1 The laboratory shall have procedures for the collection, processing where indicated and preservation of specimens. Collection and related procedures shall be available at the location where collection is undertaken.

5.7.1.2 The laboratory shall have procedures for recording relevant data and operations relating to specimen collection that forms part of the test that is undertaken, whether the collection is performed by laboratory staff or by the client. Records shall include the collection procedure used, identification of the collector, environmental conditions (if

relevant) and diagrams or other means to identify the collection location as necessary (e.g., in the case of tissue specimens) and, if appropriate, the statistics that sampling procedures are based upon.

5.7.1.3 When sampling from populations, as appropriate, the laboratory shall have a statistically defined plan for sample collection.

> **NOTE**: While the laboratory may provide relevant scientific and/or statistical input into the development of sampling plans for the testing of animal populations, the development of these plans does not fall within the AAVLD Standard.

5.8 Handling of specimens

5.8.1 The laboratory shall have procedures which ensure the integrity of specimens. These shall include transportation, receipt, handling, protection, retention and/or disposal of specimens.

5.8.2 The laboratory shall have a system for identifying specimens that ensure no confusion between specimens or derived samples. The identification shall be retained throughout the life of the specimen and its derived samples in the laboratory, and linked to the test report (5.10).

5.8.3 Upon receipt of the specimen, any abnormalities or departures from normal or specified conditions, as described in the relevant test method, shall be recorded. If there has been a departure from specifications, then the samples should not be considered fit to test.

5.8.4 When there is any doubt as to the suitability of a specimen for testing purposes, or when a specimen does not conform to the description provided, or if the test method required is not specified in sufficient detail, the laboratory shall consult the client for further instructions before proceeding and shall record the facts and results of that discussion.

5.9 Ensuring the quality of test results

The laboratory shall have quality control procedures for monitoring the validity of test results. This monitoring shall be planned and reviewed and may include, but not be limited to, the following:

a) internal quality control schemes using statistical techniques (e.g., control charts);
b) where applicable, use of international reference reagents for preparation of national and/or working standards for internal quality control;
c) when practical, replicate tests using the same or different methods;
d) correlation of results for different characteristics of a specimen or sample;
e) re-testing of retained specimens or samples;
f) participation in interlaboratory comparison or proficiency testing programs.

> **NOTE:** The validity of test results is influenced by both technical competence and assay performance characteristics. If the validity of test results is called into question, it is important to be able to distinguish between the two. A test may demonstrate appropriate process control but poor diagnostic performance or vice versa.

5.10 Reporting test results

5.10.1 The results of each test performed by the laboratory shall be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the test method or contract.

5.10.2 Unless the laboratory has valid reasons for not doing so, each test report shall include at least the following information:

a) a title (e.g., "Test Report");
b) name and address of laboratory, and, if different, the location where the tests were performed;
c) unique identification (see 5.8.2.) at the beginning and on each page of the test report to ensure that the page is recognized as a part of the test report, and a clear identification of the end of the report;
d) name and address of the client placing the order;
e) description and unambiguous identification of the specimen(s) tested;
f) unique identification of the test method(s) used;
g) date of receipt of specimen(s) and date(s) of performance of the test where relevant to the validity and application of the results;
h) test results;
i) reference to specimen collection procedures used by the laboratory or by the client where these are relevant to the validity or application of the results;
j) where appropriate and needed, opinions and diagnostic interpretations of the test results;
k) the name(s), function(s), and signature(s) or equivalent identification of person(s) authorizing the test report.

5.10.3 Where applicable, the test report shall also include:

a) date of specimen collection;
b) unambiguous identification of specimen source;
c) location of collection, including any diagrams, sketches or photographs;
d) reference to sampling plan used (see 5.7.1.3.);
e) details of any environmental condition during collection that may affect the interpretation of the test results;
f) identification of the collection procedure or technique.

5.10.4 When opinions and diagnostic interpretations are included in the test report, the laboratory shall document the basis upon which the opinions and interpretations have been made.

> **NOTE:** When the results of a battery of tests are considered in formulating an opinion or making a diagnostic interpretation, it may be necessary to describe, for the client, the rationale behind the sequence of testing and the decision making process (e.g., presumptive vs. definitive tests or screening vs. confirmatory tests).

5.10.5 When the test report contains results of tests performed by subcontractors, these results shall be clearly identified.

5.10.6 In the case of transmission of test results and/or interpretations by telex, facsimile or other electronic or electromagnetic means, the requirements of the AAVLD Standard shall be met.

5.10.7 The report format shall be designed to accommodate each type of test carried out and to minimize the possibility of misunderstanding or misuse.

5.10.8 When a battery of tests are to be performed and results reported as available, interim test reports shall be issued to the client. These reports shall indicate tests completed and tests pending. Such reports shall be uniquely identified as interim test reports, shall contain a reference to any and all preceding interim reports and shall meet all the requirements of the AAVLD Standard. Upon completion of all testing, a final test report shall be issued that is uniquely identified and shall contain a reference to any and all interim reports that it replaces.

5.10.9 When a material amendment to a test report that has been issued is necessary, a supplement to the test report shall be issued to the client. Such amendments shall be uniquely identified as a supplement, shall contain a reference to the original test report and shall meet all the requirements of the AAVLD Standard.

5.10.10 When it is necessary to issue a new test report, it shall be uniquely identified and shall contain a reference to the original that it replaces.

Document Revision Summary

Version 4.3	10/19/09	
	• Entire document:	Removed "Essential" from "Essential Requirements"
	• Entire document:	Added Table of Contents
	• Objectives of the Accreditation Program, last bullet:	Changed date of OIE document from 2002 to 2008
	• Section 1.1:	Changed "will be" to "are"
	• Section 1.3:	In the second sentence, changed "shall be" to "is" Last sentence, changed "will be" to "are"
	• Section 1.4 :	Removed section
	• Section 3.4.1:	Removed "The AAVLD Accreditation Application is provided as Attachment 1."
	• Specific Requirements:	Changed date of OIE document from 2002 to 2008
	• Attachment 1:	Removed the Application from Requirements and made it a separate document.
	• Personnel Qualifications	Removed 4th column from table
Version 5.0	9/14/10	
	• Entire document:	Grammatical corrections
	• Table of Contents	Added Appendices 1 and 2, corrected page numbers
	• Section 1.2	Added "year-round" after "a full range of diagnostic services"
	• Section 5.7.1 Note	Replaced "sample" with "specimen"
	• Appendix 1, page 3	In * replaced "medical" with "veterinary"
	• Appendix 2	Added new appendix: Glossary of Terms

Appendix 1

PERSONNEL QUALIFICATIONS

POSITION	MINIMAL QUALIFICATIONS	PREFERRED QUALIFICATIONS
Director/Chief Administrator	1) DVM*; 2) 2 years experience in diagnostic laboratory medicine; 3) Broad knowledge of laboratory disciplines.	DVM and MS and PhD and Specialty Board Certification; 5 years experience in diagnostic veterinary medicine; management training; Broad knowledge of laboratory disciplines.
Quality Manager	BS** in a biological science related field, + 2 years QA experience.	MS or PhD in a biological science related field, documented advanced QA training, QA certification.
Section Heads		
Pathology	DVM and MS or 2 years residency in pathology.	DVM and PhD; Diplomate ACVP + 5 years experience.
Clinical Pathology	DVM and MS or 2 years residency in clinical pathology.	DVM and PhD; Diplomate ACVP + 5 years experience.
Toxicology	DVM and MS, or relevant PhD, and 3 years experience.	DVM and PhD; Diplomate ABVT + 5 years experience.
Bacteriology	MS degree in microbiology + 2 years, or BS certified Medical Technician + 5 years experience in veterinary diagnostic bacteriology.	DVM and MS or DVM and PhD or PhD; Diplomate ACVM + 5 years experience in veterinary diagnostic bacteriology.

POSITION	MINIMAL QUALIFICATIONS	PREFERRED QUALIFICATIONS
Virology	MS degree in microbiology and 2 years experience in veterinary diagnostic virology.	DVM and MS or DVM and PhD or PhD; Diplomate ACVM + 5 years experience in veterinary diagnostic virology.
Serology	MS degree in microbiology and 2 years experience in veterinary diagnostic serology.	DVM and MS or DVM and PhD or PhD; Diplomate ACVM + 5 years experience in veterinary diagnostic serology.
Molecular Diagnostics	MS degree in molecular diagnostics + 2 years, or BS certified Medical Technician + 5 years experience in veterinary molecular diagnostics.	DVM and MS or DVM and PhD or PhD + 5 years experience in veterinary molecular diagnostics.
Analytical Chemistry	MS degree in chemistry + 3 years experience, or BS degree in chemistry and 5 years experience.	MS or PhD Board Certified (ABT) + 5 years experience in chemistry.
Professional Staff		
Pathologists	DVM and MS or 2 years residency in pathology.	DVM and PhD; Diplomate ACVP + 5 years experience.
Clinical Pathologists	DVM and MS or 2 years residency in clinical pathology.	DVM and PhD; Diplomate ACVP + 5 years experience.
Diagnosticians	DVM with 2 years experience in diagnostic laboratory medicine.	DVM with advanced training in appropriate discipline and 5 years in diagnostic laboratory medicine.

POSITION	MINIMAL QUALIFICATIONS	PREFERRED QUALIFICATIONS
Technical/Clerical Staff		
Laboratory Technicians Histotechnology Bacteriology Clinical Path Toxicology Serology Virology Molecular Diagnostics	High school + 2 years experience; or comply with existing state or university policy.	BS/MT/VT/HT/HTL as appropriate and 2 years experience.
Technician Medical Records	High school + 2 years experience or Associates Degree and certification.	BS + 2 years experience and certification.

*Or equivalent /comparable veterinary degree.
**Or equivalent/comparable science degree.

Appendix 2 Glossary of Terms

Accreditation: A process by which an authoritative body (accreditation body) gives formal recognition that an organization or person is competent to carry out specific tasks as outlined in accreditation requirements.

Accuracy: The level of agreement between a test value and the expected value for a reference standard, control, or known activity or titer; closeness to the true value.

Assessment: A process of collecting and analyzing data in a systematic way to determine the compliance of an organization with specific accreditation requirements.

Audit finding: The result(s) of the evaluation between collected audit evidence and audit criteria.

Calibration: The process of adjusting the accuracy of a piece of equipment to a NIST calibrated standard.

Competence: The demonstrated ability to get the correct result by possessing the required skill, knowledge, qualification or capacity.

Continuous improvement: A set of recurring activities that an organization carries out in order to enhance its ability to meet requirements. Some of these activities may include audits, management reviews, corrective and preventive actions, analyzing data and setting objectives.

Control chart: A chart with upper and lower control limits on which values of some statistical measure for a series of samples or subgroups are plotted. Control charts may be used to evaluate shifts and trends within a controlled process (e.g. test method).

Corrective action: The steps taken to reduce or eliminate the cause of an existing nonconformity or other undesirable situation. Corrective actions prevent *recurrence* of nonconformities. See also Preventive Action. Note: An initial correction is the immediate step taken to fix a detected nonconformity or get a process back under control prior to conducting the root cause analysis of a corrective action.

Document: From AAVLD Requirements pg. 23 - **"NOTE:** In this context "document" means any information or instructions, in any format or medium, that have direct bearing on or affect the quality of test results, and includes not only the quality manual, policy, procedures, and instructions, but also test methods, worksheets, forms, international standards, and regulations."

Effectiveness: The state of having produced a decided on or desired effect. The extent to which planned activities are realized and planned results achieved.

Guideline: A document stating recommendations or suggestions.

Improvement: The positive effect of a process change effort.

Internal (first party) audit: An on-site inspection of a process or quality system to ensure compliance with specific requirements. The auditors who conduct first party audits are employees of the organization being audited.

Management system: The organizational structure, responsibilities, procedures, processes and resources for implementing policy and achieving objectives.

Management review: An evaluation of the suitability, adequacy, and effectiveness of an organization's quality policy and quality objectives, address resource needs and look for opportunities for improvement.

NIST (National Institute of Standards and Technology): An agency of the U.S. Department of Commerce that develops and promotes measurements, standards and technology.

Nonconformance (noncompliance): The failure to comply with a specified requirement.

Objective evidence: The evidence supporting the existence or verity of something. It may be obtained through observation, measurement, test, or other means.

Policy: An overarching plan (direction), used for the basis of making decisions, and for achieving an organization's goals.

Precision: The aspect of measurement that addresses repeatability or consistency when an identical item is measured several times - precise does not equal accurate.

Preventive action: Action taken to remove the cause of a potential nonconformance or undesirable situation. Preventive actions prevent *occurrence* of nonconformities. See also Corrective Action.

Process: A set of interrelated work activities characterized by a set of specific inputs that make up a procedure for a set of specific outputs.

Process control: The method for keeping a process within accepted boundaries by minimizing variation.

Quality assurance: A planned program consisting of the actions necessary to provide confidence that a test or testing activity conforms to established technical requirements.

Quality control: The operational activities used to ensure that quality standards are being met.

Quality management system: A set of interrelated or interacting elements that organizations use to implement and direct quality planning, quality control, quality assurance, and quality improvement.

Quality manual: A document specifying the quality management system of an organization. A Quality Manual may vary in detail and format in order to suit the size and complexity of an organization.

Quality policy: An organization's general statement of its beliefs about quality, how quality will come about and its expected result. It should define top management's commitment to quality and describe an organization's basic intent.

Record: Any and all written materials that provide proof of compliance with the quality system and evidence that a specified activity has been performed. They may be in hard copy or electronic form and should be attributable to an individual.

Reliability: The ability of an item to perform a required function under stated conditions for a stated period of time.

Repeatability: The variation in measurements taken by a single person or instrument on the same item and under the same conditions (e.g. running a sample in triplicate).

Reproducibility: The ability of a test or method to be accurately reproduced, or *replicated.* (e.g. running a sample for a given method on two different days or by two different analysts).

Root cause: The initiating reason for the presence of a defect or problem. When removed or corrected, the nonconformance is eliminated.

Root cause analysis: The process of problem solving used to identify the underlying or initiating source of a nonconformance.

Sample: material that is derived from a specimen and used for testing purposes.

Sensitivity (diagnostic): proportion of known infected reference animals that test positive in the assay (infected animals that test negative are considered to have false-negative results).

Specification: The requirements to which a given service must conform, usually stated in a document.

Specificity (diagnostic): proportion of known uninfected reference animals that test negative in an assay (uninfected reference animals that test positive are considered to have false-positive results).

Specimen: material submitted for testing, e.g., carcass, whole blood, serum, and urine.

Trend: The measure of a variable's tendency, over time, to increase, decrease or remain unchanged. It is typically represented graphically or through statistical means.

Traceability: The ability to identify and trace the history, distribution, location, and application of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties.

Validation: The act of confirming, through objective evidence, that the requirements which define an intended use or application have been met. The process through which a test method is confirmed to be fit for the intended purpose.

Verification: The process of comparing the accuracy of a piece of equipment to a NIST calibrated reference standard.

Callan, Tara AG:EX

From: Marty, Gary D AGRI:EX
Sent: Thursday, April 21, 2011 3:32 PM
To: Callan, Tara AG:EX
Subject: FW: Requirements to notify the CFIA
Attachments: Mandatory_Notification_Directive_L_EN.pdf

-----Original Message-----
From: Kim Klotins [mailto:Kim.Klotins@inspection.gc.cal
Sent: Friday, April 15, 2011 5:59 AM
To: Marty, Gary D AGRI:EX
Subject: Requirements to notify the CFIA

Dear Dr. Marty,

Please find attached the Directive that outlines the requirement for laboratories to notify CFIA of Reportable, Immediately Notifiable and Annually Notifiable Diseases. The requirement to notify CFIA of suspicion or detection of Reportable diseases came into effect on January 5, 2011.

I believe this meets the timelines you requested with respect to notification of certain aquatic animal diseases to CFIA.

Regards, Kim

Kim Klotins, DVM, DVSc

Acting National Manager | Gestionnaire national intérimaire Disease Control & Contingency Planning | Lutte contre les maladies et planification des mesures Aquatic Animal Health Division | Division de la santé des animaux aquatiques Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments
59 Camelot Drive, 59-3W-210
Ottawa, ON, Canada, K1A 0Y9

Telephone | Téléphone: 613-773-7427
Fax | Télécopieur: 613-773-7567
Email | Courriel: kim.klotins@inspection.gc.ca

**For notification of updates to the CFIA NAAHP website, sign up for the e-mail subscription list at http://www.inspection.gc.ca/english/util/listserv/listasube.shtml

This is Exhibit "D" referred to in the affidavit of DR. GARY MARTY
sworn before me at Abbotsford
in the Province of British Columbia this
26 day of April, 2011

A Commissioner for taking Affidavits within the Province of British Columbia

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

Aquatic Animal Health Division
59 Camelot Drive
Ottawa, ON K1A 0Y9

DIRECTIVE

Effective Date: January 19, 2011

Subject: Mandatory Notification of Regulated Aquatic Animal Diseases

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This Directive is to advise laboratory personnel of the requirements with respect to regulated aquatic animal diseases in Canada. Regulated aquatic animal diseases are currently limited to finfish, molluscs, and crustaceans, and are classified as Reportable, Immediately Notifiable, and Annually Notifiable diseases.

Requirements and disease lists are in the *Health of Animals Act*, *Health of Animals Regulations*, and *Reportable Diseases Regulations*. The Canadian Food Inspection Agency (CFIA) website (http://www.inspection.gc.ca/) provides links to the *Health of Animals Act* and *Health of Animals Regulations*. The amended portions of the *Health of Animals Regulations* and *Reportable Diseases Regulations*, listing the aquatic animal diseases, were published in *Canada Gazette, Part II*, on December 22, 2010, and on January 5, 2011, respectively. They can be found at (http://www.gazette.gc.ca/rp-pr/p2/2011/2011-01-05/html/sor-dors310-eng.html), and in the Background section of this Directive.

The requirements are as follows:

> 5. (2) Immediately after a person who is a veterinarian or who analyzes animal specimens suspects that an animal is affected or contaminated by a reportable disease or toxic substance, the person shall so notify a veterinary inspector (*Health of Animals Act*).
>
> 91.2 (1) Every laboratory that diagnoses or suspects the appearance in an animal or thing of a disease set out in Schedule VII (Immediately Notifiable) shall notify the Minister immediately of the diagnosis or suspicion (*Health of Animals Regulations*).
>
> 91.2 (2) Along with that notification, the laboratory shall include
> (a) the name, address and telephone number of the person who owns or has the possession, care or control of the animal or thing;
> (b) the location of the animal or thing; and
> (c) all other information that the laboratory has in relation to the animal or thing (*Health of Animals Regulations*).

Canada

> 91.2 (3) Every laboratory that diagnoses or suspects the appearance in an animal or thing of a disease set out in Schedule VIII (Annually Notifiable) shall notify the Minister of the diagnosis or suspicion immediately after the end of the calendar year in which the appearance of the disease is diagnosed or suspected (*Health of Animals Regulations*).

The contact information for immediate notifications of Reportable and Immediately Notifiable aquatic animal diseases is as follows:

Dr. Kim Klotins
Acting National Manager, Disease Control Contingency Planning
Aquatic Animal Health Division
Canadian Food Inspection Agency
Telephone: 613-221-1398
Fax: 613-221-3173
Email: NAAHP-PNSAA@inspection.gc.ca

Laboratory personnel may wish to consider developing a protocol to retain the original aquatic animal tissue specimens, or their isolates, of the causative agent from any positive test results, so they may be shipped to a Fisheries and Oceans Canada National Aquatic Animal Health Reference Laboratory, if requested, for confirmation of the regulated aquatic animal disease.

The Aquatic Animal Health Division of the CFIA will develop a template for laboratories to report Annually Notifiable diseases to the CFIA. The template will be sent to laboratories in December of each calendar year, and laboratories should submit positive results by fax or email to the National Manager of Disease Control Contingency Planning, Aquatic Animal Health Division, by January 15 of the following calendar year.

This directive will remain in effect until the Mandatory Notification and Suspect Phase Disease Response Policy for the National Aquatic Animal Health Program is updated to reflect any changes in policy, or is superseded by another directive.

For any specific questions about this Directive, please contact Dr. Kim Klotins by telephone at 613-221-1398 or by email at Kim.Klotins@inspection.gc.ca.

Sincerely,

Dr. K. Klotins

Dr. Kim Klotins
Acting National Manager, Disease Control Contingency Planning
Aquatic Animal Health Division, Programs and Policy Branch

BACKGROUND

The Government of Canada is committed to protecting the country's aquatic animal resources and facilitating safe trade in fish and seafood. As a result, recent amendments to the *Health of Animals Regulations* have been published in *Canada Gazette, Part II*, on December 22, 2010, and to the *Reportable Diseases Regulations*, on January 5, 2011.

The amendments authorize the Canadian Food Inspection Agency to implement the National Aquatic Animal Health Program (NAAHP). This science-based program addresses finfish, mollusc, and crustacean diseases that pose serious risks to Canada's aquatic animal resources.

These changes are in line with the existing terrestrial animal health program. They will ensure Canada meets the standards set by the World Organisation for Animal Health (OIE) for preventing disease spread from the trade of live animals or high risk commodities.

The regulated aquatic animal diseases are listed in Tables 1–3.

TABLE 1 **Reportable Diseases**

Disease	Aquatic Animal
Bonamia ostreae	Mollusc
Ceratomyxosis (*Ceratomyxa shasta*)	Finfish
Epizootic haematopoietic necrosis	Finfish
Haplosporidium nelsoni	Mollusc
Infectious haematopoietic necrosis	Finfish
Infectious pancreatic necrosis	Finfish
Infectious salmon anaemia	Finfish
Koi herpesvirus disease	Finfish
Marteilia refringens	Mollusc
Marteilioides chungmuensis	Mollusc
Mikrocytos mackini	Mollusc
Perkinsus marinus	Mollusc
Perkinsus olseni	Mollusc
Spring viraemia of carp	Finfish
Taura syndrome	Crustacean
Viral haemorrhagic septicaemia	Finfish
Whirling disease (*Myxobolus cerebralis*)	Finfish
White spot disease	Crustacean
White sturgeon iridoviral disease	Finfish
Yellow head disease	Crustacean

TABLE 2 **Immediately Notifiable Diseases**	
Disease	Aquatic Animal
Abalone viral mortality (Abalone herpes-like Virus)	Mollusc
Bonamia exitiosa	Mollusc
Bonamia roughleyi	Mollusc
Brown ring disease (*Vibrio tapetis*)	Mollusc
Crayfish plague (*Aphanomyces astaci*)	Crustacean
Epizootic ulcerative syndrome (*Aphanomyces invadans*)	Finfish
Gyrodactylosis (*Gyrodactylus salaris*)	Finfish
Infectious hypodermal and haematopoietic necrosis (Infectious hypodermal and Haematopoietic necrosis virus)	Crustacean
Infectious myonecrosis (Infectious myonecrosis virus)	Crustacean
Marteilia sydneyi	Mollusc
Necrotizing hepatopancreatitis	Crustacean
Oncorhynchus masou virus disease (Oncorhynchus masou disease virus)	Finfish
Red sea bream iridoviral disease (Red Sea bream iridovirus)	Finfish
White tail disease (White tail virus)	Crustacean
Withering syndrome of abalone (*Xenohaliotis californiensis*)	Mollusc

TABLE 3 **Annually Notifiable Diseases**	
Disease	Aquatic Animal
Bacterial kidney disease (*Renibacterium salmoninarum*)	Finfish
Enteric red mouth disease (*Yersinia ruckeri*)	Finfish
Furunculosis (*Aeromonas salmonicida*)	Finfish
QPX disease (Quahog parasite unknown)	Mollusc
Seaside organism (*Haplosporidium costale*)	Mollusc
Streptococcosis (*Streptococcus iniae*)	Finfish

This is Exhibit "E" referred to in the
affidavit of DR. GARY MARTY
sworn before me at Abbotsford
in the Province of British Columbia this
26 day of APRIL, 2011

A Commissioner for taking Affidavits
within the Province of British Columbia

CHAPTER 2.3.5.

INFECTIOUS SALMON ANAEMIA

1. Scope

Infectious salmon anaemia (ISA) is an orthomyxovirus infection of sea-farmed Atlantic salmon (*Salmo salar*) (28) inducing a systemic and lethal condition characterised by severe anaemia and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05–0.1%) typically only in a few cages, but cumulative mortality may become very high. For the purpose of this chapter, ISA is considered to be infection with salmon anaemia virus (ISAV) (12).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

ISAV is an enveloped virus, 100–130 nm in diameter, with a genome consisting of eight single-stranded RNA segments with negative polarity. The virus has haemagglutinating, receptor-destroying end fusion activity (1, 5, 14, 21, 24).

The morphological, physiochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae* (5, 21), and ISAV has recently been classified as the type species of the new genus *Isavirus* (12) within this virus family. The nucleotide sequences of all eight genome segments have been described. The viral genome encodes at least ten proteins (1, 14, 24). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa surface glycoprotein with putative fusion activity, coded by genome segments 3, 8, 6 and 5, respectively. Segment 1, 2, and 4 encode the viral polymerases PB1, PB2) and PA. The two smallest genomic segments, segments 7 and 8, each contain two open reading frames (ORF). The ORF1 of segment 7 encodes a nonstructural protein with interferon antagonistic properties, while ORF2 has been suggested to encode for a nuclear export protein. The possibility of a third ORF has been discussed. The smaller ORF1 of segment 8 encodes the matrix protein, while the larger ORF2 encodes an RNA-binding structural protein also with interferon antagonistic properties (1, 6, 13, 14, 24).

Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas (1, 14, 24). According to differences in the 5'-terminal end of the HE gene, ISAV isolates have been divided into two major groups, one European and one North American group. The European group may be further divided into three major groups (1). A small, highly polymorphic region (HPR) of the haemagglutinin gene has been identified (18). This region is characterised by the presence of gaps rather than single-nucleotide substitutions. However, there is no direct correlation between phylogenetic groups and deletion patterns in the HPR. A full-length gene (HPR0) has been suggested to represent an ancient variant from which the variants with deletions in the HPR region have been derived. The presence of HPR0 has been reported in wild and farmed Atlantic salmon (1, 14), but has not been detected in diseased fish with clinical and pathological signs consistent with ISA. Variations in the HPR between virus isolates have been suggested to be important for virulence, as all diseased fish contain deletions in this region. However, other genes are most certainly also of importance in virulence because isolates with identical HPRs vary significantly in development and severity of disease (19). Recently, a potential virulence marker has been identified in segment 5 encoding the fusion protein and involving the protease recognition pattern at the cleavage site of the fusion protein (17). Furthermore, evidence for reassortment and nonhomologous recombination of ISAV has been provided (3, 17).

2.1.2. Survival outside the host

ISAV RNA has been detected by reverse-transcription polymerase chain reaction (RT-PCR) in seawater sampled at farming sites with ISAV-positive Atlantic salmon (14). It is difficult to estimate exactly how long the virus may remain infectious in the natural environment because of a number of factors, such as the presence of particles or substances that may bind or inactivate the virus, UV irradiation and temperature. Exposing cell culture-propagated ISAV to 15°C for 10 days or to 4°C for 14 days had no effect on virus infectivity (5).

2.1.3. Stability of the agent (effective inactivation methods)

ISAV is sensitive to UV irradiation (UVC) and ozon (1). A 3-log reduction in infectivity in sterile fresh water and seawater was obtained with a UVC dose of approximately 35 Jm^{-2} and 50 Jm^{-2}, respectively, while the corresponding value for ISAV in wastewater from a fish-processing plant was approximately 72 Jm^{-2}. Ozonated seawater (4 minutes with 8 mg ml^{-1}, 600–750 mV redox potential) may inactivate ISAV completely. Cell culture-isolated ISAV may survive for weeks at low temperatures, but virus infectivity is lost within 30 minutes exposure at 56°C (5). Incubation of tissue homogenate from ISA-diseased fish at pH 4 or pH 12 for 24 hours inactivated ISA infectivity. Incubation in the presence of chlorine (100 mg ml^{-1}) for 15 minutes also inactivated virus infectivity (1).

2.1.4. Life cycle

The main infection route is most likely through the gills but infection via the intestine cannot be excluded. Endothelial cells seem to be the primary target cells for ISAV by electron microscopy (1, 14, 24). This has recently been confirmed by immunohistochemical examination of several organs (National Veterinary Institute Norway, unpublished results) and by *in-situ* hybridisation (14). Virus replication has also been demonstrated in leukocytes), and sinusoidal macrophages in kidney tissue stain positive for ISAV using immunohistochemistry (IHC). As endothelial cells are the target cells, virus replication may occur in several organs. *In-situ* hybridisation studies have indicated the most extensive and prolonged replication to occur in the heart tissue (14).

The haemagglutinin-esterase molecule of ISAV, like the haemagglutinin of other orthomyxoviruses, is essential for binding the virus to sialic acid residues on the cell surface. In the case of ISAV, the virus binds to glycoproteins containing 4-O-acetylated sialic acids, which also serve as substrates for the receptor-destroying enzyme. Further uptake and replication seem to follow the pathway described for influenza A viruses, indicated by demonstration of low pH-dependent fusion, inhibition of replication by actinomycin D and α-amanitin, early accumulation of nucleoprotein followed by matrix protein in the nucleus and budding from the cell surface (1, 14, 24).

The route of shedding of ISAV from infected fish may be through natural excretions/secretions.

2.2. Host factors

2.2.1. Susceptible host species

Natural outbreaks of ISA have only been recorded in farmed Atlantic salmon, but the virus has been isolated from rainbow trout in Ireland (7) and there is a report of isolation of ISAV from Coho salmon (*Oncorhynchus kisutch*) in Chile (14). Subclinically infected feral Atlantic salmon, brown trout and sea trout (*S. trutta*) have been identified by RT-PCR (14, 23). In marine fish, detection of ISAV by RT-PCR has been reported in tissues of pollock (*Pollachius virens*) and cod (*Gadus morhua*), but only in fish collected from cages with Atlantic salmon exhibiting ISA (14). In these studies, only weak positive results were obtained, and gills were included in the tissue samples examined. Contamination of virus present in surrounding water cannot, therefore, be excluded, and corroborative studies are needed before these species can be identified as possible hosts for ISAV.

Following experimental infection, replication of ISAV has been demonstrated in several fish species, including brown trout, sea trout, rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), herring (*Clupea harengus*) (1, 14, 24) and Atlantic cod (*Gadus morhua*) (10). Mortality and histopathological changes have been introduced in rainbow trout by experimental infection, although the lesion characteristics were different from those in Atlantic salmon (16). Attempts have been made to induce infection or disease in *P. virens*, but with negative results (14).

2.2.2. Susceptible stages of the host

In Atlantic salmon, disease outbreaks are mainly reported in seawater cages, and only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (1). Furthermore, ISA has been experimentally induced in both Atlantic salmon fry and parr kept in freshwater. Genetics may also play an important role in the susceptibility of Atlantic salmon to ISA, as differences in susceptibility among different family groups have been observed (1). Furthermore, a functional association between disease resistance and major histocompatibility (MHC) class I and II polymorphism has been demonstrated (9). The use of MHC-compatible Atlantic salmon indicated that the ability to mount a strong proliferative response correlated to survival and virus clearance, while induction of a humoral response was less protective (19).

2.2.3. Species or subpopulation predilection (probability of detection)

ISA is primarily a disease of Atlantic salmon.

2.2.4. Target organs and infected tissue

Endothelial cells in many organs (heart, liver, kidney, spleen and others).

2.2.5. Persistent infection with lifelong carriers

Persistent infection in lifelong carriers has not been documented in Atlantic salmon, but at the farm level, infection may persist in the population by continuous infection of new individuals that do not cause any recognisable disease problems. Experimental infection of rainbow trout and brown trout indicates that persistent infection in these species could be possible (1, 14, 24). See Section 2.4.1 for the possible creation of virus carriers through vaccination.

2.2.6. Vectors

Passive transfer of ISA by salmon lice (*Lepeophtheirus salmonis*) has been demonstrated under experimental conditions. Although natural vectors have not been identified, several different vector groups could be possibel vectors under certain defined conditions.

2.2.7. Known or suspected wild aquatic animal carriers

Wild Atlantic salmon, brown trout and sea trout (*S. trutta*) may be carriers of ISAV (1, 23). The importance of wild marine fish (see Section 2.2.1) as virus carriers needs to be clarified.

2.3. Disease pattern

2.3.1. Transmission mechanisms

The disease is spread horizontally by water-borne transmission as shown by experimental infection studies. There is no strong evidence for vertical transmission through infected gonadal products. It has been suggested that ISAV is spread over long distances by transportation of smolt, either infected prior to shipping or by well boats contaminated with ISAV. Contamination of well boats may be due either to previous transport of infected fish or through intake water from areas with farms harbouring diseased fish.

Epidemiological studies have shown that the risk of ISA transmission is closely linked to husbandry practices in aquaculture and horizontal transmission. Geographical or hydrological (via prevailing currents) proximity (<5 km) to farms with ISA outbreaks or slaughterhouses/processing plants releasing contaminated water, numerous smolt deliveries and the use of well boats, and sharing staff and equipment are all considered significant risk factors (1, 11, 15, 25).

Other horizontal pathways have also been suggested, such as transmission through sea lice, infected wild fish and various harvesting methods (14, 24). According to Nylund *et al.* (22) vertical or transgenerational transmission may occur. Carryover or stocking of multiple year-classes on a given site, or within a region connected hydrologically, may also influence occurrence of ISA (11).

2.3.2. Prevalence

In a net pen containing diseased fish, the prevalence may vary widely, while in adjacent net pens ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations it is important to sample from net pens containing diseased fish.

2.3.3. Geographical distribution

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, report to OIE), USA (Maine in 2001) and in Chile (1, 8, 14, 24). The virus has been reported from rainbow trout in Ireland in 2002 (7) and from Coho salmon in Chile (14).

2.3.4. Mortality and morbidity

Morbidity and mortality may vary greatly within and between different net pens in a seawater fish farm, and between different fish farms. Morbidity and mortality within a net pen may start at very low levels. Typically, daily mortality ranges from 0.5 to 1% in affected cages. Without intervention, mortality increases and seems to peak in early summer and winter. The range of cumulative mortality during an outbreak is from insignificant to moderate, but in severe cases, cumulative mortality exceeding 90% may be experienced during a period of, for example, 3 months. Initially, an outbreak of ISA may be limited to one or two net pens over a long period and the spread to other net pens may take months but is slowed by

early detection and depopulation of infected pens. In outbreaks where smolts have been infected in well boats during transport, simultaneous outbreaks may occur.

2.3.5. Environmental factors

Generally, outbreaks of ISA have been recorded at various times during the year. Handling of fish (e.g. sorting or treatment, splitting or moving of cages) may initiate disease outbreaks on infected farms, especially if long-term undiagnosed problems have been experienced in advance (15).

2.4. Control and prevention

2.4.1. Vaccination

Vaccination against ISA has been carried out in North America and the Faroe Islands during the past 5 years, but the currently available vaccines do not seem to offer complete protection in Atlantic salmon. The vaccines, which are inactivated, whole virus vaccines, do not give virus clearance in immunised fish, and they may thus become virus carriers (14).

2.4.2. Chemotherapy

Not applicable.

2.4.3. Immunostimulation

Not applicable.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon in fresh water have been observed in challenge experiments and in field tests, indicating the potential for resistance breeding.

2.4.5. Restocking with resistant species

Not applicable.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs according to standard procedures is suggested as an important control measure.

2.4.8. General husbandry practices

The incidence of ISA may be greatly reduced by implementation of legislative measures or husbandry practices regarding the movement of fish, mandatory health control, transport and slaughterhouse regulations. Specific measures including restrictions on affected, suspected and neighbouring farms, enforced sanitary slaughtering, generation segregation ('all in/all out') as well as disinfection of offal and wastewater from fish slaughterhouses and fish processing plants may also contribute to reducing the incidence of the disease.

3. Sampling

3.1. Selection of individual specimens

The following is primarily for verification of suspected cases based on clinical signs and gross pathology or positive RT-PCR for ISAV.

3.2. Preservation of samples for submission

Haematology:	Heparin or EDTA (ethylene diamine tetra-acetic acid)
Cell culture:	Virus transport medium
Histology and immunohistochemistry:	Fixation in neutral phosphate-buffered 10% formalin
Immunocytochemistry (smears):	Either submitted dried, or dried and fixed in 100% ecetone
Molecular biology (RT-PCR and sequencing):	Appropriate medium for preservation of RNA

3.3. Pooling of samples

Pooling of samples is not recommended for verification of ISAV as it is usually of interest to compare results from the various examinations for each individual. For surveillance purposes, pooling of samples for virological examination (PCR and/or cell culture) may be accepted. However, the number of fish to be pooled may depend on the suggested prevalence of ISAV in the population and of the method used.

3.4. Best organs or tissues

Blood for non-lethal sampling;
Virological examination (cell culture and PCR): heart (should always be included) and mid-kidney. For surveillance purposes using PCR, gills should also be included;
Histology (prioritised): mid-kidney, liver, heart, pancreas/intestine, spleen, gills, skin/muscle;
Immunocytochemistry (smears): mid-kidney;
Immunohistochemistry: mid-kidney, heart (including valves and bulbus arteriosus).

3.5. Samples/tissues that are not suitable

For virus isolation, gills are not recommended as it is quite difficult to avoid microbial contamination of cell cultures inoculated with gill tissue homogenate even after appropriate filtration.

4. Diagnostic methods

The diagnosis of ISA was initally based on clinical and pathological findings only (14, 24). Following the isolation of the causative agent, a number of direct methods for detection of virus and confirmation of the diagnosis have been established. These include isolation of the virus in cell culture followed by immunological identification, immunological demonstration of ISAV antigen in tissues and PCR techniques. Differential diagnoses are: other anaemic and haemorrhagic conditions, and winter ulcer and septicaemias caused by infections with *Moritella viscosa*.

4.1. Field diagnostic methods

4.1.1. Clinical signs

The most prominent external signs are pale gills (except in the case of blood stasis in the gills), exophthalmia, distended abdomen, blood in the anterior eye chamber, and sometimes skin haemorrhages especially of the abdomen, as well as scale pocket oedema.

Nutritional status is usually quite normal, but diseased fish has no feed in the digestive tract.

4.1.2. Behavioural changes

Generally, naturally infected Atlantic salmon with ISA appear lethargic and may keep close to the wall of the net pen.

4.2. Clinical methods

4.2.1. Gross pathology

Fish infected with ISAV may show a range of pathological changes, from none to severe, depending on factors such as infective dose, virus strain, temperature, age and immune status of the fish. No lesions are pathognomonic to ISA, but but anemia and circulatory disturbances are always present. The following findings have been described to be consistent with ISA.

- Yellowish or blood-tinged fluid in peritoneal and pericardial cavities.
- Oedema of the swim bladder.
- Small haemorrhages of the visceral and parietal peritoneum.
- Focal or diffusely dark red liver. A thin fibrin layer may be present on the surface.
- Swollen, dark red spleen with rounded margins.
- Dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens.

- Swollen, dark red kidney with blood and liquid effusing from cut surfaces.
- Pinpoint haemorrhages of the skeletal muscle.

4.2.2. Clinical chemistry

- Haematocrit <10 in end stages (25–30 often seen in less advanced cases).
  Haematocrit <10 should always be followed up by investigation for ISA in sea-water reared Atlantic salmon.
- Blood smears with degenerate and vacuolised erythrocytes and the presence of erythroblasts with irregular nuclear shape. Differential counts show a reduction in the proportion of leucocytes relative to erythrocytes, with the largest reduction being among lymphocytes and thrombocytes.

Liver pathology will lead to increased levels of liver enzymes in the blood

A haematocrit value below 10 is not a unique finding for ISA. Fish with disease conditions such as ulcerations and erythrocytic inclusion body syndrome, may regularly demonstrate haematocrit values this low.

4.2.3. Microscopic pathology

Histological changes in clinically diseased Atlantic salmon are variable, but can include the following:

- Numerous erythrocytes in the central venous sinus and lamellar capillaries where erythrocyte thrombi also form in the gills.
- Multifocal to confluent haemorrhages and/or hepatocyte necrosis at some distance from larger vessels in the liver. Focal accumulations of erythrocytes in dilated hepatic sinusoids.
- Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and eventually haemorrhage into the lamina propria.
- Spleen stroma distended by erythrocyte accumulation.
- Slight multifocal to extensive diffuse interstitial haemorrhage with tubular necrosis in the haemorrhagic areas, erythrocyte accumulation in the glomeruli in the kidney.
- Erythrophagocytosis in the spleen and secondary haemorrhages in liver and kidney.

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

See Section 4.3.1.1.2

4.2.6. Electron microscopy/cytopathology

Virus has been observed in endothelial cells throughout the body by electron microscopy of tissue preparations, but this method has not been used for diagnostic purposes.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

4.3.1.1.2.1 Indirect fluorescent antibody test

An indirect fluorescent antibody test (IFAT) using validated monoclonal antibodies (MAbs) against ISAV haemagglutinin-esterase (HE) on kidney smears (imprints) or on frozen tissue sections of kidney, heart and liver has given positive reactions in both experimentally and naturally infected Atlantic salmon. Suspected cases (see Section 7.1) may be confirmed with a positive IFAT.

i) Preparations of tissue smears (imprints)

A small piece of the mid-kidney is briefly blotted against absorbent paper to remove excess fluid, and several imprints in a thumbnail-sized area are fixed on poly-L-lysine-coated microscope slides. The imprints are air-dried, fixed in chilled 100% acetone for 10 minutes and stored either at 4°C for a few days or at –80°C until use.

ii) Preparations of cryosections

Tissue samples from kidney, liver and heart are collected from moribund fish, frozen in iso-pentane, chilled in liquid nitrogen, and stored at –80°C. Sections are cut on a cryostat, placed on poly-L-lysine-coated slides, fixed in chilled 100% acetone for 10 minutes and stored at –80°C until use.

iii) Staining procedure

After blocking with 5% non-fat dry milk in phosphate-buffered saline (PBS) for 30 minutes, the preparations are incubated for 1 hour with an approriate dilution of anti-ISAV MAb, followed by three washes. For the detection of bound antibodies, the preparations are incubated with fluoescein isothiocyanate (FITC)-conjugated anti-mouse Ig for 1 hour. PBS with 0.1% Tween 20 is used for washing. All incubations are performed at room temperature.

4.3.1.1.3. Fixed sections

4.3.1.1.3.1 Immunohistochemistry (IHC)

Polyclonal antibody against ISAV nucleoprotein is used on paraffin sections from formalin-fixed tissue. This IHC staining has given positive reactions in both experimentally and naturally infected Atlantic salmon. Preferred organs are mid-kidney and heart (transitional area including all three chambers and valves). Suspected cases due to pathological signs are verified with a positive IHC. Histological sections are prepared according to standard methods.

i) *Preparation of tissue sections*

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 5 µm thick sections (for IHC sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for pathomorphology and IHC as described below.

ii) *Staining procedure for IHC*

All incubations are carried out at room temperature on a rocking platform, unless otherwise stated.

a) Antigen retrival is done by boiling sections in 0.1 M citrate buffer pH 6.0 for 2 × 6 minutes followed by blocking with 5% non-fat dry milk and 2% goat serum in 50 mM TBS (TBS; Tris/HCl 50 mM, NaCl 150 mM, pH 7.6) for 20 minutes.

b) Sections are then incubated overnight with primary antibody (monospecific rabbit antibody against ISAV nucleoprotein) diluted in TBS with 1% non-fat dry milk, followed by three washes in TBS with 0.1% Tween 20.

c) For detection of bound antibodies, sections are incubated with Alkaline phosphatase-conjugated antibodies to rabbit IgG for 60 minutes. Following a final wash, Fast Red (1 mg ml^{-1}) and Naphtol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) is added to develop for 20 minutes. Sections are then washed in tap water before counterstaining with Harris haematoxylin and mounted in aqueous mounting medium. ISAV positive and ISAV negative tissue sections are included as controls in every setup.

iii) *Interpretation*

Negative control sections should not have any significant colour reactions. Positive control sections should have clearly visible red-coloured cytoplasmic and intranuclear staining of endothelial cells in blood vessels or heart endocardium. A test sample section should only be regarded as positive if clear, intranuclear red staining of endothelial cells is found. The intranuclear localisation is particular to the orthomyxovirus nucleoprotein during a stage of virus replication. Concurrent cytoplasmic staining is often dominant. Cytoplasmic and other staining patterns without intranuclear localisation must be considered as nonspecific or inconclusive.

The strongest positive staining reactions are usually obtained in endothelial cells of heart and kidney. Endothelial staining reactions within very extensive haemorrhagic lesions can be slight or absent, possibly because of lysis of infected endothelial cells.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture

SHK-1 (2), ASK (4) or other susceptible cell lines, such as TO and CHSE-214 (14), may be used but strain variability and the ability to replicate in different cell lines should be taken into consideration. The SHK-1 and ASK cells seem to support growth of the hitherto known virus isolates. A more distinct cytopathic effect (CPE) may appear in ASK cells. Both the SHK-1 and ASK cell lines appear to lose susceptibility for ISAV with increasing passage level.

The SHK-1 and ASK cells are grown at 20°C in Leibovitz's L-15 cell culture medium supplemented with fetal bovine serum (5% or 10%), L-glutamine (4 mM), gentamicin (50 µg ml^{-1}) and 2-mercaptoethanol (40 µM) (this latter may be omitted).

For virus isolation, cells grown in 25 cm^2 tissue culture flasks or multi-well cell culture plates, which may be sealed with parafilm to stabilise the pH of the medium, may be used. Cells grown in 24-well plates may not grow very well into monolayers, but this trait may vary between laboratories and according to the type of cell culture plates used. Serially diluted ISAV-positive controls should be inoculated in parallel with the tissue samples as a test for cell susceptibility to ISAV (this should be performed in a separate location from that of the test samples).

i) *Inoculation of cell monolayers*

Prepare a 2% suspension of tissue homogenate using L-15 medium without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1–3 day old cultures or cultures of 70–80 % confluency) grown in 25 cm^2 tissue culture flasks or 24-well plates. Inoculate monolayers with 0.1 ml of the 2% tissue homogenate. Allow 3–4 hours incubation at 15°C followed by removal of the inoculum, and addition of fresh, fully supplemented growth medium. Alternatively, a 1/1000 dilution and direct inoculation without medium replacement can be used.

When fish samples come from production sites where infectious pancreatic necrosis virus (IPNV) is regarded as endemic, the tissue homogenate supernatant should be incubated (for a minimum of 1 hour at 15°C) with a pool of antisera to the indigenous serotypes of IPNV prior to inoculation.

ii) *Monitoring incubation*

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of a CPE. Typical CPE due to ISAV appears as vacuolated cells that subsequently round up and loosen from the growth surface. If a CPE consistent with that described for ISAV or IPNV appears, an aliquot of the medium for virus identification, as described below, must be collected. In the case of an IPNV infection, re-inoculate cells with tissue homogenate supernatant that has been incubated with a lower dilution of IPNV antisera. If no CPE has developed after 14 days, subcultivate to fresh cell cultures.

iii) *Subcultivation procedure*

Aliquots of medium (supernatant) from the primary cultures are collected 14 days (or earlier when obvious CPE appears) after inoculation. Supernatants from wells inoculated with different dilutions of identical samples may be pooled for surveillance purposes.

Supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 3–4 hours before addition of fresh medium. Alternatively, add supernatants (final dilutions 1/10 and higher) directly to cell cultures with growth medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures with no CPE should always be examined for the presence of ISAV by immunofluorescence (IFAT), haemadsorption or by PCR because virus replication may occur without development of apparent CPE.

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1 Virus identification by IFAT

All incubations are carried out at room temperature on a rocking platform, unless otherwise stated.

i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well or 24-well plates), in slide flasks or on plastic cover-slips dependent on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). SHK-1 cells grow rather poorly on glass cover-slips. The necessary monolayers for negative and positive controls must be included.

ii) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 7 days or, if CPE appears, for a shorter time.

iii) Fix in 80% acetone for 20 minutes after removing of cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. The fixed cell cultures may be stored dry for less than 1 week at 4°C or at –20°C for longer storage.

iv) Incubate the cell monolayers with anti- ISAV Mab in an appropriate dilution in PBS containing 0.5% dry skimmed milk for 1 hour, and rinse twice with PBS/0.05% Tween 20.

v) Incubate with FITC-conjugated goat anti-mouse immunoglobulin for 1 hour, (or if antibody raised in rabbits is used as the primary antibody, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity, FITC-conjugated goat anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with the decribed rinsing in between the additional step. Rinse once with PBS/0.05% Tween 20, as described above. The nuclei can be stained with propidiumiodid (100 µg ml^{-1} in sterile distilled water. If the plates cannot be examined immediately, add a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent as an anti-fade solution. Examine under UV light.

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1 Reverse-transcription polymerase chain reaction (RT-PCR)

RT-PCR may be used for detection of ISAV in tissue samples or in samples from cell cultures. Care should be taken for the interpretation of results from cell culture, as detected virus does not necessarily indicate that virus replication has taken place, but may represent virus remaining in the cell culture after inoculation with a tissue sample.

Total RNA (or total nucleic acid) is extracted from tissues, tissue homogenates or from infected cell layers. The concentration and purity of the extracted nucelic acid can be estimated by measuring the optical density at 260 nm and at 280. An alternative approach is to include internal controls directed against host RNAs. For detection of viral nucleic acids in fish tissue, primers against 18s rRNA, elongation factor 1 alpha (ELF-1A) or RNA polymerase 1 have been used successfully as internal controls.

Since the first RT-PCR for ISAV was reported in 1997, several attempts have been made to optimise the method (see ref. 20 for a review). A two-step RT-PCR can be performed whereby the RT and PCR steps are run in separate tubes. The introduction of one-step procedures, where the two reactions are run in a single tube, has been successful regarding sensitivity of the test. However, in this case, no cDNA is left for use in additional amplifications, which may be a disadvantage if several primer sets need to be included in the examination.

Several primer sets for ISAV RT-PCR have been reported and some are presented in the table below. The primer sets derived from genomic segment 8 (ILA1/ILA2 and FA3/RA3) have been used by several laboratories and have been found suitable for detection of ISAV during disease outbreaks and in carrier fish. The ILA2 reverse primer does not match isolates from North America and alternative primer sets should be used. In these cases, a modified FA3 primer together with RA3 can be used. The segment 6 primers may be useful for verification of PCR results based on segment 8 primers as an alternative to sequencing the PCR product.

RT-PCR: Primer sequences	Named	Genomic segment	Product size	Reference
5'-GGC-TAT-CTA-CCA-TGA-ACG-AAT-C-3' 5'-GCC-AAG-TGT-AAG-TAG-CAC-TCC-3'	ILA1 ILA2	8	155	(20)
5'-GAA-GAG-TCA-GGA-TGC-CAA-GAC-G-3' 5'-GAA-GTC-GAT-GAA-CTG-CAG-CGA-3'	FA3 RA3	8	211	(4)
5'-GGA-ATC-TAC-AAG-GTC-TGC-ATT-G-3' 5'-CTT-CAA-AGG-TGT-CTG-ACA-CGT-A-3'	Seg6U Seg6L	6	130	Designed by OIE Ref. Lab.

The use of real-time RT-PCR may increase the specificity and, probably, also the sensitivity of the test, especially when including e sequence-specific probe (23, 26, 27). This method is more rapid compared with conventional one-tube RT-PCR, the risk of contamination may be reduced and it is possible to estimate the relative amount of viral RNA in the sample. Primer and probe sequences that have been used for screening for ISAV by real-time RT-PCR are presented in the table below. Both primers and probes listed in this table target conserved regions of and ensure detection of all documented ISAV strains (26).

Real-time RT-PCR: Primer and probe sequences	Named	Genomic segment	Reference
5'- CAGGGTTGTATCCATGGTTGAAATG -3'	forward primer		
5'- GTCCAGCCCTAAGCTCAACTC -3'	reverse primer		(26)
5'-6FAM- CTCTCTCATTGTGATCCC-MGB *MGBNFQ-3'*	Taqman®probe	7	
5'- CTACACAGCAGGATGCAGATGT -3'	forward primer		(26)
5'- CAGGATGCCGGAAGTCGAT -3'	reverse primer	8	
5'-6FAM- CATCGTCGCTGCAGTTC *-TAMRA-3'*	Taqman®probe		

As an alternative, ILAS7-probe listed in the table below has been shown to be effective at detecting European ISAV isolates.

Real-time RT-PCR: Primer and probe sequences	Named	Genomic segment	Reference
5'-TGG-GAT-CAT-GTG-TTT-CCT-GCT-A-3'	ILAS7-F1		
5'-GAA-AAT-CCA-TGT-TCT-CAG-ATG-CAA-3'	ILAS7-R1	7	(23)
5'-6FAM-CACATGACCCCTCGTC-MGBNFQ-3'	ILAS7-probe		

4.3.1.2.4. Agent purification

ISAV propagated in cell culture can be purified by sucrose gradient centrifugation (5) or by affinity purification using immunomagnetic beads coated with anti-ISAV MAb.

4.3.2. Serological methods

Both Atlantic salmon and rainbow trout develop a humoral immune response to the ISAV infection. Enzyme-linked immunosorbent assays (ELISAs) with either purified virus or lysates from ISAV-infected cell cultures have been established for detection of ISAV-specific antibodies. ELISA titres can be very high and appear to be quite specific for the nucleoprotein in Western blots (K. Falk, pers. comm.). The test is not standardised for surveillance or diagnostic use, but may be used as a supplement to direct virus detection and pathology in obscure cases. Furthermore, the level and distribution of seroconversion in an ISAV-infected population may give some information about the spread of infection, particularly in cases where vaccination is not practised, and in wild fish.

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance and diagnosis of infectious salmon anaemia are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with

good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance for ISAV				Presumptive ISA diagnosis	Confirmatory ISA diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	d	d	c	b
Histopathology	d	d	d	b	b	b
IFAT on kidney imprints	c	c	c	c	b	a
Immunohistochemistry	c	c	c	c	b	a
Transmission EM	d	d	d	d	c	d
Isolation in cell culture with virus identification	a	a	a	a	a	a
RT-PCR or real-time RT-PCR (Sequencing for genotyping)	a	a	a	a	b	a

PLs = postlarvae; IFAT = indirect fluorescent antibody test; EM = electron microscopy; RT-PCR = reverse-transcription polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infectious salmon anaemia

None of the methods described above have been evaluated for the purpose of declaration of freedom of virus or disease, as data on prevalence and distribution of ISAV in subclinically infected fish population are lacking. Regular health inspections combined with investigation for ISA when increased mortality is associated with one of the given clinical signs and/or pathological changes consistent with ISA may be an efficient way of obtaining data on the prevalence of ISA in farmed populations. Alternatively, testing for ISAV, preferentially by PCR, at certain intervals may be carried out, in addition to regular health inspections. However, the significance of positive findings of ISAV by PCR alone for the risk of developing ISA disease is not clear, and therefore any positive findings would have to be carefully interpreted.

7. Corroborative diagnostic criteria

Reasonable grounds to suspect fish of being infected with ISAV are outlined below. The Competent Authority should ensure that, following the suspicion of fish infected with ISAV on a farm, an official investigation to confirm or rule out the presence of the disease will be carried out as quickly as possible, applying inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in Section 4.

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, as described in Section 4.3.1.2.1;

iii) Evidence for the presence of ISAV from two independent laboratory tests such as RT-PCR (Section 4.3.1.2.3) and IFAT on tissue imprints (Section 4.3.1.1.2);

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 [Section 4.3.1.1.2] or fixed sections as described in Section 4.3.1.1.3) in addition to either:

   a) isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm, as described in Section 4.3.1.2.1

   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 as described in Section 4.3.1.2.1;

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 (Sections 4.3.1.1.2 and 4.3.1.1.3).

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* * *

NB: There are OIE Reference Laboratories for Infectious salmon anaemia (see Table at the end of this *Aquatic Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).

This is Exhibit "F" referred to in the affidavit of DR. GARY MARY

sworn before me at ABBOTSFORD in the Province of British Columbia this 26 day of APRIL, 2011

A Commissioner for taking Affidavits within the Province of British Columbia

Summary of relevant diagnoses and comments among the 35 Animal Health Centre reports identified by Alexandra Morton because of concern about Infectious salmon anaemia virus (ISAV). Veterinarians that submitted cases and received case reports include Drs. Barry Milligan (BM), Diane Morrison (DM), Sonja Saksida (SS), and Peter McKenzie (PM). Animal Health Centre veterinary virologist Dr. John Robinson (JR) verified most of the PCR and virology results. Other viruses tested for include viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV).

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result – = negative test result
1	2007-1353 BM	Liver: sinusoidal congestion, acute, multifocal, moderate (consistent with viral hemorrhagic septicemia virus, VHSV; slide 2A, 1 piece)	Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus (consistent with PCR and virology results) and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	**VHSV+** [verified by a technician]
2	2007-1758 BM	Liver: sinusoidal congestion, acute, multifocal, moderate (slides 1, 2B)	Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV, and IHNV (if not already done).	not done
3	2007-1859 DM	1. Liver: sinusoidal congestion, acute, multifocal, moderate 2. Trunk kidney and head kidney: interstitial congestion and hemorrhage, diffuse, mild	Congestion of hepatic sinusoids and renal interstitium is evidence of endothelial damage. In BC Atlantic salmon, congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. I have also seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).	IHNV– VHSV– [verified by a technician] Virus isolation – [verified by JR]
4	2007-2120 DM	Liver: sinusoidal congestion, acute, multifocal, moderate (slide 5A)	Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	1 sample **VHSV+** 4 samples VHSV– 5 samples IHNV– [verified by JR] Virus isolation –
5	2007-2123 DM	Liver: sinusoidal congestion, acute, multifocal, moderate (slide 2A)	Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	1 sample **VHSV+** 4 samples VHSV– 5 samples IHNV– [verified by JR] Virus isolation –

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result − = negative test result
6	2007-2373 BM	Liver: sinusoidal congestion, acute, multifocal, mild (slides 1, 2, 4), moderate (slide 3)	Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	not done
7	2008-0533 SS	Liver: sinusoidal congestion, focal, moderate (slide 3A)	Sinusoidal congestion in the liver is nonspecific evidence of sinusoidal damage. In BC farmed salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. In fish 3 it might be a result of altered blood through the focus of vascular/biliary fibrosis. More diffuse sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider PCR for VHSV and IHNV.	not done
8	2008-2143 BM	Liver: sinusoidal congestion, multifocal, mild (slide 1A, one liver), moderate (slide 1A, one liver), severe (slides 1A, one liver; slide 2A)	Vascular congestion in the liver, kidney, and brain is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. I have not associated this change with hypoxia, but hypoxia could provide the stress needed to allow an infectious organism to replicate and cause disease. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Severe congestion is rare in the liver of farmed Pacific salmon in BC; indeed, among the 310 Pacific salmon livers I have examined as part of the BC Fish Health Auditing and Surveillance Program since January 2006, none have had severe congestion, and only 6 fish have had moderate congestion. Consider bacteriology and PCR for VHSV and IHNV.	not done
9	2008-4567 PM	sinusoidal congestion, multifocal, with focal intracytoplasmic spherical eosinophilic to amphophilic inclusions, mild (slide 5)	Sinusoidal congestion in the liver (fish 5) is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV and IHNV (if not already done). The golden to amphophilic cytoplasmic inclusions in hepatocytes vary from 0.5 to 1.5 times the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	not done
10	2008-4813 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV, but often the cause remains unknown. Consider bacteriology (if not already done). Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Sinusoidal congestion has also been described in wild fish (dab) surveyed in the north Atlantic, but the cause was not determined. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin deposits in congested foci, but nowhere else in the liver, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	IHNV− VHSV− [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result – = negative test result
11	2009-0026 DM	Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild	Sinusoidal congestion in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. PCR results rule out VHSV and IHNV as potential causes. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	IHNV– VHSV– [verified by JR]
12	2009-0109 DM	Liver: sinusoidal congestion, multifocal, moderate (slides 1, 2)	Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	not done
13	2009-0111 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slides 1, 2)	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV, but often the cause remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	not done
14	2009-0711 DM	Liver: sinusoidal congestion, multifocal, moderate, with acid hematin granules and small numbers of intracytoplasmic spherical amphophilic inclusions	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	IHNV– VHSV– [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result − = negative test result
15	2009-0805 PM	Liver: sinusoidal congestion and fibrin, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1)	Sinusoidal congestion and fibrin in the liver is evidence of circulating vasodilators; congestion sometimes occurs as a postmortem artifact. In slide 1, all foci of necrosis are associated with sinusoidal congestion, but several foci of congestion are not associated with hepatic necrosis. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	not done
16	2009-1617 DM	Liver: sinusoidal congestion, multifocal, mild	Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	IHNV− VHSV− [verified by JR]
17	2009-1714 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slides 1A, 2A)	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	IHNV− VHSV− [verified by JR]
18	2009-1766 PM	Liver: sinusoidal congestion, multifocal, moderate (slide 3A)	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).	IHNV− VHSV− [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result – = negative test result
19	2009-1932 DM	Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild	This fish has several changes that are common in cultured Atlantic salmon in BC. Although none are of sufficient severity to have killed the fish, sinusoidal congestion in the liver is probably the most significant. Hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	IHNV– VHSV– [verified by JR]
20	2009-1999 PM	Liver: sinusoidal congestion, multifocal, moderate (slides 1A, 2A)	Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider submission of unfixed tissues that can be subjected to PCR for VHSV and IHNV.	not done
21	2009-2477 DM	Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild (slide 4A)	Sinusoidal congestion (sometimes called "peliosis") in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	not done
22	2009-2492 DM	Liver: sinusoidal congestion, multifocal, moderate (slide 4)	Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	Virus isolation – [verified by JR]
23	2009-2594 DM	Liver: sinusoidal congestion, multifocal, mild (slide 5)	Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.	Virus isolation – [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result − = negative test result
24	2009-2849 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acuto, focal, mild (slide 2), multifocal, moderate (slide 1)	In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	IHNV− VHSV− [verified by JR]
25	2009-2936 PM	Liver: sinusoidal congestion, multifocal, mild (slides 2-2, 2-3), moderate (slides 2-1, 4-1, 4-2)	Vascular congestion in the kidney, liver, and brain might be evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact (especially in the kidney and brain). Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Most commonly, the cause is unknown (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology; PCR results rule out VHSV and IHNV.	IHNV− VHSV− Virus isolation − [verified by JR]
26	2009-2969 DM	Liver: sinusoidal congestion, with acid hematin granules, acute, multifocal, mild (slides 2, 3, 4), moderate (slide 1)	Sinusoidal congestion in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).	IHNV− VHSV− [verified by JR]
27	2009-3042 DM	Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions; acute, bifocal, mild (slide 1)	Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV; as in this case, the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	IHNV− VHSV− [verified by a technician]
28	2009-3272 DM	Liver: sinusoidal congestion, acute, multifocal, moderate (slide 2A)	Multifocal sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV; as in this case, the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).	**ISAV−** IHNV− VHSV− Salmon alphavirus − [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result – = negative test result
29	2009-4967 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1)	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes it occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the liver, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	not done
30	2010-0314 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia (**and PCR results rule it out in this case**). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the liver, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	**ISAV–** **IHNV–** VHSV– [verified by JR]
31	2010-0329 PM	Liver: sinusoidal congestion, with acid hematin granules focal, moderate (slide 2), and intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, moderate (slide 1)	Focal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	IHNV– VHSV– Virus isolation – [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result − = negative test result
32	2010-0799 DM	Liver: sinusoidal congestion, with cellular thrombi, acid hematin granules, and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, severe	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Scattered cellular thrombi in some foci of congestion are consistent with vascular damage. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology, if not already done. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the liver, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	ISAV− IHNV− VHSV− [verified by JR]
33	2010-1034 PM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1)	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; **PCR results point to VHSV as the cause in this case.** Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	VHSV+ [verified by JR]
34	2010-1368 DM	Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 2)	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.	ISAV− IHNV− VHSV− [verified by JR]

#	AHC Case # & veterinarian	Relevant Diagnoses	Relevant Comment in report	PCR/virology results + = positive test result – = negative test result
35	2010-1442 DM	Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, moderate, focal (slides 2, 4), multifocal (slides 1, 3)	Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV (ruled out in this case by PCR); the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).	ISAV– IHNV– VHSV– Virus isolation – [verified by JR]

undefined

Aquatic Animal Health Code

Contents | Index ?

PDF

This is Exhibit "G" referred to in the affidavit of DR. GARY MARTY sworn before me at ABBOTSFORD in the Province of British Columbia this 26 day of APRIL, 2011

A Commissioner for taking Affidavits within the Province of British Columbia

GLOSSARY

For the purpose of the *Aquatic Code*:

Aquaculture

means the farming of *aquatic animals* with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc.

Aquaculture establishment

means an establishment in which fish, molluscs or crustaceans for breeding, stocking or marketing are raised or kept.

Aquatic animal health status

means the status of a country, *zone* or *compartment* with respect to an *aquatic animal disease*, according to the criteria listed in the relevant chapter of the *Aquatic Code* dealing with the *disease*.

Aquatic Animal Health Services

means the governmental and non-governmental organisations that implement animal health and welfare measures and other standards and recommendations in the *Aquatic Code* in the territory. The Aquatic Animal Health Services are under the overall control and direction of the *Competent Authority*. Private sector organisations, *veterinarians*, aquatic animal health professionals or veterinary paraprofessionals are normally accredited or approved by the *Competent Authority* to deliver the delegated functions.

Aquatic animal products

means non-viable *aquatic animals* and products from *aquatic animals*.

Aquatic animals

means all life stages (including *eggs* and *gametes*) of fish, molluscs, crustaceans and amphibians originating from *aquaculture establishments* or removed from the wild, for farming purposes, for release into the environment, for human consumption or for ornamental purposes.

Aquatic Code

means the OIE *Aquatic Animal Health Code*.

Aquatic Manual

means the OIE *Manual of Diagnostic Tests for Aquatic Animals*.

Basic biosecurity conditions

means a set of conditions applying to a particular *disease*, and a particular *zone* or country, required to ensure adequate disease security, such as:

a. the *disease*, including suspicion of the *disease*, is compulsorily notifiable to the *Competent Authority*; and

b. an *early detection system* is in place within the *zone* or country; and

c. import requirements to prevent the introduction of *disease* into the country or *zone*, as outlined in the *Aquatic Code*, are in place.

Bias

means a tendency of an estimate to differ in a non-random fashion from the true value of a population parameter.

Biological products

means:

a. biological reagents for use in the *diagnosis* of certain *diseases*;

b. sera for use in the prevention and treatment of certain *diseases*;

c. inactivated or modified vaccines for use in preventive vaccination against certain *diseases*;

d. genetic material of infectious agents;

e. endocrine tissues from fish or used in fish.

Biosecurity plan

means a plan that identifies significant potential pathways for the introduction and spread of *disease* in a *zone* or *compartment*, and describes the measures which are being, or will be, applied to mitigate the *risks* to introduce and spread *disease*, taking into consideration the recommendations in the *Aquatic Code*. The plan should also describe how these measures are audited, with respect to both their implementation and their targeting, to ensure that the *risks* are regularly re-assessed and the measures adjusted accordingly.

Case

means an individual *aquatic animal* infected by a *pathogenic agent*, with or without clinical signs.

Case definition

is a set of criteria used to distinguish a *case* animal or an *epidemiological unit* from a non-case.

Certifying official

means a person authorised by the *Competent Authority* to sign health certificates for *aquatic animals*.

Commodity

means *aquatic animals*, *aquatic animal products*, *biological products* and *pathological material*.

Compartment

means one or more *aquaculture establishments* under a common biosecurity management system containing an *aquatic animal* population with a distinct health status with respect to a specific *disease* or *diseases* for which required *surveillance* and control measures are applied and *basic biosecurity conditions* are met for the purpose of *international trade*. Such *compartments* must be clearly documented by the *Competent Authority(ies)*.

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.

Container

means a transport appliance:

a. of a permanent type and sufficiently strong to enable repeated use;

b. specially constructed to facilitate transport of *aquatic animals* or *aquatic animal products* by one or several means of transport;

c. provided with fittings that make it easy to manipulate, particularly for trans-shipment from one kind of transport *vehicle* to another;

d. constructed in a watertight way, easy to load and unload and capable of being cleansed and disinfected;

e. ensuring safe and optimal transport of *aquatic animals*.

Contingency plan

means a documented work plan designed to ensure that all needed actions, requirements and resources are provided in order to eradicate or bring under control *outbreaks* of specified *diseases* of *aquatic animals*.

Diagnosis

means determination of the nature of a *disease*.

Disease

means clinical or non clinical *infection* with one or more of the aetiological agents of the *diseases* referred to in the *Aquatic Code*.

Disinfectants

means chemical compounds capable of destroying pathogenic microorganisms or inhibiting their growth or survival ability.

Disinfection

means the application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of *diseases* of *aquatic animals*, including zoonoses; this applies to *aquaculture establishments* (i.e. hatcheries, fish farms, oyster farms, shrimp farms, nurseries, etc.), *vehicles*, and different equipment/objects that may have been directly or indirectly contaminated.

Early detection system

means an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation by the *Aquatic Animal Health Services* with minimal delay. Such a system will include the following characteristics:

a. broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in processing, of the characteristic signs of the *listed diseases* and *emerging diseases*;

b. *veterinarians* or *aquatic animal* health professionals trained in recognising and reporting suspicions of *disease* occurrence;

c. ability of the *Aquatic Animal Health Services* to undertake rapid and effective *disease* investigation based on a national chain of command;

d. access by the *Aquatic Animal Health Services* to laboratories with the facilities for diagnosing and differentiating *listed diseases* and *emerging diseases*;

e. the legal obligation of private *veterinarians* or *aquatic animal* health professionals to report suspicions of *disease* occurrence to the *Competent Authority*.

Egg

means a viable fertilised *ovum* of an *aquatic animal*. 'Green eggs' means newly fertilised ova of fish. 'Eyed eggs' means *eggs* of fish where the eyes of the embryo are visible and that the *eggs* may be transported.

Emerging disease

means a newly recognised *infection* resulting from the evolution or change of an existing *pathogenic agent*, a known *infection* spreading to a new geographic area or population, or a previously unrecognised *pathogenic agent* or a *disease* diagnosed for the first time and which has a significant impact on *aquatic animal* or public health.

Epidemiological unit

means a group of animals that share approximately the same *risk* of exposure to a *pathogenic agent* with a defined location. This may be because they share a common aquatic environment (e.g. fish in a pond, caged fish in a lake), or because management practices make it likely that a *pathogenic agent* in one group of animals would quickly spread to other animals (e.g. all the ponds on a farm, all the ponds in a village system).

Eviscerated fish

means fish from which internal organs, excluding the brain and gills, have been removed.

Exporting country

means a country from which *aquatic animals* or *aquatic animal products*, *biological products* or *pathological material* are sent to a destination in another country.

Fallowing

means, for *disease* management purposes, an operation where an *aquaculture establishment* is emptied of *aquatic animals* susceptible to a *disease* of concern or known to be capable of transferring the *pathogenic agent*, and, where feasible, of the carrying water. For *aquatic animals* of unknown susceptibility and those agreed not to be capable of acting as carriers of a *disease* of concern, decisions on *fallowing* should be based on a *risk assessment*.

Feed

means any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to *aquatic animals*.

Feed ingredient

means a component, part or constituent of any combination or mixture making up a *feed*, including feed additives, whether or not it has a nutritional value in the animal's diet. Ingredients may be of terrestrial or aquatic, plant or animal origin and may be organic or inorganic substances.

Free compartment

means a *compartment* that fulfils the requirements for *self-declaration of freedom from disease* with respect to the *disease(s)* under consideration, according to the relevant chapter(s) in the *Aquatic Code*.

Free country

means a country that fulfils the requirements for *self-declaration of freedom from disease* with respect to the *disease(s)* under consideration according to the relevant chapter(s) in the *Aquatic Code*.

Free zone

means a *zone* that fulfils the requirements for *self-declaration of freedom from disease* with respect to the *disease(s)* under consideration according to the relevant chapter(s) in the *Aquatic Code*.

Frontier post

means any international airport or any port, railway station or road post open to *international trade*.

Gametes

means the sperm or unfertilised *eggs* of *aquatic animals* that are held or transported separately prior to fertilisation.

Hazard

means a biological, chemical or physical agent in, or a condition of, an *aquatic animal* or *aquatic animal product* with the potential to cause an adverse effect on *aquatic animal* health or public health.

Hazard identification

means the process of identifying the *pathogenic agent(s)* that could potentially be introduced in the *commodity* considered for importation.

Headquarters

means the Permanent Secretariat of the World Organisation for Animal Health (OIE), located at:

12, rue de Prony, 75017 Paris, FRANCE
Telephone: 33-(0)1 44 15 18 88
Fax: 33-(0)1 42 67 09 87
Electronic mail: oie@oie.int
WWW: http://www.oie.int

Importing country

means a country that is the final destination to which *aquatic animals*, *aquatic animal products*, *biological products* or *pathological material* are sent.

Incidence

means the number of new *outbreaks* of *disease* within a specified period of time in a defined *aquatic animal* population.

Infected zone

means a *zone* in which a *disease* has been diagnosed.

Infection

means the presence of a multiplying or otherwise developing or latent *pathogenic agent* in

a host. This term is understood to include infestation where the *pathogenic agent* is a parasite in or on a host.

Infective period

means the longest period during which an affected *aquatic animal* can be a source of *infection*.

International aquatic animal health certificate

means a certificate, issued in conformity with the provisions of Chapter 5.10., describing the *aquatic animal* health and/or public health requirements that should be fulfilled prior to export of *commodity*.

International trade

means import, export or transit of *aquatic animals*, *aquatic animal products*, *biological products* and *pathological material*.

Live feed

means live farmed or wild caught animals and algae used as *feed* for *aquatic animals*. Live feed is often fed to *aquatic animal* species at an early life-stage and to *aquatic animal* species that have been cultured for a relatively short time.

Meal

means a product derived from an *aquatic animal* that has been ground and heat processed to reduce the moisture content to less than 10%.

Notification

means the procedure by which:

a. the *Veterinary Authority* informs the *Headquarters*,

b. the *Central Bureau* inform *Veterinary Authorities* of Members

of the occurrence of a *disease*, according to the provisions of Section 1. of the *Aquatic Code*.

OIE listed diseases

means *diseases* that are referred to in Chapter 1.3. of the *Aquatic Code*. (Synonym: diseases listed by the OIE.)

Outbreak

means an occurrence of one or more *cases* in an *epidemiological unit*.

Pathogenic agent

means an organism that causes or contributes to the development of a *disease* referred to in the *Aquatic Code*.

Pathological material

means samples obtained from live or dead *aquatic animals*, containing or suspected of containing *pathogenic agents*, to be sent to a laboratory.

Prevalence

means the total number of infected *aquatic animals* expressed as a percentage of the total number of *aquatic animals* in a given *aquatic animal* population at one specific time.

Probability sampling

means a sampling strategy in which every unit has a known non-zero probability of inclusion in the sample.

Protection zone

means a *zone* established to protect the health status of *aquatic animals* in a *free country* or *free zone*, from those in a country or *zone* of a different *aquatic animal health status*, using measures based on the epidemiology of the *disease* under consideration to prevent spread of the *pathogenic agent* into a *free country* or *free zone*. These measures may include, but are not limited to, vaccination, movement control and an intensified degree of *surveillance*.

Quarantine

means maintaining a group of *aquatic animals* in isolation with no direct or indirect contact with other *aquatic animals*, in order to undergo observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent waters.

Risk

means the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health.

Risk analysis

means the complete process composed of *hazard identification*, *risk assessment*, *risk management* and *risk communication*.

Risk assessment

means the evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a *hazard* within the *territory* of an *importing country*.

Risk communication

is the interactive exchange of information and opinions throughout the *risk analysis* process concerning *risk*, *risk*-related factors and *risk* perceptions among *risk* assessors, *risk* managers, *risk* communicators, the general public and other interested parties.

Risk management

means the process of identifying, selecting and implementing measures that can be applied to reduce the level of *risk*.

Sanitary measure

means a measure, such as those described in various chapters of the *Aquatic Code*, destined to protect *aquatic animal* or human health or life within the *territory* of the OIE Member from *risks* arising from the entry, establishment and/or spread of a *hazard*.

Self-declaration of freedom from disease

means declaration by the *Competent Authority* of the country concerned that the country, *zone* or *compartment* is free from a *listed disease* based on implementation of the provisions of the *Aquatic Code* and the *Aquatic Manual*. The *Veterinary Authority* of the country may wish to transmit this information to the OIE *Central Bureau*, which may publish the information.

Sensitivity

means the proportion of true positive tests given in a diagnostic test, i.e. the number of true positive results divided by the number of true positive and false negative results.

Specificity

means the probability that absence of *infection* will be correctly identified by a diagnostic test, i.e. the number of true negative results divided by the number of true negative and false positive results.

Stamping-out policy

means the carrying out under the authority of the *Competent Authority*, on confirmation of a *disease*, of preventive *aquatic animal* health measures, consisting of killing the *aquatic animals* that are affected, those suspected of being affected in the population and those in other populations that have been exposed to *infection* by direct or indirect contact of a kind likely to cause the transmission of the *pathogenic agent*. All these *aquatic animals*, vaccinated or unvaccinated, on an infected site should be killed and the carcasses destroyed by burning or burial, or by any other method that will eliminate the spread of *infection* through the carcasses or products of the *aquatic animals* destroyed.

This policy should be accompanied by cleansing and *disinfection* procedures as defined in the *Aquatic Code*. *Fallowing* should be for an appropriate period determined by *risk assessment*.

Study population

means the population from which *surveillance* data are derived. This may be the same as the *target population* or a subset of it.

Subpopulation

means a distinct part of a population identifiable according to specific common *aquatic animal* health characteristics.

Surveillance

means a systematic series of investigations of a given population of *aquatic animals* to detect the occurrence of *disease* for control purposes, and which may involve testing samples of a population.

Susceptible species

means a species of *aquatic animal* in which *infection* has been demonstrated by natural *cases* or by experimental exposures to the *pathogenic agent* that mimics the natural pathways for *infection*. Each *disease* chapter in the *Aquatic Code* and the *Aquatic Manual* contains a list of currently known *susceptible species*.

Target population

means, for the purposes of demonstrating freedom from *infection*, the population of interest, usually made up of all *aquatic animals* of species susceptible to a specified *pathogenic agent* in a defined country, *zone* or *aquaculture establishment*.

Targeted surveillance

means *surveillance* targeted at a specific *disease* or *infection*.

Territory

means land and water under jurisdiction of a country.

Transit country

means a country through which *aquatic animals*, *aquatic animal products*, *biological products* or *pathological material* destined for an *importing country*, are transported or in which a stopover is made at a *frontier post*.

Unit

means individually identifiable elements. This is a generic concept used to describe, for example, the members of a population, or the elements selected when sampling. In these contexts, examples of *units* include individual animals, ponds, nets, cages, farms, villages, districts, etc.

Vehicle

means any method of transport by land, air or water.

Veterinarian

means a person registered or licensed by the relevant *veterinary statutory body* of a country to practise veterinary medicine/science in that country.

Veterinary Authority

means the Governmental Authority of an OIE Member, comprising *veterinarians*, other professionals and para-professionals, having the responsibility and competence for ensuring or supervising the implementation of *aquatic animal* health and welfare measures, international *aquatic animal* health certification and other standards and recommendations in the *Aquatic Code* in the whole *territory*.

Veterinary statutory body

means an autonomous authority regulating *veterinarians* and veterinary para-professionals.

Water catchment

means an area or basin of land bounded by natural features such as hills or mountains, into which all run-off water flows.

Zone

means a portion of one or more countries comprising:

a. an entire *water catchment* from the source of a waterway to the estuary or lake, or

b. more than one *water catchment*, or

c. part of a *water catchment* from the source of a waterway to a barrier that prevents the introduction of a specific *disease* or *diseases*, or

d. part of a coastal area with a precise geographical delimitation, or

e. an estuary with a precise geographical delimitation,

that consists of a contiguous hydrological system with a distinct health status with respect to a specific *disease* or *diseases*. The *zones* must be clearly documented (e.g. by a map or other precise locators such as GPS co-ordinates) by the *Competent Authority(ies)*.

CHAPTER 1.1.

NOTIFICATION OF DISEASES AND EPIDEMIOLOGICAL INFORMATION

Article 1.1.1.

For the purposes of the *Aquatic Code* and in terms of Articles 5, 9 and 10 of the Statutes, every Member of the OIE shall recognise the right of the *Headquarters* to communicate directly with the *Veterinary Authority* of its *territory* or *territories*.

All *notifications* and all information sent by the OIE to the *Veterinary Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to the OIE by the *Veterinary Authority* shall be regarded as having been sent by the country concerned.

Article 1.1.2.

1. Countries shall make available to other countries, through the OIE, whatever information is necessary to minimise the spread of *aquatic animal diseases* and their aetiological agents and to assist in achieving better world-wide control of these *diseases*.
2. To achieve this, countries shall comply with the reporting requirements specified in Article 1.1.3.
3. To assist in the clear and concise exchange of information, reports shall conform as closely as possible to the current OIE *disease* reporting format.
4. Recognising that scientific knowledge concerning the relationship between *pathogenic agents* and *diseases* is constantly evolving and that the presence of an infectious agent does not necessarily imply the presence of a *disease*, countries shall ensure through their reports that they comply with the spirit and intention of paragraph 1 above. This means that the presence of an infectious agent, even in the absence of clinical *disease*, should be reported.
5. In addition to notifying findings in accordance with Article 1.1.3., countries shall also provide information on the measures taken to prevent the spread of *diseases*, including possible *quarantine* measures and restrictions on the movement of *aquatic animals*, *aquatic animal products*, *biological products* and other miscellaneous objects that could by their nature be responsible for transmission of *disease*. In the case of *diseases* transmitted by vectors, the measures taken against such vectors shall also be described.

Article 1.1.3.

The *Veterinary Authority* shall send to the OIE:

1. Immediate *notification* (within 24 hours), by fax or electronically, of any of the following events:
   a) for *diseases listed by the OIE*, the first occurrence or re-occurrence of a *disease* in a country or *zone* or *compartment* of the country, if the country or *zone* or *compartment* of the country was previously considered to be free of that particular *disease*; or
   b) for *diseases listed by the OIE*, if the *disease* has occurred in a new host species; or

c) for *diseases listed by the OIE*, if the *disease* has occurred with a new pathogen strain or in a new *disease* manifestation; or

d) for *diseases listed by the OIE*, if the *disease* has a newly recognised zoonotic potential; or

e) for *diseases* not listed by the OIE, if there is a *case* of an *emerging disease* or *pathogenic agent* should there be findings that are of epidemiological significance to other countries.

In deciding whether findings justify immediate *notification* (within 24 hours), countries must ensure that they comply with the obligations of Chapters 5.1. and 5.2. of the *Aquatic Code* (especially Article 5.1.1.), to report developments that may have implications for *international trade*.

2. Weekly reports by fax or electronically subsequent to a *notification* under paragraph 1 above, to provide further information on the evolution of an incident that justified immediate *notification*. These reports should continue until the *disease* has been eradicated or the situation has become sufficiently stable that six-monthly reporting under point 3 will satisfy the obligation of the country to the OIE; in each case, a final report on the incident should be submitted.

3. Six-monthly reports on the absence or presence and evolution of *diseases listed by the OIE*, and findings of epidemiological significance to other countries with respect to *diseases* that are not listed.

4. An annual questionnaire concerning any other information of significance to other countries.

Article 1.1.4.

1. The *Veterinary Authority* of a country in which an *infected zone* or *compartment* was located shall inform the *Headquarters* when this *zone* or *compartment* is free from the *disease*.

2. An *infected zone* or *compartment* of a *disease* shall be considered as such until a period exceeding the known *infective period* for the *disease* in question has elapsed after the last reported *outbreak* and when full prophylactic and appropriate *sanitary measures* have been applied to prevent possible reappearance or spread of the *disease*. These measures will be found in detail in the various chapters of Section 8. to Section 11. of the *Aquatic Code*.

3. A country may again declare itself free (i.e. *self-declaration of freedom from disease*) from a specific *disease* when it complies with all the conditions given in the corresponding chapters of Section 8. to Section 11. of of the *Aquatic Code*.

4. The *Veterinary Authority* of a country in which one or more *free zones* or *compartments* have been established may wish to inform the *Headquarters*, giving necessary particulars of the *zones* or *compartments* and describing their location (e.g. by a map or other precise locators such as GPS [Global Positioning System] co-ordinates). The *Headquarters* may publish this information.

Article 1.1.5.

1. The *Headquarters* shall send by fax or electronically to the *Veterinary Authority* concerned, all *notifications* received as provided in Articles 1.1.2.-1.1.4.

2. The *Headquarters* shall notify Members through *Disease Information* of any event of exceptional epidemiological significance reported by a Member.