Commission of Inquiry into the Decline of Sockeye Salmon in the Fraser River

Commission d'enquête sur le déclin des populations de saumon rouge du fleuve Fraser

Public Hearings

Audience publique

Commissioner

L'Honorable juge /
The Honourable Justice
Bruce Cohen

Commissaire

Held at:

Asia Pacific Hall at the
Morris J Wosk Centre for Dialogue
580 West Hastings Street
Vancouver, B.C.

Thursday, December 15, 2011

Tenue à :

Asia Pacific du
Morris J Wosk Centre for Dialogue
580 rue Hastings Ouest
Vancouver (C.-B.)

le jeudi 15 decembre 2011

Errata for the Transcript of Hearings on December 15, 2011

Page	Line	Error	Correction
9	42	virus for fish	virus of fish
9	43	causes – it is called	causes a disease called
10	6	Orthomyxo virus	Orthomyxovirus
10	8	it causes communicable	it causes clinical
12	38	for	four
13	10-11	set of samples was saved	set of samples was received
13	21, 23	by the time we put a result	by the time we report a result
26	3	the also the ones	they are also the ones
26	5-6	was able several samples	was able to find several samples
26	8	and deposited to the	and blasted to the
26	10-11	able to identify some (indiscernible – rapid speech)	able to identify some homology there
31	37	my thing is that	my thinking's that
31	44	studied the disposition	studied the distribution
32	7	the disposition of this virus	the distribution of this virus
32	40	I had the methods that	I heard the methods that
37	25	The OIE Manual shows segment	The OIE Manual also lists segment
37	41	the (indiscernible – rapid speech)	the diagnostic labs
37	43	they only test	the only test

Suite 2800, PO Box 11530, 650 West Georgia Street, Vancouver, BC V6B 4N7
Tel: 604 658 3600 Toll-free Tel: 1 877 658 2808
Fax: 604 658 3644 Toll-free Fax: 1 877 658 2809
www.cohencommission.ca

43	36	it's version 1.50	it's version 1.50 (sic)
44	35-37, 47	salines, saline	cell lines, cell line
44	35, 42	S2	ASK-2
45	13, 18	saline, salines	cell line, cell lines
45	14	S2	ASK-2
58	39-47	stock code	stop code
59	4	stock code	stop code
59	37	the results of (indiscernible)	the results, it's only 71
59	47	ISA virus	Isavirus
69	4	burst	blast
69	18	pass it to	blast it to
69	20	homology; 7 to 1 basis is small,	homology; 71 bases is small,
69	25	(indiscernible – voice drops)	Orthomyxo
69	46	very high virus datas and try	very high virus titres and try
70	8	it divide,	It diverged,
70	17	this is called DNA sequencing	this is called deep -- deep DNA sequencing
70	19	without (indiscernible)	without actually virus
74	35	think it's an accrediting body	think OIE is an accrediting body
75	18	samples we	samples were submitted [retaining the notation: "(indiscernible – overlapping speakers)"]

86	9-10	and also used up Nellie Gagne's	and also used Doc -- Ms. Kibenge's
91	46	Exhibit 64	Exhibit 1464
92	43	she could check with (indiscernible) to	she could check with her supervisor to
107	2	happen	happy
112	1	(indiscernible) PB1 gene,	RNA polymerase PB1 gene,
113	8, 30	pasendrial (phonetic)	piscine reovirus
114	2	pseudochromis	pseudochromonius (phonetic)
114	7	pasendrial	piscine reovirus
117	21	hear	here
133	2	differ (sic)	defer
135	6	fish-off (sic)	fish health

APPEARANCES / COMPARUTIONS

Brian Wallace, Q.C.	Senior Commission Counsel
Brock Martland	Associate Commission Counsel
Jennifer Chan	Junior Commission Counsel
Kathy Grant	Junior Commission Counsel
Mitchell Taylor, Q.C. Mark East Geneva Grande-McNeill Adam Taylor (Articling Student)	Government of Canada ("CAN")
Clifton Prowse, Q.C. Boris Tyzuk, Q.C. Tara Callan	Province of British Columbia ("BCPROV")
No appearance	Pacific Salmon Commission ("PSC")
No appearance	B.C. Public Service Alliance of Canada Union of Environment Workers B.C. ("BCPSAC")
No appearance	Rio Tinto Alcan Inc. ("RTAI")
No appearance	B.C. Salmon Farmers Association ("BCSFA")
No appearance	Seafood Producers Association of B.C. ("SPABC")
Gregory McDade Lisa Glowacki	Aquaculture Coalition: Alexandra Morton; Raincoast Research Society; Pacific Coast Wild Salmon Society ("AQUA")
Karen Campbell Judah Harrison	Conservation Coalition; Coastal Alliance for Aquaculture Reform Fraser Riverkeeper Society; Georgia Strait Alliance; Raincoast Conservation Foundation; Watershed Watch Salmon Society; Mr. Otto Langer; David Suzuki Foundation ("CONSERV")

APPEARANCES / COMPARUTIONS, cont'd.

Don Rosenbloom	Area D Salmon Gillnet Association; Area B Harvest Committee (Seine) ("GILLFSC")
No appearance	Southern Area E Gillnetters Assn. B.C. Fisheries Survival Coalition ("SGAHC")
No appearance	West Coast Trollers Area G Association; United Fishermen and Allied Workers' Union ("TWCTUFA")
No appearance	B.C. Wildlife Federation; B.C. Federation of Drift Fishers ("WFFDF")
No appearance	Maa-nulth Treaty Society; Tsawwassen First Nation; Musqueam First Nation ("MTM")
No appearance	Western Central Coast Salish First Nations: Cowichan Tribes and Chemainus First Nation Hwlitsum First Nation and Penelakut Tribe Te'mexw Treaty Association ("WCCSFN")
Leah Pence Crystal Reeves	First Nations Coalition: First Nations Fisheries Council; Aboriginal Caucus of the Fraser River; Aboriginal Fisheries Secretariat; Fraser Valley Aboriginal Fisheries Society; Northern Shuswap Tribal Council; Chehalis Indian Band; Secwepemc Fisheries Commission of the Shuswap Nation Tribal Council; Upper Fraser Fisheries Conservation Alliance; Other Douglas Treaty First Nations who applied together (the Snuneymuxw, Tsartlip and Tsawout); Adams Lake Indian Band; Carrier Sekani Tribal Council; Council of Haida Nation ("FNC")
No appearance	Métis Nation British Columbia ("MNBC")

APPEARANCES / COMPARUTIONS, cont'd.

Nicole Schabus	Sto:lo Tribal Council Cheam Indian Band ("STCCIB")
No appearance	Laich-kwil-tach Treaty Society Chief Harold Sewid, Aboriginal Aquaculture Association ("LJHAH")
Krista Robertson	Musgamagw Tsawataineuk Tribal Council ("MTTC")
No appearance	Heiltsuk Tribal Council ("HTC")

TABLE OF CONTENTS / TABLE DES MATIERES

TABLE OF CONTENTS / TABLE DES MATIERES

EXHIBITS / PIECES

EXHIBITS / PIECES

EXHIBITS / PIECES

EXHIBITS / PIECES

EXHIBITS / PIECES

EXHIBITS / PIECES

Vancouver, B.C. /Vancouver
(C.-B.)
December 15, 2011/le 15
decembre 2011

MR. LUNN: The hearing is now resumed.
MR. MARTLAND: Mr. Commissioner, Brock Martland, appearing as Associate Commission Counsel, and I have -- sorry, just making sure that everyone can hear me properly, and I hope I'm going some thumbs up. We'll see if the thumbs up last, or not. In any event, we're here today with three days of hearing on the topic of testing for ISAV. I have with me today three other Commission lawyers, Brian Wallace, Senior Commission Counsel, Jennifer Chan and Kathy Grant.

These are hearings today with -- we're dealing with a panel of four witnesses. They'll be affirmed momentarily. Two of our witnesses are available today only, so they'll be with us through the day today but not for the first half of tomorrow when we conclude this panel's evidence. So I'll ask first, please, that these witnesses be affirmed.

Mr. Lunn, we have new technological challenges in this room. It's not our usual hearing room, so we may have to adapt on the move a little bit. Once Mr. Lunn is able to affirm these witnesses, we can do that, please.

MR. LUNN: Until I can get my microphone working I'm just going to use this one.

Dr. Nylund, can you hear me?

We'll start with the other witnesses and see if we can get sound from Norway from the audio booth.

KRISTI MILLER, recalled,
reminded.

MR. LUNN: Dr. Miller, you were here before and were affirmed at that time, so your affirmation still stands. I am going to ask the other witnesses to please state their names for the record.
DR. KIBENGE: Frederick Kibenge.
MS. GAGNE: Nellie Gagné.
MR. LUNN: Okay, thank you. I don't believe Dr. Kibenge's microphone is working. If the audio

booth could address that, please.

FREDRICK KIBENGE, affirmed.

NELLIE GAGNE, affirmed.

MR. LUNN: Thank you, both.
Dr. Nylund, can you hear me?
DR. NYLUND: Yes.
MR. LUNN: Thank you. Would you please state your name for the record.
DR. NYLUND: Are Nylund.
MR. LUNN: Thank you. And I am going to ask you the oath of affirmation. If you could answer yes if you agree.

ARE NYLUND, affirmed.

MR. LUNN: Thank you very much. Counsel?
MR. MARTLAND: Thank you. It occurs to me as we do this that we may have -- it may be of some benefit as we move through questions of witnesses to keep mikes on, if that's necessary. I'll leave that to the sound engineers. The witnesses present, as I look at them, are Dr. Kibenge, Dr. Miller and Ms. Gagné, and, of course, Dr. Nylund from Norway, and where I can do so, I will direct a question to one witness so hopefully that will assist the sound engineers in ensuring that we have the right mikes on.

By way of one preliminary matter, what we're proposing to do, Mr. Commissioner, we've listed many documents. This is a hearing where we don't have the benefit of a Policy and Practice Report, given the way this topic has arisen. So we have more documents than we otherwise might have. We're proposing that what we expect are non-controversial documents, which is to say in essence the lab reports at issues, a few manuals and policies and protocols that are not draft but final, and some media release or public documents would be put in as exhibits without -- with support, or at least without objection from other participants. I'd asked other participants to identify any objections. I haven't heard any.

I've canvassed with Canada, who is the document holder for these various documents, and

they had concerns about us trying to do that with the entirety of all of our list, but not with this short list.

I'll refer to the tab numbers from our list of documents as the following: 1, 2, 4, 5, 6, 7, 11 and 12, 16, 26, 28, 31 through 39, 46 to 51, 53, 54, 80, 93 to 95, 102, 103, 105 through 107, 116, 126, 133 to 135, and 140 and 141. Unless any counsel raises an objection, I propose simply to have those marked at the outset. I expect to go to all of them, but that will speed us up in terms of having marked exhibit numbers. They begin, Mr. Lunn, I believe, at Exhibit 1994 and following.

EXHIBIT 1994: *Curriculum vitae* of Nellie Gagné

EXHIBIT 1995: *Curriculum vitae* of Dr. Fred Kibenge

EXHIBIT 1996: Profile and list of publications for Dr. Are Nylund

EXHIBIT 1997: *Curriculum vitae* of Dr. Kim Klotins

EXHIBIT 1998: *Curriculum vitae* of Mr. Stephen Stephen

EXHIBIT 1999: *Curriculum vitae* of Dr. Peter Wright

EXHIBIT 2000: Validation Pathway for NAAHLS Diagnostic Test Methods: Molecular Analysis for Infections Salmon Anemia Virus, undated

EXHIBIT 2001: Caraguel *et al*, "Traditional descriptive analysis and novel visual representation of diagnostic repeatability and reproducibility: Application to an infectious salmon anaemia virus RT-PCR assay," Preventative Veterinary Medicine 92 (2009)

EXHIBIT 2002: Laboratory Report, November 17, 2011

EXHIBIT 2003: P. Nérette *et al*, Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anaemia virus, Journal of Fish Diseases 2005

EXHIBIT 2004: Statement from the Federal Minister of Fisheries and Oceans Canada, Keith Ashfield, on Negative Infectious Salmon Anaemia Test Results in British Columbia Salmon, December 2, 2011

EXHIBIT 2005: Content of information to provide from an OIE Reference Laboratory to inform the OIE on positive results of samples on OIE listed diseases, Dr. Fred Kibenge, October 15, 2011

EXHIBIT 2006: Testing Records: Richard Routledge samples (Sockeye smolts) VT10042011_October 12 2011 Update on virus isolation attempts

EXHIBIT 2007: Email from Fred Kibenge to Alexandra Morton, Re: update, November 2, 2011, attaching report "Alexandra Morton Samples (Sockeye Chinook and Coho) VT10142001_OCTOBER 20 2011.pdf"

EXHIBIT 2008: Testing Records: Alexandra Morton samples (Sockeye, Chinook & Coho) VT10142011 October 20, 2011, Update on virus isolation attempts

EXHIBIT 2009: Testing Records: Alexandra Morton samples (Sockeye, Coho, Pink) VT11072011 November 07 2011, Dr. Fred Kibenge

EXHIBIT 2010: Email from Fred Kibenge to Alexandra Morton, Re: Samples, November 23 2011, attaching report “Alexandra Morton Samples (HERRING and SOCKEYE) VT10312011 OCTOBER31 2011.pdf”

EXHIBIT 2011: Terms of Reference – OIE – World Organisation for Animal Health, Reference Laboratories

EXHIBIT 2012: Workenhe *et al*, Absolute quantitation of infectious salmon anaemia virus using different real-time reverse transcription PCR chemistries, Journal of Virological Methods (2008)

EXHIBIT 2013: OIE Reference Lab for ISA – Annual Reports – 2004-2010

EXHIBIT 2014: Dr. Are Nylund Report-I, October 27, 2011: Testing of gill samples from juvenile *Oncorhynchus nerka* (sockeye salmon) collected in Rivers Inlet on the central coast of British Columbia, Canada

EXHIBIT 2015: Dr. Are Nylund Report, November 2, 2011: Testing of gill samples from juvenile *Oncorhynchus nerka* (sockeye salmon) collected in Rivers Inlet on the central coast of British Columbia, Canada)

EXHIBIT 2016: Dr. Are Nylund Report, November 23, 2011: Testing of gill samples from salmonids collected in British Columbia, Canada

EXHIBIT 2017: Devold *et al*, Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection, Diseases of Aquatic Organisms, Vol. 40: 9-18, 2000

EXHIBIT 2018: Plarre *et al*, Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway, Diseases of Aquatic Organisms, Vol. 66:71-79, 2005

EXHIBIT 2019: Snow *et al*, Development, Application and Validation of a Taqman Real-Time RT-PCR Assay for the Detection of Infectious Salmon Anaemia Virus (ISAV) in Atlantic Salmon (*Salmo salar*), Vannier P, Espeseth D (eds): New Diagnostic Technology: Applications in Animal Health and Biologics Controls. Dev Bio (Basel). Basel, Karger, 2006

EXHIBIT 2020: International Response to Infectious Salmon Anemia: Prevention, Control, and Eradication, p. 25-37 and 69-73

EXHIBIT 2021: No Confirmed Cases of Infectious Salmon Anaemia in British Columbia, DFO Information Bulletin, November 9, 2011 (DFO website)

EXHIBIT 2022: Letter of Designation (28 October 2011)

EXHIBIT 2023: Mandatory Notification and Suspect Phase Disease Response Policy for the National Aquatic Animal Health Program

EXHIBIT 2024: Procedure for Receipt and Evaluation of Mandatory Notifications for the National Aquatic Animal Health Program

EXHIBIT 2025: Reportable Diseases of Finfish, Infectious salmon anaemia (ISA)

EXHIBIT 2026: CFIA, Information Bulletin

EXHIBIT 2027: Directive: Mandatory Notification of Reportable Aquatic Animal Diseases (19 January 2011)

EXHIBIT 2028: Ministers' statement (24 October 2011)

EXHIBIT 2029: News Release (8 November 2011)

EXHIBIT 2030: Transcription: News Conference (8 November 2011)

EXHIBIT 2031: Dr. Are Nylund, Report, November 8, 2011: Testing of gill and heart samples from smolt and herring collected in British Columbia, Canada

EXHIBIT 2032: News Conference, December 2, 2011

EXHIBIT 2033: Nylund, Report, 12th December 2011, Testing of gill samples from salmonids collected in British Columbia, Canada

EXHIBIT 2034: Kibenge F *et al*, Infectious Salmon Anaemia Virus (ISAV) Ringtest: Validation of the ISAV Diagnostic Process using Virus-spiked Fish Tissues and ISAV Taqman Real-time RT-PCR. J Aquac Res Development

EXHIBIT 2035: Workenhe *et al*, Infectious salmon anaemia virus (ISAV) isolates induce distinct gene expression responses in the Atlantic salmon (*Salmo salar*) macrophage/dendritic-like cell line TO, assessed using genomic techniques. Molecular Immunology 46 (2009)

EXHIBIT 2036: Laboratory Report to CFIA, December 6, 2011

EXHIBIT 2037: Laboratory Report to CFIA, December 1, 2011

MR. TAYLOR: Mitchell Taylor, counsel for the participant Canada. That all mostly sounds fine. I think I heard you including 33. I have no idea what 33 is, but it's not on the list I was given.

MR. MARTLAND: It was added to the list. There was an email exchange with Ms. Grande-McNeill, who is nodding yes, so I'm hoping that (indiscernible - overlapping speakers).

MR. TAYLOR: We're fine with it.

MR. MARTLAND: Thank you.

MR. TAYLOR: And I should say for the record, that with me -- I'm Mitchell Taylor, and with me is Mark East, to my right, Geneva Grande-McNeill to my far right, and Adam Taylor, who is an articled student that is with us, and seek leave that he be with us at table, Mr. Commissioner.

THE COMMISSIONER: Yes.

MR. MARTLAND: As we move through things today, I'm likely to be referring to tab numbers. If I can tie that to the exhibit number, I'll do that. I'll be looking to my left for some assistance in that regard.

EXAMINATION IN CHIEF ON QUALIFICATIONS BY MR. MARTLAND:

Q I'd like to begin first with some basic questions of the witnesses that we have. Dr. Nylund, good evening. It's 6:00 p.m., or a little thereafter in Norway. You've joined us by video link. We're very grateful to you for doing that. And I know you had an early start to your day and I think we've got you till 1:00 a.m., so we're grateful to have you joining us. You, sir, you serve as a professor in the Department of Biology with the Fish Disease Group at the University of Bergen in Norway; is that correct?

DR. NYLUND: Yes. I'm the Head of the Fish Disease Group at the University of Bergen.

MR. MARTLAND: And if I might ask for Tab 4 from our list of documents, which has now been marked as Exhibit 1996, to please be brought up on screen. Now, as I understand from Mr. Lunn, the business of putting documents on screen today will work for counsel, we hope, but won't work for the big screens because we're using the big screens for Norway. But I'm hoping momentarily we will see your *c.v.*, in fact, a Profile and List of Publications on ISAV.

Q Do you see that?

DR. NYLUND: Yeah, I can see that.

MR. MARTLAND: So, and I think we do, too. I'll ask -- that has been marked as an exhibit. I'll ask to have Dr. Nylund qualified as an expert in viral diseases of fish, in particular ISA or Infectious Salmon Anaemia virus, and methods for viral detection.

THE COMMISSIONER: Very well.

MR. MARTLAND: Thank you.

Q Dr. Kibenge, you serve, sir, as a Professor of Virology and Chairman of the Department of Pathology and Microbiology at the Atlantic Veterinary College at the University of PEI. You also head the OIE Reference Laboratory for ISA for the Americas, sir; is that correct?

DR. KIBENGE: Yes.

Q And Tab 2 of our list, which should be 1995, is, I hope, your *c.v.*; is that right?

DR. KIBENGE: Yes, that's correct.

MR. MARTLAND: Thank you. I propose to have Dr. Kibenge on the basis of his qualifications

qualified as an expert in viral diseases of fish, in particular ISA virus, and methods for viral detection and identification.

THE COMMISSIONER: Very well, thank you.

MR. MARTLAND: Thank you. And, Mr. Commissioner, your microphone may or may not be on for these things, so where I have said "thank you", I am simply confirming your direction that these qualifications have been made.

Q Ms. Gagné, ma'am, you serve as the Molecular Biology Scientist and Laboratory Supervisor at the Molecular Biology Unit at DFO Moncton, which is also referred to, if I have it correctly, as the GFC, the Gulf Fishery Centre; is that right?

MS. GAGNE: Yes.

Q And Tab 1 on our list, Exhibit 1994, I hope is your *c.v.*

MS. GAGNE: Yes.

MR. MARTLAND: Thank you. I ask to have this witness qualified as an expert in diagnostic methods and validation techniques for viral detection in fish and seafood.

THE COMMISSIONER: Yes, thank you.

MR. MARTLAND: Thank you.

EXAMINATION IN CHIEF BY MR. MARTLAND:

Q Dr. Miller, you previously testified, Tab 3 on our list is Exhibit 1510, your *c.v.* You testified on August 24 and 25. You are the Head of the Molecular Genetics Laboratory at DFO Pacific Biological Station in Nanaimo; is that right?

DR. MILLER: Right. Yes.

Q And your expertation, as before, it's already been made, is as an expert in molecular genetics, immunogenetics and functional genomics with a specialty in salmon.

DR. MILLER: Yes.

Q Thank you. Let me start big, so to speak. Dr. Kibenge, what is ISAV?

DR. KIBENGE: ISAV is Infectious Salmon Anaemia virus, and that's a virus for fish. It infects farmed Atlantic salmon and causes -- it is called the Infectious Salmon Anaemia, or ISA. The virus structure of this virus is similar to influenza viruses and they are both grouped together in the same virus family. The family is called

Orthomyxoviridae.
Q Thank you. How many Orthomyxo viruses are known to occur in fish?
DR. KIBENGE: I think right now Infectious Salmon Anaemia virus is probably the only known what are characterized Orthomyxo virus that affects fish.
Q And what types of fish are typically infected?
DR. KIBENGE: ISA virus is -- it causes communicable disease in farmed Atlantic salmon, but it has also been found in various species of wild fish.
Q In our previous, in particular I'm thinking of the hearings held on the disease topic, we had evidence about the very important distinction between a disease and a virus. Dr. Nylund, can I ask you, please, does the presence of ISA virus, if it is present, does that mean the disease ISA is present?
DR. NYLUND: No, there is a large difference between detection of the virus, or the viral genome, and the actual disease. And usually you will only find disease development in Atlantic salmon. And none of the other salmonid species are really suffering from ISA infection. You may have some disease developing in rainbow trout, or steelhead, as you call it, but most of the other species will be carriers or they will have a viremia, but they will not show any clear signs of disease.
Q I'm going to move into a more technical area that indeed will take some of our attention today. The terminology which we'll be using through the day, I expect is RT-PCR, reverse transcription polymerase chain reaction. RT-PCR, the RT, I've now learned, doesn't stand for "real time", but there's also both the real time, and I guess it's sometimes called the conventional RT-PCR. So first tell me, please, if I have any lingo on this or anything wrong. Ms. Gagné, I'd like to ask you, we've learned a little bit through documents. A number of these documents are now in evidence and will help us to understand, as well. But in terms of this method of using real time RT-PCR, could you please tell us what is real time RT-PCR?
MS. GAGNE: PCR is a process of specific amplification of DNA that is on specific detection of a fragment of DNA in the mixture of DNA. RT is for reverse transcription. In this case, we're working with RNA viruses, so we need to start by extracting the

RNA from, in this case, a fish tissue. And if the RNA of the virus is present in there, mixed with the RNA of the fish, where we'd try to detect it with the PCR assay.

So the assay requires primers. Primers are short custom-made segments of DNA that will anneal if there's a match with the DNA in your mixture. If the virus is in the mixture with the DNA of the fish, we would get a match, and the PCR process will amplify that segment between the two primers that you have put in your mixture.

The probe is in between those primers. The probe is linked with a reporter of fluorescent molecule. So when the PCR process goes on, if there was a match with the primers first, the PCR process amplifies what's in between those primers, so it creates a sequence, a short fragment of DNA, and the probe will be released, and what the real time RT-PCR acid detects is the fluorescence from a probe.

Q Before I move to my next question, these are sensitive mikes. I notice that as I sway back and forth. I'm going to ask all of the witnesses to please angle your mikes up and to use them as close to your mouth as you can as we go forward. That's helpful to us.

I alluded to a distinction between conventional and real time RT-PCR. Ms. Gagné, could you help us understand that distinction?

MS. GAGNE: In the conventional RT-PCR, there is no probe. We amplify what's -- the primers will anneal to a matched sequence, and the polymerase reaction will amplify what's between those primers, the primer is included. So there is no probe. But at the end of the process we will put the product in a gel, and if there was sufficient target to start with in the material, we will see the amplification product on the gel after electrophoresis.

With the real-time assay it's different because you have the probe, you don't need to use a gel, you just rely on the fluorescence produced by the probe.

Q I'd like to move into a series of questions that focus on -- sorry, moving into a series of questions that look first and if you will distinctly at the RT-PCR test results that each of

you variously have obtained. We'll turn to some other testing related to some ultimate conclusions a little later on.

Dr. Kibenge, my first set of questions are for you, sir. At Tab 31 on our list of documents, Exhibit 2005, is your report on ISAV, RT-PCR tests bearing a date of October 15th, 2011 -- I'm sorry, that's the date that it's signed. Do you see that on screen, sir?

DR. KIBENGE: Yes.

Q And in the course of that paper on page 2, I don't think I need to take you there, you make reference to a paper by Workenhe, W-o-r-k-e-n-h-e, and a Workenhe paper. I hope that Tab 38 of our list of documents, now marked Exhibit 2012, is that Workenhe paper; is that correct?

DR. KIBENGE: Yes, that's the paper.

Q In turn, this is following the rabbit down the hole, I suppose, but the Workenhe paper in turn makes reference to the use of primers and probes as described in Snow 2006, Tab 51 of our list, Exhibit 2019, I hope is the Snow 2006 paper.

DR. KIBENGE: Yes, that's the paper.

Q Tab 33 on our list, Exhibit 2007, is your report on ISAV RT-PCR tests bearing the date October 20, 2011.

DR. KIBENGE: Yes.

Q That is the second test report. The third I expect is Tab 36, Exhibit 2010, report with October 31 as the date.

DR. KIBENGE: Yes, that's correct.

Q And the fourth, I hope is Tab 35, Exhibit 2009, a report dated November 7 of this year.

DR. KIBENGE: Yes.

Q So having blitzed through those report documents, in a nutshell, what were the results of your RT-PCR tests for ISAV on those various samples?

DR. KIBENGE: Well, so in total we received for submissions, the very first one was the 48 hearts of sockeye smolts. And in that testing we found two positive samples out of 48.

Q And the 48 I believe have been described as being from Rivers -- sockeye from this coast from Rivers Inlet on the British Columbia Coast. Is that your understanding?

DR. KIBENGE: Yes, that's correct.

Q When material is shipped to you, do you know

anything about where it's from, or are you relying on what you've been told about its provenance?
DR. KIBENGE: We rely on what the submitter tells us, of where they collected the samples and when they were actually even taken from the fish and submitted to the lab.
Q all right. So you've described your results on the 48. Can you tell us about other results, please?
DR. KIBENGE: Yes. So the second set of samples was saved, we have from a different submitter and in that case I think we found in total three positive samples. And then in the third and the fourth, those samples were negative.
Q Mm-hmm.
DR. KIBENGE: On the same test.
Q Okay. Could any of the positive results obtained in your view be attributed to contamination, or could they be false positives?
DR. KIBENGE: You know, in -- the way we work in my lab, by the time we put a result, we would have ruled out all possible causes of contamination, or if it's a false positive. So by the time we put a result, we are confident that is a true positive result.
Q Dr. Nylund, I'll turn my next set of questions to the testing that you have done. I understand you have tested several batches of Pacific salmon, as well as the one group of samples for herring, testing for ISAV; is that correct?
DR. NYLUND: Yeah, that's true.
Q Tab 46 on our list. Exhibit 2014, I believe is your initial report on ISAV RT-PCR tests dated October 27 this year?
DR. NYLUND: Yeah, that's a preliminary report on the first 48 samples.
Q All right. And tissue from the same 48, although perhaps different tissue, is that your understanding?
DR. NYLUND: Yeah, they are gill tissues and Kibenge was testing heart tissues.
Q With respect to your -- that document that's on screen, you make reference to Snow 2006. Was that the Snow paper that we had on screen a few minutes ago that's been marked as an exhibit?
DR. NYLUND: Yes.
Q We also -- you also make reference to, I may

mispronounce, Plarre 2005 paper, Tab 50 of our list, Exhibit 2018, I expect is the Plarre paper.

DR. NYLUND: Yes.

Q Tab 47, so we now see the Plarre paper on screen here. Do you see that as well, Dr. Nylund?

DR. NYLUND: Yes.

Q Thank you. Tab 47 on our list of documents, Exhibit 2015, is your report on ISAV RT-PCR tests dated November 2nd this year; is that correct?

DR. NYLUND: Yes, all 48 samples.

Q All right. The next, Tab 48, Exhibit 2016, your report dated November 23 this year; is that correct?

DR. NYLUND: Yes.

Q Tab 133, and this is a document that you may not have, given what we have been sending over, you may need to look at on the screen if we can, Exhibit 2033 in our proceedings now. But I expect you'll recognize that as being -- I'm sorry, the dated of December (sic) 12, your report on ISAV RT-PCR tests, as well as other viral tests; is that correct?

DR. NYLUND: Yes. The report, the 12th -- it says 12th of November --

Q 12th of November.

DR. NYLUND: -- 2011.

Q That's my mistake, 12th of November of this year.

DR. NYLUND: Yeah. But it's probably from December, so it's my mistake.

Q Oh, there we go. In brief, what were your results? Did you obtain positive -- any positive RT-PCR tests for ISAV in those samples that you tested?

DR. NYLUND: Yeah, the -- among the first 48 I had one positive, and it was sample number 36.

Q Mm-hmm.

DR. NYLUND: Yeah.

Q Thank you.

DR. NYLUND: But I was not able to repeat it, and I tried to repeat it several times.

Q And as among the other samples that you tested.

DR. NYLUND: Among the others, I don't remember them. I have to look at it. There was one positive in the report from the 23rd, sample H10 and that was repeatable.

Q And that should be Tab 48 of our list.

DR. NYLUND: And I also got sample 14 heart positive,

but that was not possible to repeat.
Q For the record, Tab 48 is Exhibit 2016. I'll ask the same question I put to Dr. Kibenge. Do you believe any of the results that you found positive test results could be attributed to contamination or understood, should be understood as false positive for any other reason?
DR. NYLUND: I had no sign of contamination. I mean, we have a specially designed lab for this kind of work, and I have also been running just as many negative controls as positive tissues. And it was only these tissues that came up positive. But of course I was not able to sequence any ISA virus from these samples. So I was not able to verify that this was actually ISA virus I was picking out. But you know that the assays that we are using, the real time assay we're using are very specific, so they should only be picking out ISA virus, and maybe not all ISA virus, but most of the ISA viruses that we know.
Q Ms. Gagné, I have next first to move through some documents and then ask about the testing that you have done with the DFO lab in Moncton. We understand that in relation to a number of the same salmon, we understand the same salmon that have been tested by Dr. Kibenge and Dr. Nylund, you have indeed tested many of the same tissue, same fish.
MS. GAGNE: Sometimes we tested the same material, other times we tested other tissue from the same fish.
Q Okay. At Tab 142, on our list of documents, Tab 142 isn't an exhibit at this point. I'll be asking for it to be marked momentarily. This is probably a useful document for many of us in getting an understanding about the different testing that has been done. It's titled "Technical Information for DFO Moncton", et cetera. Do you recognize that document?
MS. GAGNE: I didn't produce it, but I recognize it.
Q All right. Is it an accurate summary, to your understanding, of the testing?
MS. GAGNE: Yes.
MR. MARTLAND: I'll ask this be marked as the next exhibit, please.
THE REGISTRAR: That will be Exhibit 2038.

EXHIBIT 2038: Technical Information for DFO Moncton based on sample sets for lab assessment regarding ISA in BC salmon

MR. MARTLAND:
Q What are the RT-PCR result reports in this document?
MS. GAGNE: They are negative.
Q At the bottom of the document there's a row which has -- it's greyed out or highlighted, I suppose, "Interpretation of DFO testing" is the heading, and then we see "inconclusive" or not applicable, depending. Were your RT-PCR results inconclusive?
MS. GAGNE: We reported them as inconclusive based on our policy. Samples are tested additionally for the quality of the RNA tissue, and in this case all samples submitted show extensive to total degradation of RNA. So for that reason we would not reject a positive result if we had found one, we would have investigated and followed our own policies, but in the case of negative results, because of the possible degradation of any material in there, we have to declare the samples inconclusive.
Q Tab 15, Mr. Lunn, please, is our -- of our list of documents, I expect you'll see an email that you were c.c.'d on from Anne Veniot, section head of the Aquatic Animal Health Group, which reads at the start there. It's addressed to Stewart Johnson, but you and Peter Wright are c.c.'d:

> Absolutely every sample we received showed signs of degradation.

It goes on to say:

> ...much more than what allows for conclusive testing.

And I take it from the answer you've just given, for you that's an accurate description of the sample quality?
MS. GAGNE: Exactly.
MR. MARTLAND: If I might move to Tab 21, please. Oh, and I'm sorry, if I might ask that be marked as an exhibit, 2039, I think.
THE REGISTRAR: That's correct, thank you.

EXHIBIT 2039: Email exchange between Anne Veniot and Stewart Johnson, November 17-18, 2011

MR. MARTLAND:
Q Tab 21 of our list of documents is an email -- 21, I'm sorry, Mr. Lunn, we're doing this at highway speed today.
Do you recognize that email?
MS. GAGNE: Yes.
Q Does it describe a positive RT-PCR result?
MS. GAGNE: It was one well of duplicate wells showing at 38, and normally our policy would -- we would not report that as is. We have to do follow-up, and try to repeat at least, because we start from calling that suspicious, and need to repeat, and need to confirm the initial result. And in this case, we had to -- everybody was asking for results daily, in our case, so I think my email shows that, and normally I would not report this. We would have to do the follow-up, which we did and we were not able to confirm it by re-extraction.
We also attempted, and I think it's explained here. It's explained in one of the other emails probably. We attempted to use a portion of that material and put it in a fresh master mix to try to amplify again the signal. Couldn't do that. And after three attempts, we just called it negative. It was not reproducible.
Q So was that -- is that the result you would class as a false positive?
MS. GAGNE: In our hands, this is -- this can be false positives, and the company employed by our system can confirm this, they have document about that. You can occasionally see a signal in one well, close to the limit of the assays, which can be due to the reporter, the fluorescence being present due to priming between your primers and probes, and the probe gets degraded and that creates fluorescence, but it doesn't mean you have a specific result.
Q I'd like to ask you about some of the text in that email, and if we have a look at the third or fourth paragraph of your email dated November 4th, "I am not convinced" -- first of all, if we jump

down and read the email to you from - sorry, Mr. Lunn - Crystal Colette writing in French on November the 4th "j'ai eu un Ct de", and then she goes on to say that she's received that result from heart tissue. Her response, which I think is bilingual, is "hummmmm". Above that, in your email responding in the last paragraph, you write:

> I am not convinced it should reported to our friends in Ottawa, guess why! We do not like to see a Ct like this, but this is the type of Ct that is equivalent to the finding by Nylund, i.e. can't conclude anything from it.

Could you help us -- could you explain that, please.

MS. GAGNE: Okay. I wrote that because, as I said, normally this is not even going out of the lab. It stays between ourselves because we're not done with that sample. We would not have reported it immediately like that, as one Ct. And it was not in both wells, another indication that something was not proper with that sample. And about the -- yeah, that's why I knew, like, showing this result would trigger a lot of, like, tons of emails, tons of stuff. For me it was too early to even report it, that's why. That's why I wrote that.

But the other thing I wanted to mention is this sample was not even one of the -- this lot of sample, this case that we were testing, was where in Kibenge's lab there were three positives by PCR, and this one was not one of the positives in Kibenge's lab, as well. So there was several indications at this stage, it was too early to report anything.

Q Who were the -- who were the friends in Ottawa that are referred to?

MS. GAGNE: Oh, it's just colleagues, no friends in particular, just --

Q Okay.

MS. GAGNE: -- that I didn't want to trigger another ton of calls and emails. We were already quite busy at the time.

Q Tab 11 from our list of documents, Exhibit 2000 is the DFO ISAV Validation Pathway. Is this a document that you've been intensively involved in? Oh, and I'm sorry, Ms. Chan points out I forgot to

mark the last email, Tab 21, from our list as an exhibit. If that could be Exhibit 2040, please. Thank you.

MR. LUNN: So marked, thank you.

EXHIBIT 2040: Email exchange between Nellie Gagné, Crystal Collette and others, November 4, 2011

MR. MARTLAND:

Q So I was bringing up on screen the Validation Pathway.

MS. GAGNE: Mm-hmm.

Q Could you tell us of your involvement in this, please?

MS. GAGNE: I wrote this.

Q Has it been finalized, and if so, when?

MS. GAGNE: It is in review right now.

Q All right. If I could move, please, to page 9 of this document, and if we look on the last paragraph which is sort of a yellow -- text on yellow, if you will:

Using the real-time version of the ISAV assay, we analyzed the effect on repeatability versus the amount of target in the samples. As can be seen in the figure below, when samples are lightly infected, the repeatability decreases. On average, at Ct 38, samples are less likely to repeat...

MS. GAGNE: Running -- if you have a group of fish, this was -- we did a validation, we had access to 400 fish with coming from infected cages, so these were real cases of ISA, and there there was a proportion. The final proportion of positives in that lot was about 50 percent, ranging from heavy to light infection. So the fish that were lightly infected, the ones that are at the highest Ct - a high Ct means a light infection - those fish that are above the Ct of 35, which you try to repeat the result blindly, using the same, like, a set of tissues from those same fish again, That's where you show that your repeatable it is not so good, because the light infections are -- you pick it once, the next time you don't, et cetera. So this is what we're saying here. And at 38, really it's

becoming very difficult to repeat results starting back from the same fish, but another sample of that fish.

Q In terms of the 37.79, do I understand you to say that that could point to a light infection?

MS. GAGNE: In our labs, yes, 37 is a light infection. It's the limit of the assay.

Q Dr. Miller, I'd like to now turn to you and move through initially some RT-PCR tests that you've done. You've recently conducted RT-PCR tests for ISAV. Why did you do that?

DR. MILLER: When I testified here before, I talked about running tests for various different known viruses, in association with our mortality rated signature, and I had testified that we had tested for ISA and it was negative. And so when I heard about these initial potential positive results, I went back to what we had done previously, and looked at what assay we had used, and realized that we had used an assay to segment 6, which does not necessarily pick up all strains of ISA.

Q I'm sorry to interrupt you, but when you say "we had used", who are you referring to there?

DR. MILLER: My lab. My lab.

Q At PBS.

DR. MILLER: At PBS, yes. So I was concerned that, you know, we hadn't done enough due diligence to make sure that indeed our fish were negative. So I embarked to try to obtain the primers that Dr. Kibenge used, and that our DFO validation assay, as well. I was not able to obtain any of those primer probe sets, so we went to the published literature and we got the papers from Plarre, and from Snow and Christiansen paper that was a revision of a segment 8. We ordered five different TaqMan assay primer probe sets, and we started running those on our own fish that we had run on microarrays previously, because of course our question was do we see any indications of ISA in our fish, and do they have any association with our signature?

And so we -- we embarked in five different primer probe sets and we did initially obtain a number of PCR positives. We -- I tried to get a positive control from DFO, and I wasn't able to get a positive control. So we ran the assays with no positive control, which it can be problematic

in that you don't know if your assay doesn't work. But on the other end of the spectrum, there's nothing -- nothing to contaminate your assays with, because we don't have ISA in our lab, we've never worked with ISA, and we don't have a clone of ISA. So if we obtain a positive and are able to sequence a positive, it is a real sequence positive.

So we -- we did embark and we obtained products from four of the five primer sets that we used, and we sequenced from all of them, multiple individuals, and we did indeed obtain ISA sequence. However, the sequence is especially from the ISA segment 7, and this is using a Plarre primer set, is divergent from all known ISA strains. It's 95 percent similar to all known ISA variants.

Q If I could move first to document 117 on our list of documents, I'll go after that to 137. But 137 -- I'm sorry, 117, when we see it, Dr. Miller, can you tell me if this gives us the primers and probes that you just described.

DR. MILLER: Yes, that shows the primers and probes, and the publications that they arose from.

MR. MARTLAND: If this might be marked, then, as Exhibit, I think 2042 (sic).

MS. PANCHUK: So marked.

EXHIBIT 2041: Primers and probes for ISAV

MR. MARTLAND: Thank you.

Q With respect to Tab 137, I'll ask that that be brought up on screen and ask, Dr. Miller, if you recognize that as being your presentation titled "Prevalence of ISAV using five distinct TaqMan assays".

DR. MILLER: Yes, that's correct.

MR. MARTLAND: If this might be marked as Exhibit 2043 (sic), please.

MS. PANCHUK: So marked.

EXHIBIT 2042: Prevalence of ISAV identified using 5 distinct TaqMan assays in gill tissue from 2007-2010

EXHIBIT 2043: Garver results by experiment

MR. MARTLAND: Thank you.
Q What does this presentation indicate?
DR. MILLER: Well, since we've actually sequenced from a number of individuals that we ran this assay from, and every time we have sequenced from positives we have obtained an ISA sequence. To me it suggests that these primers are not amplifying all -- the primers are amplifying -- or there are nulls in some of the primers. So the ISA 7, P7 primer set amplifies the most positive samples. It seems to -- it probably matches the ISA variant that we are amplifying in our B.C. sockeye salmon better than the other primers and probes. The other primers and probes are mostly from segment 8. A lot of the work that has been done in DFO in the validation and by, I believe, Nylund and Kibenge, have centred on segment 8, and we find quite a lot of variability in our ability to pick up positives with segment 8 with various segment 8 primers. But when we do pick them up, they sequence as being ISA.

So I believe that what we have in B.C. is a somewhat divergent strain of ISA that is not universally picked up with all -- with the assays that are presently in use. So, you know, when you develop one of these assays, you usually develop the assay and a lot of them were developed in, I guess, Nylund's lab, and he could speak to this better than I could in terms of their development. But you have a backdrop of knowing all of the strains that you know about, all of the sequences that you know exist and you try to develop an assay that will amplify all known strains. But you can't know things that you don't have a sequence for, and so there is always the possibility that you will develop an assay that doesn't pick other variants that you didn't know about. And I believe that that's what's happening here.

Q Did you provide any of your samples to other scientists in order to either -- to see whether they confirm or dispute your findings, and if so, to who?
DR. MILLER: Initially we provided a set of positive and negative blind samples on to Dr. Kyle Garver, who is a virologist that I testified with previously. He's at the Pacific Biological

Station, And he ran an assay -- he ran basically the same assay that Nellie Gagné has run, the validation assay, and he also ran our ISA-7, the Plarre-7 primer sets that we use, and he -- he ran it under two different conditions under their -- using the protocol that is part of the validation protocol, and then also using the protocol that we use in our lab.

And I should say that we have different instrumentation and a slight -- a slight variance in the protocol that we use for RT-PCR, in that we use a -- we use a high throughput Fluidigm system, which allows us to amplify 96 different biomarkers on 96 samples at once. It's a microfluidic system. And in order to be able to do that, because the volumes are very small, the volume in each well is only ten nanolitres, it's very, very small, it requires a pre-amplification step. And so we take all of the primers that we will be using on one chip, and go through 15 cycles of pre-amplification at a very low primer concentration, basically about one-twentieth of what you would use in a typical assay.

And there are studies that show that that actually increases the sensitivity of these assays, so that the cycle threshold that we pick up on the Fluidigm system will be lower than what one could pick up with another system. So we can pick up lower copy number of viruses more effectively.

So he ran basically the validated assay that Nellie uses, and the ISA-7 Plarre assay and he was able -- he was not able to pick up any positives using the DFO validated assay, but he did pick up a positive of ISA-7 using our assay with our pre-amplification.

Q If we could look at Tab 114, please, Mr. Lunn. And I appreciate that Dr. Garver has testified, but he's not here today, but is your understanding when you see it, that this document is a description of Dr. Garver's RT-PCR results? It's just coming up. Is that your understanding of that document?

DR. MILLER: Yes.

MR. MARTLAND: I'll ask this be marked as the next exhibit, and in doing that, I brilliantly am trying to guess it, exhibit numbers skipped a

number so if this could be Exhibit 2041, I think we'd then be on track, Ms. Panchuk and Mr. Lunn.
MR. LUNN: I just want to verify for the record, I have Tab 117 as 2041, Tab 137 as 2042, and this document, Tab 114 as 2043.
MR. MARTLAND: Oh, thank you.
MR. LUNN: Thanks very much.
MR. MARTLAND:
Q Were there others, Dr. Miller, that you provided samples on to?
DR. MILLER: Yes, we sent a 96-well plate of liver tissue samples to Nellie Gagné's lab to use their validated assay. That was done a few weeks ago.
Q Dr. Gagné (sic), could you tell us about that? Were you able to reproduce the results that Dr. Miller had obtained from what she provided to you?
MS. GAGNE: This was a plate of RNA, not amplified RNA, and using our assay they were negative.
Q I'm sorry, they were...?
MS. GAGNE: Negative.
Q Negative. Do you --
DR. MILLER: Can I say -- sorry, can I say one thing. We've actually since that time, just last week, tried to amplify with Nellie Gagné's primers, not the probe, not a TaqMan assay, but basically we used the pre-amplification procedure that we normally use, and then did conventional PCR with her primers, and we have been unable to pick up any positives using her primer sets from our samples.
DR. NYLUND: May I ask a question?
Q It's always dangerous when that happens to a lawyer, but I think you should. Go ahead.
DR. NYLUND: Since Miller is running a pre-amplification, I would like to know what kind of primers she is using.
DR. MILLER: What kind of primers?
DR. NYLUND: Yes.
DR. MILLER: They're the same primers that you use in the assay. It's not nested, it's the same. You use a 120th concentration of all primers that will go into all assays on each chip.
DR. NYLUND: So you are using the real time primers.
DR. MILLER: Yes.
DR. NYLUND: Yeah.
Q We will -- we will come back and I'll be asking for your views on eventually conclusions to be

drawn from these different testing results that we're covering right now. Tab 68 on our list of documents. Dr. Miller, when you see this, can you tell me if you recognize this as being the notes you've prepared dated October 21, 2011, describing the Rivers Inlet sockeye and your involvement in that sampling.

DR. MILLER: Yes, I prepared this document.

MR. MARTLAND: If this might be the next exhibit, please.

MS. PANCHUK: Exhibit 2044.

EXHIBIT 2044: Rivers Inlet Sockeye Notes by Dr. K. Miller, Oct. 21, 2011

MR. MARTLAND: 2044, thank you.

Q Dr. Kibenge, your wife, Dr. Molly Kibenge, who has expertise in this very same area, also conducted tests for ISAV on Pacific salmon; is that correct?

DR. KIBENGE: Yes, that's correct.

MR. MARTLAND: Tab 29 of our list of documents, I expect we'll see really two things together, first an email and attached to it a draft manuscript that Dr. Molly Kibenge prepared. And so perhaps we can just flip along a little bit. We see the email on the first page or two. We then see a title page with Molly Kibenge as lead author, Simon Jones, Garth Traxler, and yourself listed as co-authors. This was a draft manuscript that Dr. Molly Kibenge prepared; is that correct?

DR. KIBENGE: Yes, that's correct.

MR. MARTLAND: And if I could ask that this please become Exhibit 2045.

MS. PANCHUK: So marked.

EXHIBIT 2045: Cover email and draft report Asymptomatic infectious salmon anaemia in juvenile *Oncorhynchus* species from the North West Pacific Ocean, Kibenge Molly T. et al

MR. MARTLAND:

Q What were the RT-PCR results described in that paper and in the course of that testing?

DR. KIBENGE: Well, this work, at the time it was being carried out, there was no real time RT-PCR, so the testing that was done used the conventional RT-PCR, and the primers that were used were targeting

segment 8 and they are the standard primers for testing for ISA that are described by Devold, 2001, I think, and the also the ones that are in the OIE Manual. And the results, I think, as far as I recall, were that Dr. Molly Kibenge was able several samples positive for ISA virus, and some of those samples were sequenced, the products that we amplified were sequenced, and deposited to the gene bank, and again they were able to -- we were able to identify some (indiscernible - rapid speech) there with the ISA virus sequences that are deposited in the gene bank. So this was clearly a positive amplification of ISA virus in those samples.

Q I don't want Mr. Lunn to rest for a nanosecond. I'm going to ask him if he can bring up Tab 49, Exhibit 2017, simply just to tie the loose ends together and confirm if this is the Devold paper you just described.

DR. KIBENGE: Yes, those, yeah, the primers that are used -- that were used in the conventional RT-PCR are from that paper.

Q Now, if I return to the Dr. Molly Kibenge and others draft manuscript, I take it to be your understanding that didn't move past being a draft manuscript. Are those results that were ever published, or a paper that was completed?

DR. KIBENGE: No, they were not published, and the reason that was given was that the results that we obtained were considered to have been due to contamination, and the decision to submit the paper was denied.

Q All right. You say the results were considered to be attributed to contamination, considered that way by whom?

DR. KIBENGE: Dr. Molly Kibenge was working in the lab of Dr. Simon Jones. Dr. Simon Jones was the supervisor of this project, and it was his call as to how to proceed with the results of that work.

Q Ms. Gagné, for your part, did you have involvement in the year 2004 or thereabouts dealing with this very question of ISAV tests by Dr. Molly Kibenge?

MS. GAGNE: Yes. We received a call in -- I received a call from Molly and I was informed by my section head at the time, who was Dr. Gilles Olivier, that there was some positives for ISA she was finding in her samples and at the time we were interested

to first get samples and to confirmation and help out figure what this was as much as we could. So we received 93 samples, I believe they were kidney, preserved in RNALater from Molly and tested then.

Q And tell me about that testing, please.

MS. GAGNE: We exchanged information with Molly as much as we could. We figured that we were using the same kits and pretty much the same techniques as she was using. We were using FA3/RA2 primer, if I recall, and this is also what she was using, and she was finding positives with these primers. We ran the samples and didn't find positives in them. So there was a long strong of emails that was provided, but I -- from these emails, what I can figure is that we tried several different things and exchange of information with her to figure, because in my view, it was almost to the point where why can't we find anything. You seem to be quite sure of what you're finding.

But then I -- I remember seeing pictures of her gels, and for us when -- because these are conventional PCR, the products are put on gel and they're supposed to be at the same height as your controls, if they're positive. I remember seeing pictures and in our lab we would not call positives when they are not at the same height as the positive control. So I just mentioned these things, and but then she said that she had sequences for that particular segment 8 that she was working with. So still we reran several times many of the samples. At some time we were just focusing on a selection of the samples she was telling us to use. And we have not been able to find it.

Q So in -- tell me if I have this right, in around 2004, if I have your -- understand what you're saying, you were effectively surprised not to get equivalent results.

MS. GAGNE: Because we had the same primers, we had the same kit that she was using, everything was matching. There was minor differences at some points, but we reran things. We had done it so many times, it was -- it was not possible.

May I add, if it's not already something you plan to ask, but recently because of all this issue, we returned, we had -- we had still the

kidneys preserved in RNALater, the backup, the tissues that were left. So we reprocessed these samples. We extracted them and did the real time PCR that we use now, and we also ran the Snow primers, segment 8 that are used at the moment in AVC's lab and they were still negative. Quality was sufficient, in my view to have -- to be confident in the result.

Q So this question of different labs, perhaps unexpectedly getting different results, I'd like to in fact draw your attention to Tab 26, Exhibit 2003, a paper that you're one of the co-authors with. Nérette is the lead author for that paper. And I won't need Mr. Lunn to go to it, but you'll recall, I'm sure, from that paper, the reference is page 109, referring to a substantial difference in repeatability of RT-PCR among different labs as being something that this paper indeed comments upon. I wonder if you're surprised in '04 but in '05 you've written a paper like this, that indeed picks up on this difficulty or unlikelihood of repeatability in a predictable way, does that change your view, or does that...

MS. GAGNE: This study from the -- it was done in 2004, I think, this study was -- the labs were using their own method, and there was no obligation to run any protocol. Everybody was using what they were comfortable with. And it did show differences in the different labs. And some labs, in this case it was a relatively large set of sample that was provided, like split in three and provided to the different labs, and we were looking at the capacity of the lab to repeat the results within -- like blindly tried to repeat the results from different samples, and match, like, each other labs. And indeed it showed that it's -- there is some level of disagreement, some level of agreement between the labs. And obviously, this was not quantitative assay. These were not real time assays, so we don't have the Ct values, which is -- which informs us on the level of virus and the samples, but it's always when you get those samples that produce the faint bands, like the samples where you have low viral load, where you find most disagreement between the labs.

Q If I could move to Tab 115, please, on our list of documents. This is entitled "QA/QC Summary" it

bears your name on it and a date from November of this year, Ms. Gagné. Do you recognize that document?

MS. GAGNE: Yes.

MR. MARTLAND: If this could become, please, Exhibit -- I should check if it's 2046?

MS. PANCHUK: So marked.

EXHIBIT 2046: QA/QC summary by Nellie Gagné dated November 14, 2011

MR. MARTLAND:

Q If we could go to page 3 of that document, please. So we see a heading at the bottom of that page, heading (4) "Additional steps and controls in place to insure results accuracy". You see that subheading (4) at the very bottom of page 3. And then under, on the next page, Mr. Lunn, if we just simply go almost to the end of the document - there we are - we see, we read as follows:

> b) It is also possible to confirm presumptive results by other techniques:

They include a few things. The third is:

> iii. Amplification of an alternate portion of the pathogen genome (and sequencing of this region if desired).

Has this confirmation of presumptive results that's described there, has that been achieved by amplification of segment 7 and segment 8?

MS. GAGNE: On what samples you are...

Q On the -- through the work that we've heard described today by others, at any level.

MS. GAGNE: In our case, no. In this, like, in the -- in the -- I think my mike's not working. In the samples provided --

Q And I've just been asked if you're able to speak louder. I think your --

MS. GAGNE: In the samples provided by -- during this ISA amplification, since there is no initial positive to confirm, we have not attempted amplifying another segment, except that we have run the Snow primers, which are different than our primers, the Snow assay primers and probes, and

they - okay - and have not confirmed either using this assay that is validated also, and in the OIE Manual, we have not confirmed positives.

DR. MILLER: Can I just make one comment?

Q Yes.

DR. MILLER: Of the various primer sets or TaqMan assays that we have used, the Snow-8 picks up the fewest of positives. So it isn't -- in our view, it isn't the best match to the sequences that we're seeing in B.C. And I think it's very important to look at another segment, because the Snow primer probe set overlaps with the ones that Nellie is using already considerably. So if there were mismatches in that area, it's not really an alternate portion of the genome.

Q Dr. Kibenge, did you -- could you describe the segments you've been working with?

DR. KIBENGE: Yeah. In our lab we use segment 8 and the Snow primers. But if I could just comment on the samples that the -- Nellie Gagné used from Nanaimo, B.C. from Dr. Molly Kibenge. In the work of Dr. Molly Kibenge, they actually used -- they were targeting two segments, that was segment 8 and segment 7, and in each of those segments we had positive results. More or less like what we are seeing with the real time PCR results of this most recent samples, where Dr. Miller has found positives both with segment 8 and 7, and the -- Dr. Nylund has found positives with segment 7. I have found positives with segment 8, just because I use it on segment 8.

Q I suppose, Ms. Gagné, I'm wondering, do you simply define away the question here by saying well, they're not initially treated as positives, so therefore we're not -- there's nothing to confirm.

MS. GAGNE: We use our validated assay, which to our knowledge, and again, we use the current information we have, the current strains of ISA that we know. We used this assay and in theory this assay is made to be universal, taking all the ISA that we know of. So to -- the test is designed to be fit for a purpose. It has a good sensitivity. It picks down to 17 plus or minus seven copies, if you look at the validation dossier, this is what it says. It is a sensitive assay. It has been used in our program and has been producing positive results. It's not because

I'm reporting only negative results right now, that's not ever the -- it's not always the case. We've confirmed cases of ISA. We've confirmed HPR0 in the region, it's -- at the moment the assay is working for what it was designed to do. And in this situation it's difficult.

I recognize that we always -- we are not trying not to detect anything. We're doing our best to find something. And the other thing we've done, because it's been reported by others, we have used the OIE primers. In some labs they don't seem to be working the best but they were the ones, the Plarre primers and Snow are the ones that were used initially to report the first positives in Dr. Kibenge's lab. So we used them also on the samples we received.

So at this point I think we've done a reasonable effort to detect what was -- what was claimed to be there.

Q Is the test that you used one that's designed to detect novel ISAV?

MS. GAGNE: It's a -- when it's novel, it's like you at the moment you're testing, you don't know it's there. So we don't know what we're missing, if it's the question.

Q Let me move into some questions that try and address different components of why different RT-PCR test results could differ. There may be different reasons for that. A fairly obvious starting point is different labs might be testing whether it's different fish or different organs or tissues from the fish. Dr. Kibenge, if I could ask you about that. Do you have a situation where -- could you describe the implications of using different fish or tissues, and what that does for the comparability of results?

DR. KIBENGE: Well, yeah, my thing is that comparing labs or lab results, particularly when you're using field samples it's very difficult to be sure whether you are really comparing the same issues in each lab. The tests that we are using for ISA detection right now, we have actually developed based on the virus infection in farmed Atlantic salmon. In that case, we studied the disposition of this virus in those fish and we know the best tissue to take, which has the most amount of virus, and therefore we are confident when we use

those tests that they will either be a true positive or a true negative.

In the case of the fish samples that we've been talking about since October, these are the sockeye salmon and other wild fish in B.C., we really don't have a very good idea of the disposition of this virus in the tissues. We don't know which is the best tissue to take, at what time, and what's the amount of virus that is likely to be in this fish. In the farmed Atlantic salmon, this virus causes disease, so it's very clear that you can get very high levels of virus in target tissues.

In the sockeye salmon, if these fish are carriers, the amount of virus in the various tissues or the virus load in the various tissues would be different. We don't know which is the tissue in which this virus is persisting for this fish to be a carrier fish.

So clearly the tests we are using are designed for farmed Atlantic salmon, and we are applying them to tissue samples from wild fish, where we don't have very good information. But even if it was for farmed Atlantic salmon, the distribution of virus in the different tissues cannot be expected to be the same. In my case, for example, I received the samples that were heart, and the other labs were getting gills or kidney, and it's very difficult to expect that all those labs will have exactly the same results. So just on the basis of the tissues alone, it's very difficult to expect that you have agreeable results, let alone when you introduce the variations in the testing methods for the primers, probes, the different targets and so on.

And if I could just actually add one more point, even as far back as when Dr. Molly Kibenge was working in the Nanaimo, that test that she was using was the conventional RT-PCR, segment 8, primers from Dr. Devold. I had the methods that were being used in DFO Moncton were more or less the same.

But actually from the documents that I've seen in submissions now, I know that actually the method that was being used in DFO Moncton, they had an extra ten cycles of what they call touchdown PCR, that was at the front end of the

forward cycles of PCR. That is a significant different -- difference from the methods that were being used in Nanaimo. So when there was no repeatability or confirmation of the same results, to me it's together. The two methods were different. The primers may have been the same, but the actual cycling conditions were completely different.

MS. GAGNE: Can I add a comment?

Q Yes.

MS. GAGNE: Molly -- my mike is not on.

Q Yes, and Ms. Gagné's mike, please. Thank you. There we go.

MS. GAGNE: Molly after -- I remember the touchdown issue, that's why I want to comment. Molly used it on her samples after finding out that we were using this technique. The touchdown actually just reduces non-specificity, and it's not -- it's not doing more than that. And she found the same results in our hands, using this technique. So she was not -- so I don't think that affected what we were doing.

May I say also that at the moment, to my knowledge, we have not dismissed yet the capacity of our assay to pick up samples of ISA in B.C. We're not yet sure and there is additional work to be done on our -- in our lab and in Kristi's labs, probably, because we don't have enough sequence information to dismiss anything at this point. We know that the probe and reverse primer of this assay we're used -- we're using are matching - correct me if I'm wrong - and we don't have the information yet to dismiss that the forward primer is not matching.

DR. MILLER: They're matching the limited amount of sequence data that we get from segment 8. We get a lot more positives from segment 7 that we're not able to pick up. So my guess is they don't match all the variants that we're seeing in B.C.

MS. GAGNE: But at the moment it's early to say that the issue is the assay itself.

Q Dr. Kibenge.

MS. GAGNE: Except for sensitivity, maybe.

Q Dr. Kibenge, thank you.

DR. KIBENGE: Well, I wish to comment on the real time RT-PCR assay that is being validated by -- by DFO Moncton, and I looked at that information again

based on the documentation that we are supplied with, and that real time RT-PCR assay is actually quite different from Snow and the Plarre, and so on. It is targeting segment 8, but the primers are different, and the probe is different and the fragment length is different. So when we are saying that we can't reproduce the results, it should be clearly understood that actually we are not using the same primers and probes, and that alone can create a difference in the results, particularly when you are using field samples where there is no standard amount of virus.

In my view, the best way to compare labs, if that was an issue in terms of repeatability or reproducibility of results, would be to have an experimental sample in which there is a known amount of virus, that sample to be distributed blind, so that each lab can use their methods, and that way that will be a very effective way, a very objective scientific way of comparing the labs. In which case, if they can't have the same results, then there is a problem. But to compare labs based on field samples and particularly in this case where even the virus may be so variable that using real time on two separate segments you can't even pick up the same fish, it becomes a bit difficult to...

Q Let me pick up on that very point with respect to using different primers and probes. Tab 130 of our list of documents, Ms. Gagné, I'd like to see if I can confirm that this is your, if I have it right, a draft ISAV RT-PCR Protocol; is that correct?

MS. GAGNE: Yes.

MR. MARTLAND: All right. If this could please become, I think 2047, Ms. Panchuk.

MS. PANCHUK: So marked.

EXHIBIT 2047: DFO Draft RT-qPCR Test Method Protocol using TaqMan Universal PCR Master Mix for the Detection of Nucleic Acids from Infectious Salmon Anaemia Virus

MR. MARTLAND: Thank you.

Q It says very clearly "Draft". I take it, it hasn't been finalized at this point?

MS. GAGNE: It's near finalized, yes.

Q All right. Is that a protocol that is applied other than in DFO Moncton whether in Canada or abroad?
MS. GAGNE: DFO PBS have this document and we have also -- I'm not sure, I think we've already -- we've provided the documents to probably other labs, but I'm not sure if they're using the method.
Q is the protocol for real time RT-PCR one that's been published in a peer-reviewed journal?
MS. GAGNE: It's the method -- that method was used for samples in a study that was done recently, and that paper is going to be resubmitted after revision, so it's coming up.
Q It's in the system, so to speak.
MS. GAGNE: Yes. And the non-real time version of the assay was published in other papers.
Q Okay. So the conventional -- the conventional version of the assay.
MS. GAGNE: Yes.
Q When you validated this assay, did you perform laboratory experiments as opposed to computer experiments in order to test its ability to detect European strain ISAV?
MS. GAGNE: On some, we tested some isolates of ISA, not all known isolates of ISA, a lot -- part of the specificity of the primers were -- was done what we call in silico, by looking at the alignments of the known ISA sequences and verifying that there was a match.
Q Dr. Miller, you have used different -- some different primer and probe sets, and you've told us about that today. What can you tell us about the relative sensitivity of the different primer and probes in terms of the tests that you've done?
DR. MILLER: I believe that the sequence we have in B.C. is a closer match to the ISA-7 primer probe set from Plarre. We are able to pick up in both gill and liver tissue the most positives with that primer set, and again they sequence as positives. But there are three fixed bases that are different in the sequence between the primers, which suggest that this is a -- that are different from any known isolates, which suggest that there is a degree of divergence in what we're picking up in B.C. The various primers and probes for the segment 7, we -- whereas we'll pick up between 13 and 20 percent positives, depending on the tissue

in -- for ISA-7 we pick up between 1 and 1-in-4 percent positives, with the segment 8 primer set. So we're losing a lot, we have a lot of individuals where we pick up positives with ISA-7 and we do not pick them up with ISA -- with the ISA-8 primer sets. Suggesting that there's underlying variation in segment 8 that is precluding their ability of these tests to pick them up.

Q Dr. Nylund, with -- you have a long background in ISAV research and work. What primer and probe sets can you describe as being used, both in your lab and other labs generally, that equally -- what's the international picture here in terms of primers and probes that are used?

DR. NYLUND: I think we have to remember that the ISA virus consists of eight segments. And if you have infected particles, you can actually use an assay delegating - I just have to turn down the (indiscernible - background noise) - you can actually make assays targeting each of the eight segments, and they could be more or less equally sensitive. But if you look at tissue, it's completely different. Because in the tissues segment 7 and 8 are much more highly expressed compared to the other segments. So you may find maybe 30,000 copies of segment 7 and 8 in a cell, infected cell, while you may only find 3,000 copies of the Hemagglutinin-Esterease segment.

So this would mean that the sensitivity of the assay will depend on if you are looking on infected particles or tissue. And if you are looking at tissue, segment 7 and 8 would be the best. But then again, you would have to see if the primers or assays are targeting a certain area where you have folding of the RNA. If you have folding of the RNA, that could -- reduces the sensitivity of the assays.

So there are several different factors you have to look at. And in silico testing on the computer is one thing, but in -- when you do the actual testing in vivo, this may give a completely different result.

So if you look at the primers and probes, and the real-time assays available today, they will have more or less the same detection level if they are targeting segment 7, or if they are targeting

segment 8, they will have also more or less the same detection level. But they may vary a bit, depending on where on the RNA that they are targeting.

And we also know that if the assay is targeting, and at the 5 prime end of the RNA, it may be more prone to digestion of the RNA than if it's in the 3 prime end of the RNA. Because when the RNA is broken down, it starts in one end, and it is digested towards the other end.

So these are all very important to remember that one assay in one end may not give the same result as an assay in the other end. There may be several cycles in difference due to digestion of the RNA.

Q Thank you. Dr. Kibenge, I have just one last question, and I think, Mr. Commissioner, we may then move to break. Could you describe which published primers and probe sets are commonly used, or used internationally?

DR. KIBENGE: Well, yeah, we consider the primer probe set that was described by Snow et al in 2006, the document you showed, as being the universal primer probe set. And actually that's -- it's in the OIE Manual. The OIE Manual shows segment 7 and 6, but in terms of the three I think the segment 8 is the most commonly used, the primer probe set. There is a new description by Debes 2011 of segment 8 of a different primer probe set, but that is not as widely used. But I know that the Snow primer probe set is probably the most commonly used.

Q Whereabouts, where, what countries?

DR. KIBENGE: Oh, for example, I use it in my lab, and I am an OIE reference lab, so all the samples I receive use that primer probe set. But I also know that in Chile the government agency that is responsible for aquaculture, you know, this is Sernapesca, which would be the equivalent of, say, for DFO, they all accept the Snow primer probe set as the -- for testing real time PCR for ISA virus. And all the (indiscernible - rapid speech) labs that are testing field samples in Chile, that's they only test that they can use. So that's universally sort of commonly accepted primer probe set. I know they use that in Scotland, as well, or U.K., because that's where Mike Snow is from.

MR. MARTLAND: Right. I think on the note of Snow and

Scotland we can move to break. Mr. Commissioner, because of our logistics and the set-up in the new room we were a bit slow starting. I don't know if I might prevail upon you to suggest about a ten-minute break, and then we reconvene, please.

THE COMMISSIONER: Yes.

MR. MARTLAND: Thank you.

MR. LUNN: The hearing will recess for ten minutes. Please remain standing in place while the Commissioner exits the room. Thank you.

(PROCEEDINGS ADJOURNED FOR MORNING RECESS)
(PROCEEDINGS RECONVENED)

MS. PANCHUK: The hearing is now resumed.

MR. MARTLAND: Mr. Commissioner, as we resume, we're actually changing our system on the mikes, and if I could ask witnesses to please push on and then push off your mikes as you wish to speak, I hope that will work a little better as we move forward.

EXAMINATION IN CHIEF BY MR. MARTLAND, continuing:

Q I'd like to bring up, Mr. Lunn, two documents, if you're able to do this simultaneously, I'd be grateful for that, Tabs 44 and 57. I expect that - and, Dr. Miller, I'll ask you about this - I expect that Tab 44 you'll recognize when we see it as being an email, and an email that was sent to you by I think your lab assistant October 25, 2011; is that correct?

DR. MILLER: Yes.

Q And if we're able to bring that up on the split screen with Tab 57, is it right that the email describes the Provincial Protocol for ISAV testing and the Tab 57 indeed is that protocol on top?

DR. MILLER: Yes. We haven't actually applied this particular assay, because by the time we'd asked for it quite a bit earlier, and when we were just gathering the TaqMan assays that we would be using, and this arrived quite a bit later after we'd already had the other ones working. So we never actually used this assay.

MR. MARTLAND: All right. Now, if I deal first with the email on the right screen, Tab 44, I'd ask that be marked as Exhibit 2048, please.

MS. PANCHUK: So marked.

EXHIBIT 2048: Email from Karia Kaukinen to Kristi Miller-Saunders re "ACRDP Creative salmon array information", October 25, 2011

MR. MARTLAND: And on the left Tab 57, as Exhibit 2049, please.
MS. PANCHUK: So marked.

EXHIBIT 2049: Infectious Salmon Anemia Virus - AHC (Real-Time Assay)

MR. MARTLAND:
Q Dr. Kibenge and Dr. Nylund, I don't know to what extent you've had the opportunity to review the provincial protocol or these documents. Can you comment at all on this protocol, whether it's one you recognize? If you can't, that's fine, but...
DR. KIBENGE: No, I have not had a chance to read these documents.
Q And if we -- and Dr. Nylund, for your part?
DR. NYLUND: Well, I don't remember the sequence of primers and probes, so it's very difficult to verify anything.
Q Let me ask it a little differently. On the left document you see at the bottom, if we could try and zoom in, there are names there, and perhaps you can comment as to whether the names that are given are names of people that you recognize from ISAV research you've done, Lisa Wegener and Julie Bidulka, I think it is.
DR. NYLUND: No, I never tested those primers or probes.
Q Do you recognize those people, those names?
DR. NYLUND: No, sorry.
Q And, Dr. Kibenge, you're shaking your head no?
DR. KIBENGE: No, I don't recognize the names.
Q I'm going to move into asking some questions about amplifying smaller or larger fragments of viral genetic material. To do that, I'd like to bring up Tab 130. Ms. Gagné, in this document, first of all, do you recognize this document? And I think we've previously marked this as Exhibit 2047. I'm seeing nods of yes, so this is Exhibit 2047. You make reference in it to the expected product length being 179 base pairs; is that correct?
MS. GAGNE: Yeah, and it's a mistake, it's 169.
Q It's a typo?

MS. GAGNE: No, it's a basic mistake. We calculate -- we give a number based on positions, which are primers, and we just subtract primer positions to determine the length of the product. But in this case, the alignment of gaps in, so we included the gaps, so it's actually 169, and it's -- it's a counsel that noticed the mistake, but thank you.

Q Okay. It wasn't me, that was Ms. Chan, for the record. How large is the genetic fragment or amplicon that your test is trying to amplify, that is the number you've just given us.

MS. GAGNE: 169, yes.

Q Dr. Kibenge, what is the size of the amplicon that your test is trying to amplify?

DR. KIBENGE: The Snow probe primer target is 104 base pairs, one-zero-four, 104.

Q Dr. Nylund, same question.

DR. NYLUND: Well, we have used at least two different assays, one for segment 7 and one for segment 8, and I think the segment 8 is larger than the segment 7 assay. The segment 8 I think is around 100 nucleotides, while the segment 7 would be around 60, 70 nucleotides.

Q And we had a document, I think, that suggested segment 7 producing an 84 base pair long segment; is that right, or can you comment on that?

DR. NYLUND: You want me to comment on it?

Q If you're able.

DR. NYLUND: Well, the shorter the assay, the targeting assay, the higher the sensitivity would be. So what we would prefer is an assay around 60 nucleotides, because that's as sensitive as you can get when you have two primers and a probe. The larger the assay, the less sensitivity you will have.

Q Ms. Gagné, and as I do this, I've been passed a note, if you're able to speak up, if you're yelling I won't take it personally, but anything you can do to amplify your voice is helpful. These mikes are not as sensitive always as they might be. And mine is awfully close to my face.

Tab 131 of our list of documents, if we could have that on screen, please. It's entitled, I think, or at least it should be, the PCR Primers and Probes Design protocol, so to speak, is that right?

MS. GAGNE: Yes.

MR. MARTLAND: And I don't believe this has been marked. If I might ask this be marked as the next exhibit.
MS. PANCHUK: Exhibit 2050.

EXHIBIT 2050: DFO Moncton Primers and probes Design and Usage

MR. MARTLAND:
Q At page 3 of the document, at the top of the page, the lab protocol says that:

> For Taqman probe assay, amplicon size of 50-150 should be targeted.

If you're using an amplicon that's over 150, does that run contrary to what's set out here?
MS. GAGNE: We are -- when you design primers and probes, in this case we need an assay that's going to be able to detect all sequences. So sometimes you have no room to manoeuvre because you want to be able to have a primer in the regions that will conserve, and when you are looking for three segments that are well conserved primer, probe and other primers, sometimes you have not much room to manoeuvre there. So in our case we had to select best regions and this is the compromise we made. But we also validated these pair of primer and probes extensively before determining that they were working properly by measuring their -- the limit of detection of the assay with this, and the limits of detection was satisfactory, so...
Q I'm going to move next into some questions that focus on RT-PCR machines and in particular software. Dr. Kibenge, I'd like to start with you. As a starting point, what -- let me in fact try and do this through a document. Tab 134 of our list of documents is a paper that you're lead author on. Do you recognize that? Well, the first page is the cover sheet, I suppose. Do you recognize that paper as your own?
DR. KIBENGE: Yes.
MR. MARTLAND: If I might ask this be marked as 2051.
MS. PANCHUK: This was previously marked as --
MR. MARTLAND: Oh, it may be. Thank you. It may well have been --
MS. PANCHUK: -- 2034.

MR. MARTLAND: Thank you.
Q Let me take you to page 7 of this paper. And, I'm sorry, page 7 on the bottom, there's two columns, the bottom right column, last paragraph, so one page up. There's a paragraph that begins "An interesting observation" and I'd like to simply read that out:

> An interesting observation that could easily be overlooked is the effect of the software in the different thermocyclers on the threshold fluorescence, the value that the fluorescence intensity has to exceed in order to register a Ct value.

It goes on to say:

> ...it is apparent that the seven laboratories that used the Stratagene software MXPro...

Skipping ahead:

> ...all reported relatively high Ct values compared to the other participating laboratories for the same samples...

You go on on the next page, then, if we zoom out, flip a page, and look at the continuation of that same paragraph on the left column, about three lines down:

> This indicated to us that a significant factor influencing the Ct values obtained and therefore the diagnostic sensitivity, might be the software used.

Could you describe that concern and confirm that, tell us about that conclusion about the software, the role the software can play?
DR. KIBENGE: Yeah. This, as the paper indicates, this was actually a real surprise to us, because we were running a Ringtest in which we used I think 12 or 13 labs, and each lab had its own equipment, used its own kits and so on, and each equipment had its own software in terms of coming up with the Ct values. And what we found was that there were seven labs which had consistently very high

Ct values, and in some cases they were actually reporting false negatives. And we couldn't understand that, because all the labs had received the same samples.

And one of the labs was actually very, very good lab. So they had to question their practices. And we went back and forth trying to figure out why was this the case. And what we found actually was that all the seven labs that we flagged were using the Stratagene real time machine, which has the software, I think it's MXPro. And we worked it out that actually when you use that software, you end up with very high Cts, in fact, the -- it varied from 3 to about 7 Cts above what we would expect. And normally, our rule of thumb was that a difference of 3 Cts indicates a ten-fold difference in the amount of template in the original -- the original amount of template in the sample, which is significant.

So this was something that was unexpected, but actually it came out as a result of this study that using Stratagene machine with that software, you get very high Cts values, and that would actually reduce the diagnostic sensitivity of your lab. So when we found that out, and we were able to adjust the Ct values, you know, these labs were able to say that they were doing the right thing. But without knowing that, you know, we thought they were actually -- their practices were wrong, because with the samples that had low amounts of virus, they were being classified as false negatives.

Q Now, what software is it that the AVC, your lab in PEI uses?

DR. KIBENGE: We use the LightCycler which has the software, I think it's version 1.50, and that's -- LightCycler is made by Roche, so that's the software we use. But there were other labs that had listed ABI system.

Q Yes.

DR. KIBENGE: And their Cts were within the same line as our machine.

Q The ABI was one that didn't cause concern.

DR. KIBENGE: Exactly, that's the Applied Biosystems, I think.

Q Dr. Nylund, what software do you use, sir?

DR. NYLUND: We are using ABI 7500 and the software

included.
Q Thank you. Dr. Miller?
DR. NYLUND: Actually, we have several different ABI machines, and the results are always reproducible between the different machines.
Q And Dr. Miller, then Ms. Gagné, please, the same question.
DR. MILLER: We have two instruments and we've validated our results on each of them. We have the ABI 7900 with its accompanying software, and the Fluidigm BioMark with its accompanying software.
Q Ms. Gagné.
MS. GAGNE: We have a Stratagene machine with the Stratagene software.
Q These things have been happening awfully quickly in terms of our hearing process, I think in the last day or two is when we first learned of and provided on Dr. Kibenge's paper that raises these concerns about the software. So appreciating that you haven't -- you may not have had much if any opportunity to digest it, but are you able to respond to those concerns around the software?
MS. GAGNE: No, not at the moment.
Q Let me move to the few questions on cell culture results. Dr. Kibenge and Ms. Gagné, were either of you, and I'm looking back obviously to the testing that we've been learning about in the course of the day, was either of you able to culture the virus using a cell culture? Dr. Kibenge, then Ms. Gagné, please.
DR. KIBENGE: Yeah, the samples we received, actually, the 48 hearts that we received, we put them on cell culture. And in our lab we use four different salines for fish viruses. We use the S2 saline, SHK-1, TO and CHSE-214, and these samples were inoculated on all the four salines. And we did the same thing for the other samples that we had picked up as positive in the second submission. And in the first one I think we thought we saw CPE in CHSE. There wasn't any CPE in S2, SHK and TO, but I think we thought we saw CPE in CHSE-214 after 14 to 17 days, but that CPE was not characteristic of virus there, and we quickly ruled it out when we ran conventional RT-PCR and the results we were then getting. So we are certain that the CPE we saw on that saline was

not corresponding to the possible virus in these samples.
Q So ultimately is it the case then you were not able to culture the virus?
DR. KIBENGE: Well, yeah, you could say that. Yes.
Q Jumping to the --
DR. KIBENGE: Yes.
Q -- layperson's conclusions, of course.
DR. KIBENGE: Yeah. Now, I could also add that actually normally to call a sample negative on a virus culture, we usually need to do at least three blind passages and the duration in passage depends on the saline you use. For example, for the S2, SHK-1 and TO, we normally pass it, give it up to ten days, whereas for CHSE, it takes a bit longer to get the CPE, so we're passing after three weeks, 21 days. So in some cases, I think we've gone up to P2 or P3 in any of those salines, but we have not yet done the PCR to confirm that they are truly negative. But I think based on what I know now, I don't think that we are likely to.
Q Now, Ms. Gagné, similarly, were attempts made to culture the virus and what were the results?
MS. GAGNE: Yes. When the samples were submitted as homogenous or tissue provided in some -- like in a frozen state or fresh state, we do attempt culture. And it was -- it was not successful.
Q Is cell culture reliably successful at isolating ISAV?
MS. GAGNE: In our validation work what we have determined is that when Cts are above 30, we don't -- we don't normally -- we're not normally able to detect ISAV by cell culture.
DR. KIBENGE: A comment?
Q Yes.
DR. KIBENGE: Yeah. In our lab, actually, my experience has been that if virus is from a clinically sick fish, for example, Atlantic salmon with ISA, usually you are able to culture that virus. But in the reports I have seen so far, it has been very rarely shown that you can actually culture virus from wild fish. Most of the confirmations of virus infection of wild fish have been based on RT-PCR, and in some cases it's been based on weak positive RT-PCR. So in my view it hasn't been common to culture virus from wild

fish.
Q Are there strains of ISAV that are not culturable?
MS. GAGNE: Yes.
DR. KIBENGE: Yes. Now, the most famous one is what we call the ISAV virus HPR0 that is known to be non-pathogenic or non-virulent. This virus in fish does not cause any clinical disease, and you can only detect it by RT-PCR. But in some cases we have seen samples which are clearly RT-PCR positive, and when you put them in cell culture, we cannot culture them. And this has been even some clinical cases.

I must add that in our experience in Chile it was not very easy to use cell culture as a diagnostic method. In fact, people tried earlier on and most of the cases were always negative. So the principal method in that outbreak was actually real time RT-PCR, it was the most reliable. We could not rely on cell culture.
Q Dr. Miller, moving to a different area, and I'll just simply ask all witnesses, bearing in mind we all operate under real challenges in terms of the time we have available, if you're able to, all of you, do your level best to keep us to the two instead of the five-minute answer, I'm grateful to you if you can do that.

Dr. Miller, we touched -- I think you touched, in your previous testimony on genetic sequencing of products obtained through RT-PCR. I'm wondering if you could help us to understand that and what it indicated to you? In asking that really sort of a general question, I'll have brought up, please, Tab 40, and if it's of assistance to use that document, that would be fine.
DR. MILLER: Ultimately, gaining a genetic sequence is an ultimate validation that what you're picking up by PCR is a real product and it's the product that you're expecting to be picking up. Now, it's possible if you contaminate a PCR to sequence a positive, you know, from a contaminated product. But again, in our case, we did not have ISA virus in our lab; we had no positive control. So if the reasoning is if we're able to pick up a PCR product and we are able to sequence it from wild fish and it sequences as ISA, it is a real ISA product from wild fish. There is no other way for

us to get ISA product sequence in our lab, other than it coming from those wild fish.

So that really was the ultimate validation for us, and we were able to do that with all four primer sets that amplified product in our fish.

Q Maybe within the segments, let's have a look, please, at page 3, Mr. Lunn, followed by page 5. So first on page 3, without trying to decipher those long sequences, but we read the heading there, and if you could tell us what the finding is here?

DR. MILLER: Okay. Basically, this shows an alignment of the sequence of our amplified product, so the smaller sized products there are what we amplified, and they're aligned with known ISAV isolates. In this case, it's an ISA-8 using the Plarre primers, and there was a hundred percent match of that sequence to some known isolates in Europe. But there are, if you look over all ISA isolates, there are mutational sites within that sequence. So there's some variability within the region that's being amplified between various ISA isolates. But what we have picked up did, one hundred percent, match some known isolates for this particular segment of this sequence.

You have to be -- one thing, just to be clear, this is a very small product, so there's only 16 bases between the two primers here, and you won't know if there's minor variation under the primers, because when you sequence you get the primer sequence back. So all you can really say with this is that the 16 bases between those primers absolutely match known isolates and that there's obviously enough consistency underneath the primers to also match.

So this was the first sequence that we came up with, and when I put this sequence forward to our Fish Health Group, it was felt that it was only one fragment and we needed more sequence information to confirm whether this was, in fact, ISA, because no one had really sequenced ISA out of sockeye salmon before. So we went back and sequenced products from other primer sets after we did this, and a week later we had the sequences from three other segments of the virus.

MR. MARTLAND: On page 5 of that document -- I'd forgotten to mark this document, if I could please

do that. I've lost track; I don't know if we're 2052.

MS. PANCHUK: 2051.

EXHIBIT 2051: Presentation to Fish Health Group on status of molecular screening for Orthomyxoviruses performed by the Molecular Genetics Laboratory, November 24, 2011

MR. MARTLAND: I have a note that we may have marked 2051. No? I'm wrong. Thank you.

Q With respect to what's shown at page 5 of this document, if we can flip onto that, please, could you tell us what this describes, ISA-7, here?

DR. MILLER: Yeah. This takes the ISA-7 PCR products that we generated and the sequence and we do what's called "blasting" it, so we send that sequence to a large sequencing database that contains all known isolates, and what we find on the far right-hand corner is that the top hit is 95 percent similar to the sequence that we obtained, so there's a five percent divergence in the sequence that we obtained compared to all known isolates. That's the minimum level of divergence. And that equated to three bases that were fixed differences in the sequences that we saw in sockeye salmon in B.C.

Q Let me go to Tab 138 of our list of documents, please. I'm moving, now, into asking about functional genomics results, Dr. Miller. When we see Tab 138, this has the name Brad Davis and the title's there on the screen. In brief, what is this?

DR. MILLER: Brad Davis is a post-doc in my lab. You know, we're basically, after testifying at the Cohen previously and listening to all of the dialogue on how we actually study disease in wild fish, I came away with that, really, with the feeling that we just didn't know very much about what pathogens wild fish even carry, and there was a general arm-waving that it was really pretty impossible to study disease in wild fish, because we didn't see them die.

And so, you know, I went back and decided to start looking a this a little bit more carefully, and the first thing that I felt was needed was a good characterization of what pathogens actually

exist in wild migrating salmon. And that doesn't tell you what causes disease, necessarily, but it tells you what's there. By doing those in a quantitative way, you can look at how much virus is present, so you can -- if you have very low CT values using quantitative assays you know that there's a high abundance of that pathogen and it's not a low abundance. So that's another way to gauge how important that might be at that particular time in the life cycle of the salmon.

A third way, however, which is a bit of a novel approach, is to go back to our microarray data, which we already have, and say, once we've -- if we characterize the same tissues in the same fish that we've already run and we determine basically the intensity of infection and the presence of different pathogens, we can go back to the microarray data and analyze it and find out what is the genomic signature, what is the host response to carrying that pathogen from our genomic data?

And this is exactly what Brad Davis did for me, using the ISA-7 positives. So we contrasted fish that we'd already run on microarrays, we ran what's called a regression, so we looked for genes that are correlated to the CT values that we see associated with ISA-7, this is the Plarre-7 primer set, and basically what we found was that there was a very strong genomic response to fish that carried this ISAV-7 sequence. And if we did a functional analysis, we looked for what kinds of pathways were being stimulated in that functional response. We found that the very top hit was influenza infection.

So this is an influenza virus, and that really speaks to the fact that these fish are responding in an influenza-like response to this virus.

Q And the document alludes to that influenza-like response. What is the import of that? Is that a response documented previously in fish?

DR. MILLER: No one has actually done the kinds of statistical analysis that we've done. We have very large datasets and we, because of that, we have a lot more ability to use advanced statistics on them. A lot of people who study disease, they look at four fish that have been exposed to a

virus and four controls, and you don't have a lot of statistical support with very small sample sizes. This has over 50 or 60 fish in it, so we have really good statistical support for our data.

The influenza infection, as a pathway, is a curated reactome pathway, and so it's all of the genes that are involved in that pathway have been manually curated is -- I went back and looked at other papers and I can't see anybody who applied this type of statistical approach to their data, so I can't say for sure. And we've only had this data for less than a week, so I haven't had the time to go back, gene by gene, to see how similar our signature is compared to other published studies.

MR. MARTLAND: I don't want to forget to have this marked as an exhibit; I don't think I've done that yet.

MS. PANCHUK: Exhibit 2052.

EXHIBIT 2052: Identification of the ISAV-7 genomic expression profile in the 07/10 44K Liver Microarray data, by Brad Davis, December 7, 2011-12-15

DR. MILLER: Just to conclude, what this approach tells us is that these fish are not respond -- they are responding to the presence of this virus. This doesn't necessarily mean that we've demonstrated that there's disease and mortality, but we have demonstrated that it's not doing nothing. There is some level of damage that is occurring to the host, even at these high CT values that we're seeing in these wild fish.

MR. MARTLAND:

Q Dr. Miller, you've been conducting, as you've described, even in recent days, ongoing tests. Could you tell us about some of those tests? And one of them I'd like to try to do this by having a look at Tab 136 of our list of documents, which I think, in turn, may have two parts to it.

So the first, that's what I'll call 136B - we may mark these as separate documents - but that's 136A, and there's a 136B. This describes, I take it, some recent testing on salmon from the Pacific. Could you tell us about -- first of all, let me just confirm that those documents describe

that testing; is that correct?
DR. MILLER: Yes, they do. This is testing that we performed just last week.
MR. MARTLAND: I don't want to forget to do this, so if I could ask that, Mr. Lunn, I'm at your disposal as to whether we mark -- if it's two documents, that they be marked separately? You're nodding, "Yes," so if 136A could be the next exhibit, please?
MS. PANCHUK: Exhibit 2053.

EXHIBIT 2053: Creative Salmon ISA Test Results

MR. MARTLAND: And 136B, I take it, 2054?
MS. PANCHUK: As marked.

EXHIBIT 2054: Request 8 BCwt ISAV Prevalence in 1980s

MR. MARTLAND: And next, I think there's a covering e-mail that went alongside those -- went along, or was provided at least to us, along with those results. Mr. Lunn, I don't know if you have that e-mail. 136.
Q And again, Dr. Miller, do you recognize that as an e-mail -- the date, I think, must be wrong, January 1/01, but in any event, a recent e-mail, I infer?
DR. MILLER: I don't know why it says January 1/01, but yes, it was just a few days ago.
Q Okay. If that might be marked, then, as 2055, I think?
MS. PANCHUK: As marked.

EXHIBIT 2055: E-mail dated January-01-01, from Kristi Miller-Saunders to Stephen Stephen and Mark Saunders, Subject: more results for Orthomyxo primers, with attachments

MR. MARTLAND:
Q Tell us, in a short way, please, about this testing and --
DR. MILLER: Because of the result that we had with ISA-7 showing the three fixed differences in the variant that we'd been observing in B.C. compared

to all known isolates, one of the things that we wanted to do was say -- ask the question, "How long has this been here?" And I think that's a really, really important question to all of this, all of these issues. We have a large genetic baseline of samples in our lab, because we do stock ID for the Pacific Salmon Commission, and we had liver samples dating back as far as 1986 sitting in our archives, so we went back to our older archived liver samples and extracted RNA from those, and we ran those with these various primers. And we basically, we found that we could amplify PCR products from samples of Fraser River sockeye salmon - these were returning adult salmon - in 1986 and thereafter and that the patterns of PCR that we observed between the different primer sets were very similar to what we had seen now, where we see a lot of positives for ISA-7 and fewer positives for the ISA-8 primer sets.

We have, since then, sequenced from these 1986 samples and found that the three fixed base differences that we see, today, existed in 1986 as well, which suggests that not only has this been here for at least 25 years, but it's been here probably quite considerably longer than that, given that there were already fixed differences that existed in 1986.

Q So are you effectively finding positive ISA PCR test results relating to Fraser sockeye from the '80s?

DR. MILLER: Yes. And actually, there was a subset of pink salmon in this as well, and we did observe them in pink salmon as well.

Q In addition, has there been other testing of other species of Pacific salmon that you've done recently?

DR. MILLER: Yes. We have a project, and I brought this up the last time I testified with Creative Salmon, to look at a jaundice-disease syndrome that they experience over winter mortality in one of their farms on the west coast of Vancouver Island, and they've been really great company to work with and quite willing to work with us and allow us to test for Parvovirus and other things on their fish. It's all Chinook salmon.

And so I went ahead and ran their fish through the battery of different pathogen on the

Fluidigm that we've been employing for our wild fish, and we did identify some positive ISA fish among their fish. I should say these are fish that were sampled in the wintertime last winter. I believe that they were close to market-size fish. And the CT values were very similar to what we see in out-migrating sockeye salmon, as were the prevalence levels of positives.

And so I don't think that there's -- and there's no indication that what we're picking up as ISAV positives has any correlation with their jaundiced syndrome. There's no indication that it's causing disease, necessarily, in those fish, but we basically picked up a similar prevalence level and CT values that we see in wild migrating sockeye.

Q You've described Creative as being quite willing to work with you in this testing, including for Parvovirus. Is that true of other companies?

DR. MILLER: So far, they're the only company who's been willing to provide us samples.

Q Now, if memory serves, when you testified in August you described that there was work underway to engage in testing for Parvovirus among those farming Atlantic salmon in the Pacific. Is there an update that we need to have there?

DR. MILLER: Yes. I had a meeting with the B.C. Salmon Farmers' Association after the aquaculture sessions in the Cohen, and we agreed, in principle, on a tack to take and we were writing a co-proposal for ACRDP, which is a DFO grant, and the very last minute they basically took out all testing of Atlantic salmon in that proposal and they proposed that I, instead, look further back at sockeye salmon and before -- until I had information on how long this virus - this is the Parvovirus - has been here, they did not want their samples to be tested.

Q With respect to - you can tell this isn't my question - can you test fish farm audit samples?

DR. MILLER: So when this occurred, we approached the people in DFO that are in charge of the audit program, and the audit program is now run through DFO, but those samples are still sent to the provincial lab, the same lab that's been doing it for the province. The histology work and the PCR work is all done in the provincial lab. And we

asked, we signed a material transfer agreement with the provincial lab, and that transfer agreement only enabled us to test for Parvovirus and nothing else.

The very unfortunate thing is that we were sent tissue homogenates in water that were not kept frozen and they were sent to us on ice, and anyone who knows anything about molecular biology knows you cannot send tissue samples that are not kept frozen or they degrade very, very rapidly. So by the time they got to our lab, they were quite degraded, and the DNA was of no use. There is RNA, we could use the RNA to test, but we had to sign an agreement to say we would not test for anything but Parvovirus.

So it's useless for Parvovirus, because Parvovirus is a DNA virus, and we needed the DNA and we have completely degraded DNA.

Q With respect to reporting of the results of your testing, if I can frame that rather broadly, what I have in mind is, and I'll try and use a document to frame this, Tab 42 of our list of documents, Dr. Miller. I take it, when you see these in a moment, I think you'll recognize notes that you prepared relating to November 18 and 24 meetings, very recently; is that right?

DR. MILLER: Correct.

MR. MARTLAND: If these could be marked as the next exhibit.

EXHIBIT 2056: Notes from November 18 and 24, 2011, meeting with Miller, et al, re: Briefings on ISA testing results being conducted in the Molecular Genetics Laboratory

MR. MARTLAND:

Q There's a description, here, of some of the discussion at those meetings. Over and above that, what were you told at those meetings?

DR. MILLER: I had two meetings with our Fish Health Group, and the names of the people are listed there, as well as Mark Saunders, who's the division manager. He called the meetings.

These were meetings to let them know what we were doing and what our results were, and on the November 18th meeting it was simply that first

positive sequence that we -- I had identified and the PCR results that we had. The second meeting we had more sequence information. Between the first and second meeting, Kyle Garver had taken 10 of our samples and done some testing as well, so he had some results to report.

At the end of the second meeting, because we had had the second segment of ISA that had been sequenced as positive, it was decided that we should contact Ottawa about this, and so Stephen Stephen in Ottawa was contacted, and there was another person in the NAAHP program, but I didn't get the name of that person, that was on the phone call, and we basically told them the results that we had.

There was an ensuing discussion about whether this was really ISA or simply an Orthomyxovirus of some other sort, and a discussion about how one defines an ISA virus compared to, you know, other Orthomyxoviruses. You know, this is not -- that is not my particular subject area of expertise, although we do have sequences that are at least 95 percent similar to known isolates of ISA. So from a scientific perspective, you know, it looks like ISA, and we don't have other Orthomyxoviruses from fish, that we know of. So anyway, this is an ensuing discussion, but I believe it was decided that if it was the -- by definition of the definition that CFIA uses, that it needs to be both cultured and culturable and it needs to validate with their validated primer set. If it doesn't meet those criteria - and now they can probably speak to that better than I can - then it's not classified as ISA.

Q And appreciating those may be their -- those might be their criteria, but to your mind, had your work achieved the validation, effectively?

DR. MILLER: You know, I mean, we know that this HPR0 is not -- it doesn't appear to be culturable, and it's the one strain that is considered to be a virulent, so if one is going to define ISA as a disease rather than ISA virus, then I'm open to the interpretation that if this is found to be a virulent and if, through the regulatory framework, you know, virulent viruses don't count as being ISA, then that's their call. In terms of being an ISA virus, I would say this is an ISA virus, based

on the information that we have.
Q In the course of the discussion you describe, was any direction given to you or comment made as to whether you should continue or stop with the testing you were doing?
DR. MILLER: I don't think that Stephen Stephen, in Ottawa, was very pleased that we were doing this testing, because we are not the validated lab. You know, we're -- and I tried to explain, you know, we're doing this in a research context, we're looking at a variety of different pathogens, ISA being one of them, and I fully agreed that anything that we get that's positive should be validated in one of their testing labs. But I -- basically, there was the feeling that the labs that are not NAAHP labs should not be looking at disease.
Q Was there anything said to -- that made that clear that you should -- was it -- I'm just wondering what the discouragement was, if it was --
DR. MILLER: There was the general feeling that we shouldn't be looking so closely at disease if we didn't -- if we weren't one of the NAAHP labs and didn't understand the ramifications.
Q Was there any discussion as to whether you should or should not share test results with others?
DR. MILLER: Well, certainly we discussed the need to share results with Nellie Gagné's lab, but it was told to me that the decision on whether or not to share this with CFIA was Stephen Stephen's decision to make, not -- not certainly mine.
Q Was there any comment or discussion around implications for your lab and its work as a result of having been engaging in this testing?
DR. MILLER: One of the issues that had been brought up, and it had been brought up with Fish Health previously and it was brought up again in these discussions, is that if something is classified as being ISA that CFIA will come and basically take all the samples in the lab away, and as a way -- as their way to control for disease spread.
I have a very large genomics program that relies on the very extensive sampling inventory that we have, and I was very concerned that that would be one threat if this was classified as ISA, that I could lose the samples that I rely on for my genomics program.

Q Through the course of this morning, at times we've been into a high level of detail with respect to particular tests and particular work that you've all been doing. I've quite deliberately held off in trying to ask you ultimate opinion or ultimate conclusions kinds of testing, in part in the hopes that we can learn to what extent there may be agreement or disagreement, and also reflecting on the evidence that you've heard from the other panel members about the different testing that's been done, the different results that have been achieved.

So I'd like to ask a fairly general question and I'd like to move through the witnesses, and I'll start, Dr. Nylund, with you, and then ask the others for the general -- for your comments. Do you believe -- the question is this: Do you believe that there is ISAV or a related virus present in Pacific salmon? Dr. Nylund?

DR. NYLUND: To be quite honest, I published a publication saying that the ISA virus could be vertical transmitted to transport of embryos of Atlantic salmon from Europe to Chile. And, of course, the same could happen in British Columbia; you could import the ISA virus to import of embryos from Europe or from eastern Canada to British Columbia. But if you look at the situation in wild Pacific salmon that we've seen so far and the result presented by Miller here, I don't think we have seen evidence of ISA virus in Pacific salmon, so far. No hard evidence.

We have a lot of indications that the virus could be present in Pacific salmon, but there is no hard evidence. And I really would like to discuss the results presented by Miller, because I find them a bit strange, some of the results. So I hope that maybe she could clarify something for me, because it's something I'm wondering about, if I'm allowed to ask her about how the results were obtained?

Q Well, formally, I ask the questions, but I think, why don't you go ahead and identify the concern you have. I'll give her the opportunity to respond to it.

DR. NYLUND: Yes. First of all, I understand that she's done some pre-amplification, and in that case I understand that she's been using the same

primers as the real-time assays. And, of course, this would -- could introduce artificial genome that could match part of the assay. So my question is: Has she been sequencing on the real-time PCR products or has she been running a separate PCR, a separate RT-PCR and a separate PCR with the real-time primers without sequencing the real-time products?

Q Dr. Miller?

DR. MILLER: They are conventional PCRs that we clone, so we take the preamp and then we run a conventional PCR, no probe, and there's never been a probe in any of those assays, and then we size it to make sure that it is the correct size, we clone it, and we sequence it. And we've done this over multiple individuals, multiple years, and multiple species, now.

Q I'd like to --

DR. NYLUND: Yeah, so the segment 7 sequence that you are presenting on all your presentation, that it's numbered 63-56-7, the sequence, and you aligned it with European strains, that's the sequence you obtained?

DR. MILLER: That is the sequence we've obtained. The three fixed differences with known isolates we've seen in every sequence, and then there are single-based mutations that we see in only some individuals.

DR. NYLUND: Yeah, but most or part of that sequence is actually hundred percent identical to the primers and, of course, a part of the primer should actually have been removed from that sequence.

DR. MILLER: That's absolutely correct, but we have four different assays where the intervening sequence between the primers match ISA.

DR. NYLUND: But if you look at the sequence between the primers, there are actually some errors in the sequence, because you are -- you don't have the open reading frame, you have a stock code on that part and there shouldn't be a stock code on that.

DR. MILLER: We did obtain one sequence with a stock code, and that's correct.

Q I'm afraid --

DR. NYLUND: Yeah, and the one you presented here is with a stock code.

DR. MILLER: There's -- there's --

DR. NYLUND: So how can there be a stock code on that

sequence?
DR. MILLER: I don't -- it's not in front of me, but there was one of the clones that we sequenced that had a stock code on.
Q I'm afraid we're going to, in the interests of --
DR. NYLUND: Yeah, and that's the one (indiscernible - overlapping speakers) --
Q I'm sorry to do this, but I do need to ensure that we carry on in our schedule. I'd like to basically try to conclude, in as much as I can, I'd like to ask, now, Dr. Kibenge, Dr. Miller, and Ms. Gagné the question I asked before: Is ISAV or related virus here - let me try and expand that a bit further - if so, can you tie that? Do you have comments on the connection to Fraser sockeye? And what should be done? Dr. Kibenge?
DR. KIBENGE: You know, in my view, based on the information I've had this morning and from the test results I came with beginning in October, I think there's evidence that there are ISA virus sequences in the fish samples from B.C. and some of that information actually ties back to the work that Dr. Molly Kibenge was doing here way back in 2002, 2004, where she had that type of information, but the data was not allowed to go forward because it was considered to be -- because of contamination.

So the information we're getting now seems to actually suggest that probably it wasn't contamination and that probably there are some sequences here that can be picked up when you use the ISA virus primers and probes. I respect the comment by Dr. Nylund that maybe the sequences may not indicate ISA virus here in B.C., and part of that is simply because probably they are very small sequences, you know, in the case of Dr. Miller's -- the results of (indiscernible) nucleotides. But I think the fact that they were obtained without any positive control and when we have blasted the GenBank, which has most of the published ISA virus sequences, I mean, I think that result is credible.

Now, whether it's ISA or ISA virus-like, you know, that depends on probably to need some more work. I know that in the virus classification, you know, ISA is put in the family Orthomyxoviridae. There's one genus ISA virus and

there's one species, ISA -- infectious salmon anaemia virus.

So within that genus, I would expect that there may be ISA virus-like sequences that could be homologous - we've got to get picking up here - so I cannot exclude the fact that the virus that we're detecting here may be within the genus ISA virus. It may be ISA virus sequences or it may be ISA virus-like, but I think the evidence is, to me, it's overwhelming that there's Orthomyxovirus here.

Q Dr. Miller and then Ms. Gagné, please.

DR. MILLER: I wouldn't disagree with that. I mean, I think that I clearly believe that there is a virus here that is very similar to ISA virus in Europe, but we really do need to get a fuller sequence to get more information about how similar it is, given the level of discrepancy between the various different primers that we're using.

So yes, I do think that there is sequence validation that there is an ISA-like virus here. How it gets classified I think will be determined both based on a fuller sequence and also obviously we have not established that it causes disease.

Q Ms. Gagné?

MS. GAGNE: We discovered ISA on the east coast in the late 1990s, and prior to that it was found in Norway. But we found, also, due to the divergence in sequences from the North American -- what we call North American strains and European strains, we found that actually those viruses were probably coming from an original common source that separated physically, geographically, at least a hundred years and had time to evolve separately to create those two big branches if ISA; the North Americans and the Europeans.

And the viruses were there in nature for more than a hundred years naturally. They were there for thousands of years and they have evolved with their host. In this case, I don't know where we are at this point, because we don't have enough information, but it could really be that we're looking at another ISA that was there for a long time. And it's an interesting theory that I would -- I'm keen to see more work done on that.

If it's ISA, there's several things that don't match the picture we have right now with ISA

as it is in Atlantic salmon aquaculture, because we're talking of all below normal level that we detect in carriers, at this stage. We're talking of an unsusceptible species. Atlantic salmon are the susceptible species of diseases. Right now, what we see, there's none reported in Atlantic salmon, in cultured Atlantic salmon.

The immune response provided is interesting. We do work also looking at the immune response of salmon to various strains of ISA, and what we see is that salmon respond and they respond quickly, like in two weeks after the initial -- when you have a naive salmon, never exposed, remember ISA is a bit like the flu. You get the flu for the first time you will respond, your organisms will defend itself. And then, if that fish survives with low strain -- low pathogenic strains of ISA, the response disappears in about two months. Then, the fish is back to normal, but it's still carrier of the virus in some cases. And we have looked at some that were exposed to ISA. Eighteen months later, compared to naïve fish, there was absolutely no difference. So there were carriers, but their organisms were not responding compared to normal fish side-by-side.

MR. MARTLAND: I appreciate for all witnesses there's a lot more to be said. I suppose, luckily or unluckily, there are a lot more lawyers to come. I need to conclude my questions of you. I want to thank you very much.

Mr. Commissioner, I gather we may have some leeway. We've had these longer breaks, not through any fault except that we've had media photographs and things arranged, and we may have some ability to press a little past 12:30, till 12:40, and I'm grateful for that. We are trying to use the time with Dr. Nylund and Dr. Miller, who cannot be here for tomorrow's session.

I have, next, Mr. Taylor, for Canada, 70 minutes. Thank you.

MR. TAYLOR: Thank you. Again, Mr. Commissioner, Mitchell Taylor for Canada. With me Mark East, Geneva Grande-McNeill, and articled student Adam Taylor.

CROSS-EXAMINATION BY MR. TAYLOR:

Q To properly understand the tests that have been done by you, the witnesses, and the other scientists, it is important to have a good understanding of the fundamentals of PCR and the test protocols, and you've already given some evidence in that regard, but I do have some questions that I'm going to call ISA 101, ISA Testing 101, or PCR 101, and I'll come to those in a moment.

I'm also going to ask some questions to round out the evidence that you've given in answer to the Commission Counsel's questions to ensure that he understands with precision what it is that each of you have done and what you found and the strengths and weaknesses of the various tests and methodology that you've used.

So I'm going to, though, begin, Ms. Gagné, I'll start with you and just confirm and pick up on what was said a few moments ago. ISA is an Orthomyxovirus, as I understand it, which is flu-like; all of you, or most of you have testified to that. And am I correct, Ms. Gagné, that ISAV is the only species that's known, so far, in the aquatic world as an Orthomyxovirus?

MS. GAGNE: Yes.

Q And is that -- does anyone disagree with that or have another view?

DR. KIBENGE: That's correct.

Q All right. Now, I'm going to continue with you for a few moments, Ms. Gagné, and just have you explain the function of your laboratory and where it fits within the DFO Department and within the regulatory regime that exists on reportable diseases. I understand that you are the head of the laboratory that you're in; is that correct?

MS. GAGNE: Yes.

Q And again, as Mr. Martland has said, you're going to have to -- unfortunately, those mics don't extend, but I don't want to make you overly uncomfortable, but to the extent you can get close to the mic, it will be helpful.

Can you just explain what the object and purpose of your lab is and where and how it fits within the DFO structure, briefly?

MS. GAGNE: Our lab is part of the Aquatic Animal

Health Unit, and we have two sides, a research component, but the diagnostic component is our main -- that's what we do, mainly. We do diagnostic using molecular assays. We have other labs in the section doing virology, serology, et cetera. We do these molecular assays for shellfish and fish disease, so we have a list of pathogens that are reportable in Canada and we design and validate and run these assays. We have a list of about 25, I would say, assays to run.

Q And is your lab a diagnostic lab?

MS. GAGNE: Yes.

Q And it's a separate lab, is it, that's the research side?

MS. GAGNE: The research is half of my group, basically, that's research-oriented people. The rest is our technicians dedicated to the assays that we run.

Q Is the diagnostic lab and the research lab physically separated or together?

MS. GAGNE: They're not physically separated.

Q And what means do you have in place to avoid any contamination or cross --

MS. GAGNE: The research people, well, there's research on ISA, but there are pathogens in our group, but the research people are using -- they have their bench and their own pipettes and their own regions and projects, but they have to run everything they do under the same ISO 17025 regulations we use in the lab. So they use SOPs, they use the same procedures that if they have to use a piece of equipment they have to follow the procedures as we do under ISO 17025.

Q Are there other labs within DFO that are part of - and I've already forgotten the name that you gave to it, but you'll remind us - the --

MS. GAGNE: Aquatic Animal Health Unit?

Q Yes, thank you. Are there other labs within DFO under that umbrella?

MS. GAGNE: Yes. There's Freshwater Institute in Winnipeg, there's PBS, who has the equivalent section as ours, and we have a biocontainment laboratory in PEI.

Q All right. And more specifically in Pacific Region of DFO, is it Dr. Kyle Garver that's the equivalent to your lab but out here?

MS. GAGNE: The structure is a little different.

There's Kyle Garver for fish diseases and Cathryn Abbott for mollusc diseases.
Q All right. Is it correct that your lab is the DFO lab for ISA?
MS. GAGNE: We're responsible for the development and we're the reference lab for ISA, yes.
Q And is that, I'm presuming here, but is that because ISA has, as you described earlier, been found on the east coast and, therefore, the expertise has been put there?
MS. GAGNE: Good presumption.
Q Thank you. Had you had occasion to test west coast or B.C. water fish for ISA before this fall? I have to mind that you've already talked about 2004, and you may speak to that, but has there been testing done by your lab of B.C. or Pacific salmon before?
MS. GAGNE: Apart from the samples sent in 2004 and this present notification, no.
Q And is there any particular reason why you wouldn't have tested before?
MS. GAGNE: I think there's been surveillance done in the past using cell culture as is traditional for FHPR and this aligns using the PBS are susceptible to ISAs or by -- by this fact they would if there was ISA in cell culture, they would have seen it. But recently, to my knowledge, there is beginning of surveillance that was done by the PBS lab, the Aquatic Animal Health section --
Q All right.
MS. GAGNE: -- the Fish Health --
Q Is testing that you do done on a referral basis?
MS. GAGNE: It used to be surveillance of wild fish, but with the work that started with the NAAHP in 2005 and the ISO implementation, et cetera, so we have kind of moved away, temporarily, from surveillance of wild fish to get the lab up and running up to the ISO standards, which is a big task. But we keep having -- we keep receiving samples from like wild salmons collected for various reasons and we have done regular testing for ISA.
Q All right. You mentioned ISO. In terms of your lab, are you presently certified in any international way?
MS. GAGNE: We're not -- we didn't get the certification. We're working towards this. We

are far, like I would say 80 percent, there.
Q Am I right that that's a multi-year process in order to achieve international certification?
MS. GAGNE: It is a huge, huge endeavour, yeah.
Q What's the significance of international certification and who is whoever international is?
MS. GAGNE: For trade, your import and export, the countries that wants to do trade with you, import and exportation, will require, at some point, that you can provide these type of qualifications which we're testing.
Q All right. And is that because one country wants to know what the host country or the --
MS. GAGNE: It wants to have insurance or assurance of the quality of the test, they want to know what assay. They might prescribe the assay they want you to use. But basically, they want to be sure that they won't import accidentally something they don't want, for example.
Q All right. And which international body is this that you're seeking certification from?
MS. GAGNE: That's a good question. It's questions for our quality assurance officer, basically.
Q I'll ask the next panel, that's fine. Is your methodology that you've described that you were -- used for validating samples to determine if there is a confirmed case of ISA, has that methodology been published?
MS. GAGNE: The end point -- okay, during validation we were at the transitioning stage from end point PCR to real-time PCRs, and for validation we needed to involve external labs to test or reproduce ability of assays, and our external partners may not have been ready to run real-time PCR assays, so we validated both the end point RT-PCR assay as we were using it then, but we had, in the meantime, started to use the real-time version of this assay, so we validated both assays, but the paper published up to now compare mainly the end point RT-PCR that we were using at that time, and our real-time assay is described in one paper that's coming out soon.
Q All right. Does end point, is that another name for "conventional"?
MS. GAGNE: Yes.
Q And does the -- although the published methodology was created for end point PCR testing, does your

move to real-time testing change the applicability of what you've already published?
MS. GAGNE: No, because in some of those papers you will see side-by-side the results from both -- using both techniques. When we were working on the development of the real-time version of that end point PCR we just made sure we were at least as sensitive using both. The real-time assays are nicer to use, for various reasons. So I don't think it changed -- and we have done the phase one validation of these real-time assays, so the characteristics of the assays are well known and their sensitivity and reproducibility, et cetera, are well know, too.
Q All right. I wonder if we could have Commission Tab 52 up on the screen. And I should say, Mr. Lunn, I regret I haven't given you a list of what I might go to, but I can tell you that it will be within either the Commission's books or Canada's books.
What I think is going to come up, Ms. Gagné, is the manual of diagnostic tests for aquatic animals, which is already an exhibit. It's Exhibit 1676, by the way, as well as Tab 52 from the Commission. Do you recognize that --
MS. GAGNE: Yes.
Q -- it's up on the screen now? And just briefly, what is that?
MS. GAGNE: That's the OIE chapter. I cannot see the year of publication. Probably the latest version.
Q Okay. And OIE is the World Organization, is it?
MS. GAGNE: Organization of International Epizootics.
Q All right.
MS. GAGNE: Yes.
Q And does your methodology meet what's required in that manual of diagnostic testing?
MS. GAGNE: The description there is not complete. Like you get partially what you should do if you want to run the assay, but there's no typical -- there's no detailed description of assays. They will recommend -- they will refer to some papers and you can read the papers, but -- and we use an assay that looks a bit like the Snow 2006 paper. We use chemistry and technology that's standard like that.
Q Maybe I can rephrase the question along the lines of: Is the methodology you use consistent with

the guidelines that are set out in this?
MS. GAGNE: I would say yes.
Q Dr. Kibenge, do you have a -- are you familiar with the methodology that Ms. Gagné's lab uses? I think you are.
DR. KIBENGE: No, actually, I'm not.
Q Okay. All right. Dr. Nylund, I'm going to presume that you're not familiar, but you may correct me. Do you know the methodologies that are used by Ms. Gagné's lab?
DR. NYLUND: No.
Q Okay. The tests that you do, Ms. Gagné, you've given some evidence on this, and as I understand it, they're designed to detect ISA known strains and will pick up some other strains potentially, but not every strain. Do I have that right or, if not, can you correct things?
MS. GAGNE: It detects known strain, and if we're not detecting some strains, well, we don't know, that's the problem. But at the moment, we selected Segment 8 for a reason. Like Dr. Nylund said, Segments 7 and 8 are well expressed, like during replication of the virus there's a level of those segments in the tissue. But the other reason to choose Segment 8 by many people who design assays is that it's well conserved. You have good regions where you have very well conservations of the sequences amongst the various strains of the virus. So that's the reason we selected that.
Q All right. Just picking up, though, on one aspect of this, and that is whether it's going to pick up necessarily every unknown strain. Will it, or can it miss things?
MS. GAGNE: Only the future, I think, will prove that.
Q All right. It's the sort of what you don't know you don't know?
MS. GAGNE: Yes, mm-hmm.
Q Now, I'm going to ask this question of each of the panellists. I'll start with you, Ms. Gagné, and this is picking up on one of the last questions that Mr. Martland was asking you about what do you make of all of this and what might we have that's coming onto people's plate, and you've each given some answers in evidence on that already. But with these positive indicators and positive results that have been found by scientists,

including some of the panellists, is it assured that it must be something in the Orthomyxovirus gene, if that's the right word, or could it be something else?

MS. GAGNE: You mean if there's something in the current suspicious ISA findings --

Q Yeah.

MS. GAGNE: -- that the sequences --

Q Is it necessarily Orthomyxovirus, or could it be another?

MS. GAGNE: You need the sequences for that, and especially sequences in regions where you have less conservation. We're working, still, in sequences that are relatively well conserved, but still we see, apparently, some differences, so the level of conservation between all the segments should be looked at before we can conclude if we're looking at a different virus in the ISA virus general or outside.

Q All right. I think I'm hearing you say that it's an open question whether it's --

MS. GAGNE: Yes.

Q -- necessarily Orthomyxo or something else; is that right?

MS. GAGNE: I'm not ready with the information now, and I don't think anyone, with the information we have now, is able to answer this at the moment.

Q All right. I'll go to you next, Dr. Nylund. With the results that are being shown, do you have a view on whether it's necessarily an Orthomyxovirus or could it be something else, or what?

DR. NYLUND: Well, if you're using an ordinary real-time PCR, I would say that the chances for picking up something else is very, very small. So I would say that an ordinary real-time PCR would be picking up ISA virus, but only the known ISA virus. So the chances of getting a false negative is larger than getting a false positive.

But if you're using pre-amplification, like Dr. Miller has done, then you may increase the chances for getting arbitrary RNA or DNA instead of specific ISA virus --

Q All right.

DR. NYLUND: -- RNA.

Q Dr. Kibenge, do you have a view on this?

DR. KIBENGE: Yeah, my thinking here is actually the information that we just seen this morning, the

genetic sequence, as I mentioned, from Dr. Miller, it may be small, but this was amplified without any positive control so that the risk of contamination is zero. And when you burst that sequence and pull it out from the GenBank where people deposit these sequences from all over the world, and you come out with that type of homology, I think that's a signature that cannot be ignored.

To say it's Orthomyxovirus, you need to isolate the virus and look at its structure in terms of electron microscopy, because the characterization of a virus, Orthomyxovirus, is not only on the sequence, it includes the envelope and all those other things. But basically what we have now, I think that whatever virus is here would be either Orthomyxovirus or Orthomyxovirus-like. It's unusual to get sequence and pass it to the GenBank, you know, and pull up that type of homology; 7 to 1 basis is small, but in my view it is significant. What we need, now, is either to get more sequence and be able to conclusively classify this virus, but based on what I know, I think that there's a strong possibility that it's (indiscernible - voice drops).

Q All right. And I'll come to you in a few minutes on this very point, Dr. Miller, Just continuing with you, Dr. Kibenge, and what you were saying, you just said something of what should be done, you should do some more sequencing, or someone should, and it does seem to me, based on the evidence that all of you are giving, that while you differ in detail, all of you are, I think, of one mind that -- or the same mind that there's something that needs to be looked into. If anyone disagrees with that summing up, you can say at some point in your evidence.

But with that, proceeding on that basis, Dr. Kibenge, do you have some more specific suggestions, what now? You say more sequencing, and presumably the general answer is more work to do, but do you have anything specific that you would be suggesting?

DR. KIBENGE: Well, in my view, the ideal situation would be to find some very good samples with a very high virus datas and try to isolate this virus. If you can isolate a virus, you have a

very clear picture of its structure, the electron microscopy will tell you in a heartbeat whether it's actually Orthomyxovirus. And with that virus isolated, you should be able to sequence all the eight genome segments and compare them to the eight genome segments of the known ISA virus right now and even put, actually, a timeline as to when it divide, if there are two different species of this virus.

Q All right.

DR. KIBENGE: But I'd also add that right now there's technology that can give you that sequence without virus isolation, and this has already been proven in Norway, where they were able to produce some -- to identify a virus that was causing disease that has been known for long time but which didn't have a name. You know, this is called DNA sequencing, or second generation sequencing.

That can be done without (indiscernible) isolation, but it can generate enough sequence information to give us a complete answer to this virus.

Q All right. I'll turn to you, Dr. Miller, and the question is to whether you have a view on if the virus, whatever it is that might be being indicated, is necessarily Orthomyxo or might it be something else, or what?

DR. MILLER: Well, I mean, we have three ISA virus experts here. I think that they are the ones who should be answering that question. I would totally agree that a fuller genome sequence of multiple segments would be definitely helpful in terms of making that determination.

I think one thing that -- there's a couple things that aren't very clear, that haven't really been brought out here, but we're doing these analyses largely on wild fish, and all of the samples -- most of the samples that we've been working with in our lab and that we're -- the Rivers Inlet samples that were provided both to Dr. Kibenge and Nylund, these are samples of smolts coming out from freshwater into the marine environment and very early marine environment samples, and if you look at the literature, and again, these -- my colleagues here would be able to speak more about ISA than I, but ISA outbreaks of disease on farms don't usually occur until

those salmon have been in the ocean for a longer period of time. I've read a few papers that have suggested, you know, eight months in the ocean is when they start seeing evidence of disease.

The samples that we're talking about looking at wild fish, those fish have been in the ocean no more than three months. So we're not talking at about a time point of sampling in the ocean when we would expect to see, at least if you looked at Atlantic salmon, large amounts of -- large copy numbers of viruses and evidence of disease.

So I think that's just one context to put here. We're not sampling dying fish when we're looking at wild fish, we're looking at young fish and fish that have only been in the marine environment for a short period of time. And so expecting to find samples with very low CT values and evidence of disease, even if you had a virulent strain, you may not find that at this early stage of ocean entry.

So weird question, sorry, I just wanted to get that out there. I would -- I think once we have more sequence information we can more adequately classify exactly what these sequences belong to. But certainly all indications are, so far, consistent with it being ISA-like.

Q Are you suggesting, in what you were just saying, that there should be some testing of older fish?

DR. MILLER: I definitely believe that there should be, yes.

Q They're sometimes hard to find, of course, but --

DR. MILLER: And testing of aquaculture fish as well.

Q All right. Now, the regime for reportable diseases in the aquatic sphere is relatively new, and I expect all of the panel members are aware of that. As I understand it, the reportable aspect came into play in the context of aquatic approximately a year ago.

Dr. Kibenge, did you receive notice at some point in the last year of a change in regulatory regime where the reporting of reportable diseases, of which ISA is one, had some changes made and you had to report in, or anyone, that is, who is finding a suspect case had to report into the Canadian Food Inspection Agency? I can see the microphones are a challenge.

DR. KIBENGE: I remember seeing an e-mail to that

effect --
Q All right.
DR. KIBENGE: -- I think sometime in January of this year.
Q All right. And was a similar notification put through or distributed within DFO, Ms. Gagné and Dr. Miller?
MS. GAGNE: Yes.
Q Dr. Miller?
DR. MILLER: I don't actually know if what I presented was formally notified with the CFIA. I know that I had a conversation with the CFIA twice now, but I don't know if anything formally was put in. I'm not privy to that information.
Q No, what I mean is, back about a year ago, and Dr. Kibenge says January, was there distribution made throughout DFO that there's a new regime in place and reportable diseases have to be reported?
DR. MILLER: Okay, I'm sorry, I misunderstood your question. I believe there was, but it wasn't sent to me.
Q All right. I'm going to, in sequence, call up Canada's Tabs 2, 3, and 11, if I may. I lost you, Mr. Lunn. And we'll start with 2. And each of these is an introductory document on PCR. And I'm just going to ask Ms. Gagné if you're familiar with what I think is going to come up here.
MS. GAGNE: I've seen it in the list of documents disclosed. I wasn't sure if it was in the OIE chapter or not.
Q All right.
MS. GAGNE: It's taken out of its original document, so...
Q Okay.
MS. GAGNE: But this is, I think, general information, yes.
Q All right. Have you had a chance to look at that, and can you say if it's an accurate account of this?
MS. GAGNE: Oh, I'm sorry, I didn't read through that document. I didn't have time.
Q All right. Well, I'm going to ask that it be marked as an exhibit, unless there's any objection. And I'll try to make a note of the numbers as we're going. I hear no objection.
MS. PANCHUK: Exhibit 2057.
MR. TAYLOR: Pardon me?

MS. PANCHUK: Exhibit 2057.

EXHIBIT 2057: Appendix 1.1.4.3 Nucleic Acid Detection Assays, February 3, 2011, Development and Optimisation of Nucleic Acid Detection (AND) tests

MR. TAYLOR: Thank you. And Tab 3.
Q I understand this is something that was taken off the web. Can we get a bit more information up on the screen? And do you recognize this? Yeah, that should help. Have you seen that before, Ms. Gagné and do -- have you had a chance to look at that and formulate a view whether it's an introductory statement on PCR describing the principles and what it is and so forth?
MS. GAGNE: I went through it quickly, and there is several of those beginner's guide on the web, and this is one of the ones you can look at, and it was clear and concise, so I did recommend that it could be used for someone who doesn't have any background --
Q Okay.
MS. GAGNE: -- in this very technical issue.
Q And as well as clear and concise, do you find it accurate?
MS. GAGNE: Oh yeah. Yeah.
MR. TAYLOR: All right. Could that be the next exhibit, please, 2058?
MS. PANCHUK: So marked.

EXHIBIT 2058: Beginner's Guide to Real-time PCR, by Primerdesign

MR. TAYLOR: And next, Tab 11.
Q Do you recognize that, Ms. Gagné?
MS. GAGNE: I've seen that in the document disclosure, but the source of it, I don't know where it came from.
MR. TAYLOR: All right. Well, again, I'll ask that it be marked as an exhibit, unless there's an objection, as being an introductory document on PCR.
MS. PANCHUK: Exhibit 2059.

EXHIBIT 2059: Draft Document: Interpretation of Infectious Salmon Anaemia (ISA) Positive

Results Obtained Using Real-Time PCR

MR. TAYLOR: Thank you.
Q Now, Dr. Kibenge, your lab is known as the Atlantic Veterinary College, I understand; is that right?
DR. KIBENGE: Yes, my lab (inaudible - microphone off).
MR. TAYLOR: Just as a reminder, I guess where we're at right now, each witness has to, themselves, start the mic, do they? Okay. I think there's a repeated problem with Dr. Kibenge's mic, which is now on.
DR. KIBENGE: Yes, I was saying that my lab is located at the Atlantic Veterinary College.
Q But there's more to the Atlantic Veterinary College than your lab, of course, right?
DR. KIBENGE: Yes.
Q Yeah. And that's part, or within the University of Prince Edward Island, in Charlottetown --
DR. KIBENGE: Yes.
Q -- I understand?
DR. KIBENGE: Yes.
Q Is your lab primarily a research lab?
DR. KIBENGE: Yeah, you can say that. It's a -- I would say that my function, probably, as a faculty member, has a big component of research activity and, therefore, that reflects on my lab, yes.
Q And you, yourself, are a reference scientist for the OIE with regard to ISA; is that right?
DR. KIBENGE: That's correct.
Q And a reference scientist, that's not an accreditation, as I understand it; am I right on that?
DR. KIBENGE: Well, it's not an accreditation, as such. I don't think it's an accrediting body, but it's a designation that is accorded to the lab based on the experts in the lab. So in my case, my lab is called an OIE reference lab for ISA, infectious salmon anaemia virus, and I'm the OIE expert for ISA.
Q And does that mean that people or organizations in other parts of the world outside of Canada refer to you samples for testing for ISA and other things as you describe?
DR. KIBENGE: Essentially, yeah, that's right. Right now there are actually only two OIE reference labs for ISA virus in the world; there's my lab on this

side of the Atlantic, and there's another lab in Norway that would cater to the European and Asian regions.

Q All right. And the testing that you did that has brought you here, today, that was done based on a referral to you, was it?

DR. KIBENGE: It was done based on a submission from someone to my lab, yes.

Q Were you retained for a fee to do that?

DR. KIBENGE: Well, actually, in fact, the testing we do in my lab we test is a service for a fee, yes. So we bill out the people submitting the samples --

Q All right.

DR. KIBENGE: -- for the costs of that test.

Q And who was it that retained you?

DR. KIBENGE: I don't know whether I can describe it as being retained, but samples we (indiscernible - overlapping speakers) --

Q Who asked you to do it for a fee, then?

DR. KIBENGE: The samples were received from a graduate student of Dr. Richard Routledge out in Simon Fraser University.

Q All right. So was it the case that Simon Fraser retained you, or got you to do the work?

DR. KIBENGE: Well, actually, there was an e-mail exchange with the student asking if we could test her samples to rule out ISA virus, and I suppose she came to us based on what she was able to find, that our lab could do it.

Q All right.

DR. KIBENGE: Yeah. And she submitted the samples and we tested them.

Q Okay. Dr. Nylund, is your lab a research lab or diagnostic, or both?

DR. NYLUND: We're only a pure research lab.

Q All right. And your lab is attached to a university in Norway, as I understand it; is that correct?

DR. NYLUND: Yes; University of Bergen.

Q All right. Dr. Nylund, I'd like to ask you about techniques for avoiding cross-contamination or other problems. Can you briefly explain or tell the Commissioner how a lab should go about preventing contamination occurring? What should be done?

DR. NYLUND: Well, the major source of contamination

are usually PCR products and plasmates; that is, multiplication of the genome in bacteria. So what you would like to avoid are all kind of PCR products and plasmates or vectors or bacteria that have been multiplying the target gene. And, of course, if you have a very highly infected fish, that could also be a source of contamination.

So what we have done in our lab is that all samples from fish are taken in a separate location in the building, far from the other work, which means that we can't contaminate from that room to the room where we are working with the screening.

Then we have a separate clean area, where we do the RNA and DNA extraction, and in the same area we have a separate room for the master mixes that we use for making the PCR and real-time PCR and so on.

And then we have a third clean room for adding template to the reaction; that is, adding the RNA that they extracted in a separate clean room to the master mixes that are made in another room.

And then we have a third area away from this area that we call "Contaminated Area". That's where we keep the PCR machines. That's where we are working with PCR products. That's where we are doing cloning, and so on. Very far from the area where we do an extraction.

And all this is, of course, designed to prevent contamination.

Q All right.

DR. NYLUND: So you have to be very careful with how you design the lab to avoid contamination, and you have to know the major sources for contamination.

Q Now, Dr. Gagné (sic), do you have -- or, sorry, Ms. Gagné, do you have anything that you want to add to that?

MS. GAGNE: We do have an extensive set of measures to prevent contamination, including the use of controls that are distinguishable from the real ISA. We create inserts artificially with an insert in them, so they can be distinguished.

I would add that we have done extensive, also, environmental testing of the persistence of DNA and the environment, and you would be surprised of the -- like autoclave, flaming your tools, these are not measures that are sufficient

to destroy DNA. We have done several testings where we take an infected tissue, cut it with your scalpel blade, flame that blade, test the blade after, and it's still positive for that material. So you have to be extremely careful. And even products that are sold sometimes and claim to be destroying traces like Viralex and other products like that, were not efficient enough.

The only product that really works to destroy DNA is bleach, and that's what we use on every surface we can.

Q All right. So at the end of the day, bleach solves is, you're saying,, or bleach is what you use to try to solve it as best you can?

MS. GAGNE: We have an extensive set of tests done that are -- and I would say that it was, myself, surprise of the resistance of DNA. We know that you can find something that's been frozen and there for thousands of years and they can get DNA out of that, so DNA's really resistant. You can go to the scene after a fire and extract DNA from incarcerated material, so DNA is extremely resistant, and in our hands the only thing that really works efficiently is bleach. So you have to bleach things, you have to clean your pipettes, you use separate sets of pipettes. So there are really, in our hands, there are really several types of measures you can take, but you have to take them systematically to control your contaminations.

Q A big part of what I heard Dr. Nylund speak about, when I asked him the question, and the answer was "physical separation". Do you have physical separation of material?

MS. GAGNE: We have separate rooms and we have areas in rooms designated, and we have rules that stuff that goes in a room never comes back to the other room, et cetera. Even lab coats, gloves, like we have extensive set of procedures to avoid cross-contamination.

Q All right. Now, Dr. Miller has, Ms. Gagné, explained that she tested and found the results she did, and then they went to you and you did some more testing. Did you, at some point, deliver your assays or primers and probes to Dr. Miller?

MS. GAGNE: Yes, but only, I think it's last week, or

probably last week.
Q All right. And then, did you receive them last week, Dr. Miller, or your lab did?
DR. MILLER: She sent me the sequence for the primers and probes that she uses last week.
Q All right. So that's after you did much of the testing that we're talking about here?
DR. MILLER: That's correct. We didn't have time to order the probe to do any testing with her probe. We did do some testing with her primers, and we were unable to get product using our approach with her primers alone.
Q All right. Now, earlier, in answer to Mr. Martland's questions, Ms. Gagné spoke about ISA being determined to have come to the east coast in the order of a hundred years ago. Firstly, is that a commonly accepted fact in the science community? I'll go to you first, Dr. Kibenge, that ISA -- well, I shouldn't use the word "came". ISA, on the east coast, diverged from any European form about a hundred years ago?
DR. KIBENGE: Yes. There's literature to that effect. It may even be more than a hundred years.
Q Okay. And is that your understanding, Dr. Nylund?
DR. NYLUND: Well, to do that kind of dating you need a molecular clock and, of course, looking at the genome of the ISA virus, you don't have a molecular clock.
Q Right.
DR. NYLUND: But in a way you have a relaxed molecular clocks, and judging from that, it will be more than a hundred years that they separated.
Q Okay.
DR. NYLUND: But we don't know if they came to Canada or they came from Canada to Europe, but we know that the European ISA virus and the north Canadian ISA virus separated more than a hundred years ago, according to the relaxed molecular clock.
Q Now, Dr. Miller, you gave evidence that whatever it is that's been detected you think it's been here for quite a long time, and I think you said, "At least 25 years, and maybe more than that." Is there anything more you want to say on that, first, before I go to the other panellists about that?
DR. MILLER: Well, I mean, it's clear that what we're detecting is present in 1986, which gives it 25

years that, at least, that it's been here, and given that those samples in 1986 show the same level of divergence that -- for ISA-7 that we see today, would suggest that it's been here longer than that.

Q All right. Dr. Kibenge, and I see you're wisely keeping your mic on at all times, which is probably a good practice with that particular mic, do you have a view on how long whatever might be being found or seen would have been around?

DR. KIBENGE: You mean in terms of the work that --

Q In terms of the -- B.C.

DR. KIBENGE: Well, that's the only evidence to go on. I think the view here was that she has archival samples that go back to 1986, and just finding these sequences in those samples is enough evidence to say that the virus has been here since then.

Q All right. Ms. Gagné, did you have anything you wanted to add on that?

MS. GAGNE: No, I think it's to be --

Q Okay.

MS. GAGNE: -- to be elucidated eventually.

Q All right. Dr. Miller, does the recent findings that you have seen in the tests you've been doing and/or findings or results that you're seeing other scientists speaking of, does that inform the genomic signature research that you're doing right now?

DR. MILLER: We do not see a correlation in the positives that we're seeing with ISA with our genomic signature.

Q All right. So this is just two separate things going on, in terms of the work you're doing?

DR. MILLER: It doesn't appear to be related.

Q On the genomic signature work, have you recently received funding for that work?

DR. MILLER: Yes, we received DFO Genomic Research Development Initiative funding for three years.

Q And what range of money has been given through that?

DR. MILLER: It's, I believe it's about $450,000 over three years, which is about 150,000 a year, which is a little bit more than half of what we had before.

Q All right. And what program did you say?

DR. MILLER: Genomic Research and Development

Initiative, GRDI.
Q And that's a DFO fund, is it?
DR. MILLER: It is a DFO fund.
Q And that's a recent notification that you've been given of that, is it?
DR. MILLER: Last week from Stephen Stephen.
Q All right. Ms. Gagné, are you familiar with that same fund?
MS. GAGNE: Yes, I am.
Q Do you receive money -- does your lab receive money through that fund?
MS. GAGNE: We have in the past, and we have also, in the same round of proposal, a project on HPR0, ISHPR0.
Q All right. And what range of money and for what have you received for the going forward period of time?
MS. GAGNE: I know it's less than Dr. Miller, but I don't remember the amount.
Q All right. What's it for?
MS. GAGNE: We will look at -- there's a -- HPR0 is a hard virus to work with, so, first of all, we will try to determine if it can be -- if we can have challenges going on with positive tissue for HPR0. But we want to demonstrate that fish, they have the same resistance we see with low pathogenic forms of ISA to other forms of ISA, meaning the fish has cross-resistance to any other forms of ISA after being first exposed to HPR0.
Q All right.
MS. GAGNE: Because we have done similar work using low pathogenic forms of ISA and have shown that fish have a resistance, like they develop immunity against any other form of ISA -- not any other form, I cannot say that, but against violent forms of ISA, after being exposed.
MR. TAYLOR: All right. I'd like to go to four tabs in sequence, and I may be told that they've been marked as an exhibit, but I can't be certain that they are. Tab 19 in Canada's book, and then 20, 21 and 22. I believe these, Dr. Miller, are your test results. And this is the first one. Is this Tab 19, Mr. Lunn? And I know Mr. Lunn can always tell me these things. Have these been marked as an exhibit?
MR. LUNN: Unless they're duplicated on the Commission's tabs, I don't believe so.

MR. TAYLOR: All right. We'll proceed on that basis for now.
Q Dr. Miller, are these some of the test results that you were generating recently?
DR. MILLER: This appears to be the test results for gill tissue in sockeye salmon smolts.
Q And is that the 48 -- which batch is this?
DR. MILLER: This would be 96 samples, so one plate worth of samples with multiple different primer sets, with all of the five primer sets we've been using --
Q All right.
DR. MILLER: -- run in duplicate.
Q All right. Could that be the next exhibit, please.
MS. PANCHUK: Exhibit 2060.

EXHIBIT 2060: Test results of 96 samples with all five primer sets, by Dr. Miller

MR. TAYLOR: 2060, thank you. Mr. Commissioner, I see the hour. I heard Mr. Martland saying we may go later. I'm in your hands. Keep going? All right.
MR. MARTLAND: Mr. Commissioner, as you appreciate, we're doing what we can to cover all the ground in the time we have. If we're able to sit till 12:40, we really would appreciate that extra bit of time, thank you.
MR. TAYLOR: I don't think Mr. Martland is thinking I'm finishing then, but I have 70 minutes and I think that will take me to the 55-minute mark.
MR. MARTLAND: Sounds right. Hoping, not thinking.
MR. TAYLOR: All right. Tab 20.
Q Are these more of the results that you obtained, Dr. Miller?
DR. MILLER: Yes, these are results that come from the 7900, so it's a different -- it's a different system.
MR. TAYLOR: Okay. Could this be the next exhibit, please.
MS. PANCHUK: Exhibit 2061.

EXHIBIT 2061: Test results from the 7900, by Dr. Miller

MR. TAYLOR: Now, could we have all of it there at

once? I'm just going to focus on the graph that's over on the right side. Others may focus on that quite colourful quadrant in the upper left. But you'll see that the graph has a flat line and then it goes up.

Q What's the vertical column and what's the horizontal column showing, Dr. Miller?

DR. MILLER: It's basically showing at what cycle number, or CT, you're beginning to see a fluorescent signal, and there's two different groups in this. These, the ones labelled in purple, were samples that were pre -- that underwent our pre-amplification step, and the ones in, I would say, teal or green were the same samples that were run without a pre-amplification step on the 7900, and you can see that there is a consistently lower CT with the pre-amplification step than there is without it.

This, you know, we didn't make this pre-amplification step up, by the way; this is something that was developed for use in the Fluidigm system, but we thought we would try, since we can't get these same sorts of plots in this way from the Fluidigm, we thought we would try this test on the 7900.

Q Okay.

DR. MILLER: So you can see that your sensitivity to detect positives is at -- at a lower cycle number is greater with the pre-amplification, which is not generally very surprising.

Q Okay. And I'll barge ahead with my next question, and you correct me if I'm getting off on a wrong track here, but in that graph, if it had gone vertical, closer to the left column, would that show a greater strength of positive?

DR. MILLER: If it had -- if the curve had started --

Q Earlier?

DR. MILLER: -- more to the left --

Q Yeah.

DR. MILLER: -- it would show that you were starting to see product at a lower cycle threshold. So the --

Q And that would mean what?

DR. MILLER: That would mean that there's more product.

Q Right. And more product meaning more virus?

DR. MILLER: More virus.

Q All right. And Tab 21. Sorry, did I mark Tab 20 as an exhibit? Thank you. 21, is this more of

your results?
DR. MILLER: Yes.
Q What exactly is this telling us, in very brief?
DR. MILLER: This is the sequence data from our -- the Christiansen primer probe set for ISA-8, which we've called ISA 2010, and we -- there were actually -- it turns out that in 2003/2004 we had actually performed some of the sequencing for Molly Kibenge. In our lab we do most of the sequencing for the Fish Health Group. And we had -- we found these sequences on our computer, and we are not sure exactly where -- what she was sequencing, but we aligned them with the sequences that we've been obtaining, and they're shown here. Unfortunately, this is in colour, and it would have all shown up, which ones are hers and which ones are ours, if it had still been in colour, but it's not, so her sequences are starting on row 4. So row 4, 5, 6, 7, 8, those are all her sequences; ours are -- you see the whole product for the 2010 primer set below.
MR. MARTLAND: I wonder if I can assist. Tab 139, I think, will be the colour -- of Commission Counsel's list of documents ought to be the colour version of this, we hope.
MR. TAYLOR: All right. Can we bring up 139?
DR. MILLER: As we're speaking, I can tell you a couple things about this. Yes, this is much better. So ours are highlighted in blue. So the middle blue section are what's obtained -- I'm sorry, those are the Snow8 ISA-8 primers, and the ones on the bottom, the smaller, shorter sequences are the ISA 2010 sequences. I had them backwards. So the 2010 is a much smaller product size, and the ones above the blue ones are Molly Kibenge's sequences. And the bases that are highlighted in yellow are places where she saw a fixed base and we did not see that. And so there were four fixed differences between the sequences that were on our computer from her, compared to the sequences that we are -- that we have been sequencing in our lab currently.
MR. TAYLOR: All right, thank you. And if we could, then, we'll mark Tab 139 in the Commission binder, as the next exhibit.
MS. PANCHUK: Exhibit 2062.

EXHIBIT 2062: ISA Snow8 and ISA-8 2010 Sequences

MR. TAYLOR: Finally, in this little group, Tab 22 back in Canada's binder.
Q This is more of your results, is it, Dr. Miller?
DR. MILLER: Yes, this is livers from sockeye salmon smolts.
Q What does this tell us, in brief?
DR. MILLER: Well, again, it's the same sets of primers that we used, and in here we ran those on two different instruments, so we're -- no, actually, this, I believe, was a rerun on the Fluidigm system. I'm sorry. So we ran -- we basically ran one of the plates that we had run previously, a second time, and these are the results of a second analysis of all the same samples.
MR. TAYLOR: All right. In the couple of minutes before we, I think, are going to break for lunch, Mr. Lunn, could we bring up, in this order, Canada Tab 24 and Canada Tab 23. Oh, and as we're going to that, I'm reminded that I didn't mark what's on the screen right now, which is Canada Tab 22, if that could be the next exhibit, please.
MS. PANCHUK: Exhibit 2063.

EXHIBIT 2063: Test result of second analysis, by Dr. Miller

MR. TAYLOR: Is this 24? Could I see 23, then? Yes, thanks. Is it feasible to put 24 on the left and 23 on the right?
Q And my question, Dr. Miller, when it comes up, is whether you can identify these two documents as a memo and a statement of survey goals that DFO Pacific is seen in a potential research plan to pursue work following on the results that we're now seeing from the various testing?
DR. MILLER: I did receive this e-mail, but I was not involved in the development of this proposal. I was not involved in any conversations in regards to this proposal, but it was sent to me at the date of this e-mail.
MR. TAYLOR: All right. Well, maybe we could just mark those two documents as the next two exhibits, then. If document 24, which is the left side, the memo dated December 8, 2011, could be the next

exhibit, and then the Survey Goals document, is what it's entitled, which is Tab 23, be the following exhibit.

MS. PANCHUK: Tab 24, Exhibit 2064; Tab 23, Exhibit 2065.

EXHIBIT 2064: E-mail dated December 8, 2011, from Mark Saunders to Kristi Miller-Saunders et al, Subject: Research and Monitoring Plan related to ISA

EXHIBIT 2065: DFO Pacific Region ISAV, IHNV and IPNV Survey Goals

MR. TAYLOR: I see we're at the 12:40 mark, Mr. Commissioner.

THE COMMISSIONER: Thank you, Mr. Taylor.

MR. TAYLOR: Just for my own benefit, at least, if Mr. Lunn is able to say when we're actually returning when we -- he announces the adjournment?

MR. LUNN: At 1:30.

MR. TAYLOR: Thank you.

MS. PANCHUK: The hearing will now adjourn until 1:30 p.m. Please remain standing in place while the Commissioner exits the room. Thank you.

(PROCEEDINGS ADJOURNED FOR NOON RECESS)
(PROCEEDINGS RECONVENED)

MS. PANCHUK: The hearings will now resume.

MR. TAYLOR: Thank you, Mr. Commissioner. I have 15 minutes remaining, I'm told.

CROSS-EXAMINATION BY MR. TAYLOR, continuing:

Q First, Tab 31 in Canada's book, if that could come up on the screen? I'll ask you a question about this, Dr. Miller. Do you recognize this as the test results that Dr. Garver did when he took the samples you gave him and did some testing?

DR. MILLER: Yes, I do.

Q All right.

MR. TAYLOR: Could that be the next exhibit, please?

MR. MARTLAND: I think it already is. We'll just look to correlate it to the right number.

MR. TAYLOR: Okay. Well, we'll proceed on that basis for the moment.

MR. MARTLAND: 2043 is our note of the exhibit.
MR. TAYLOR: All right. Perfect, thank you.
Q Now, I'm going to do this very quickly, and I have limited time available remaining, of course. As I understand it, you gave him 10 blind samples, and he then took those sample -- but you told him that five were positive and five were negative, and you -- and he then took those samples and used an assay similar to what you had and also used up Nellie Gagné's assay and did tests and obtained the results that you see in this exhibit; is that what happened?
DR. MILLER: He used Nellie Gagné's assay.
Q Not yours at all?
DR. MILLER: No, he used mine, but not Ms. Kibenge's.
Q Oh, I'm sorry. Yes, thank you. So with that one correction, what I said is right?
DR. MILLER: That's correct.
Q Thank you.
MR. TAYLOR: Now, could we have Exhibit 2027, please, which is Commission's Tab 26. I apologize, Mr. Lunn, having given you a list, I've now taken you off the list. And I'd like to go to page 109 of that document, which is the second-last page.
Q And if you look under conclusions, you'll see there it says, towards the third down, "Conclusions:"

> There was a substantial difference in repeatability of RTPCR among the three laboratories and, consequently, only a moderate reproducibility between those laboratories suggesting that diagnostic protocols and the interpretation of RTPCR should be standardized across laboratories.

And stopping there, this is really calling for a yes or no answer, but in fairness to panellists, if you have something briefly to add to yes or no, I'll invite you to say that. Do you agree with that statement, Dr. Kibenge?
DR. KIBENGE: I agree with it in principle.
Q Dr. Miller?
DR. MILLER: Yes, in principle.
Q Ms. Gagné?
MS. GAGNE: Yes.
Q Dr. Nylund, did that come up on your screen?

DR. NYLUND: Conclusion, there will be large differences between labs, yeah.
Q Okay.
DR. NYLUND: For several reasons, not only the assay and interpretation, but for several other reasons.
Q All right. And you'll see the last sentence in that same paragraph, it says:

> The assay should be performed by highly-trained personnel to read the sample consistently.

Do you agree with that, Dr. Kibenge?
DR. KIBENGE: Yes.
Q Dr. Miller?
DR. MILLER: Yes.
Q Ms. Gagné?
MS. GAGNE: Yes.
Q And Dr. Nylund, too?
DR. NYLUND: Yes.
Q All right. And would you say that given that labs, different labs using the same tests can come up with different results, underlines, that the operationalizing of a testing methodology is as important as the methodology, itself? Dr. Kibenge?
DR. KIBENGE: Yes, that's correct.
Q All right. Anyone disagree? Hearing none, I'm going to move on. Now, I just want to pick up on one point you said earlier, Dr. Miller, you said, as I heard you, that even at high CT values, which means a weak signal, as I understand it, those are my words, there's no demonstrated disease or mortality, but it's causing -- or there's still damage being caused to the fish. And if I've got your evidence right, I wonder what you mean by "damage" in that context?
DR. MILLER: The fish are still responding to something being present, okay? So there's a lot of studies on multiple different species that show that organisms, when infected with a pathogen, their level of host response to that pathogen will largely be coincident with the level of damage being done, and the level of virulence of that pathogen. So if you contrast pathogens, I know this has been done in IHN, but in a variety of different pathogens of low virulence and high

virulence, you will find the strongest immune response and the strongest basic host-transcriptional response to a pathogen that is causing disease and damage, rather than one that is not. And I only showed you one pathogen, and we're doing this on a variety of different pathogens, but the only point from that wasn't necessarily that we have evidence of disease and mortality by any stretch, but that it's clear that salmon that are carrying the CT values for ISA7, there is a change in the transcription of those fish. They are responding in some way and really interestingly, they are responding similarly to the response that has already been shown to exist in response to influenza infection in mammals because those pathways are curated from mammals.

Q Okay.

DR. MILLER: So it's biologically consistent that they are responding to a virus that causes an influenza kind of response. That's not to say that they're suffering disease and mortality.

Q Okay. Thank you. Now, I just want to pick up on something else quite quickly. Samples for testing for your lab. I understand that DFO has fish in freezers in Courtenay that are from fish farms that the audit people have obtained from the fish farms. You're aware of that, Dr. Miller?

DR. MILLER: I am aware of that.

Q And in normal times, those fish would be available to you for testing as I understand it; is that right?

DR. MILLER: I have asked for access to those fish when I realized the samples that were provided by the Province were degraded. And they were reluctant because they were worried that they didn't want chain of custody issues and they thought that the CFIA might want those samples for ISA testing. So at the time that I asked, they weren't comfortable releasing them.

Q Right, and that's because of the current situation where CFIA is doing an investigation given the recent reports; is that right?

DR. MILLER: That is correct. As I understand, for the last couple of months, they have been collecting an extra kidney sample for all of the fish that they have, which will come straight to me.

Q But again, you know that in normal times, firstly,

they're collected, the fish are collected from the farms by DFO and secondly, absent a CFIA investigation, there would be fish available for you for tests?

DR. MILLER: It hasn't been entirely clear that they'll be available for me, but I would hope so, yes.

Q Tabs 8 and 16, and this is a question of Ms. Gagné. I wonder if we could bring those up together, just in the interests of time, or at least in rapid fire. We're now moving beyond Mr. Martland's highway speed to warp speed. 8 and 16.

MR. LUNN: Would you like to mark Tab 26?

MR. TAYLOR: Oh, I see. Thank you, Ms. Panchuk. I'm sure I would.

MR. MARTLAND: The last document is, I believe, is Exhibit 2003 already, our 26 on the list.

MR. TAYLOR: I don't even know. This will be one of Tab 8 or 16.

Q Essentially, Ms. Gagné, I just want you to identify if these are lab reports that you've prepared, and if you can identify them, we'll mark them as an exhibit. Lab reports on the fish that you were doing testing on.

MR. MARTLAND: And I think -- Mr. Taylor, I think both of these are already in as 2036 and 2037 already.

MR. TAYLOR: Thank you.

MR. MARTLAND: They're on a list of consent documents.

MR. TAYLOR:

Q Well, while we're here, though, can you identify those?

MS. GAGNE: Yes, they are reports done by our section.

Q All right.

MR. TAYLOR: And I realize you can't bring these all up on the screen at the same time, but I'm going to Canada's Tabs 12, 13, 14, and this is a question of Dr. Miller and/or Ms. Gagné.

Q And really, what this -- what I think these are are documents showing that Dr. Garver's lab passed proficiency for coming within the National Aquatic Program. Is that what these --

MS. GAGNE: Yeah, we sent them.

Q They're going so fast.

MS. GAGNE: Part of the procedures is to have -- to send proficiency panels to labs who wants to run an assay and we verify that they can match the results we obtain in our lab.

Q And Dr. Garver's lab passed?

MS. GAGNE: Yes.
MR. TAYLOR: All right. At the risk of being told they are exhibits, I'm going to ask that 12, 13, 14 be the next exhibits, please.
MS. PANCHUK: 12 will be Exhibit 2066, 13, Exhibit 2067, and 14, Exhibit 2068.

EXHIBIT 2066: Email from Laura Hawley to Kyle Garver, dated November 21, 2011, re Sequencher project, with attachment

EXHIBIT 2067: Email from Laura Hawley to Kyle Garver, dated August 30, 2011, re ISAV Proficiency Panel, with two attachments

EXHIBIT 2068: Email from Crystal Collette to Laura Hawley, dated September 9, 2011, with attachment

MR. TAYLOR: All right. Tab 25 of Canada's documents is an email confirming what I referred to earlier, I think, which is that Ms. Gagné delivered assays to Dr. Miller.
Q Can you identify that, Ms. Gagné, as what I just said?
A Yes.
MR. TAYLOR: Next exhibit, please.
MS. PANCHUK: Exhibit 2069.

EXHIBIT 2069: Email from Nellie Gagné to Kristi Miller-Saunders, dated December 6, 2011, re: Shipment, primers and probe, with attachment

MR. TAYLOR: Canada's Tab 1 is the *CV* of -- I think it's Rick Routledge of SFU. I realize he's not a witness, but I think it's important to have this as an exhibit, and I'll ask that that be the next exhibit.
MS. PANCHUK: Exhibit 2070.

EXHIBIT 2070: *Curriculum vitae* of Rick Routledge

MR. TAYLOR: And then in these orders, these are OIE diagnostic and reference material, Tabs 34, 35, 36, 37. Mr. Martland is going to tell me that at

least one of those is an exhibit, but I'm asking that they be exhibits.

MR. MARTLAND: These are the Commission Counsel List, is that --

MR. TAYLOR: No, Canada's 34, 35, 36, 37, they're OIE material that -- when you put exhibits in, I think one of them is this, but in the interests of time --

MR. MARTLAND: Well, 35 is an exhibit, the others should get exhibit numbers.

MR. TAYLOR: Okay. 34, 36, 37, then, if we may? No one's objecting. I don't need them on the screen.

MS. PANCHUK: Tab 34 will be Exhibit 2070, 36 will be 2071, and 37 will be 2072.

MR. TAYLOR: Thank you. Tab 46 of Canada?

MS. PANCHUK: I apologize. Tab 34 will be Exhibit 2071, Tab 36 will be 2072 and Tab 37 will be 2073.

MR. TAYLOR: Okay.

EXHIBIT 2071: OIE Validation and Certification of Diagnostic Assays, Validation Pathway for NAAHLS Diagnostic Test Methods, Molecular Analysis for Infectious Salmon Anaemia Virus

EXHIBIT 2072: Document entitled, "Principles and methods of validation of diagnostic assays for infectious diseases"

EXHIBIT 2073: OIE Validation and Certification of Diagnostic Assays, Validation Pathway for NAAHLS Diagnostic Test Methods

MR. TAYLOR: Okay. Tab 46 of Canada is a article that speaks to the relative resistance of Pacific Salmon to infectious salmon anaemia. One or more panellists have spoken to this question before. I think I recall Dr. Kibenge speaking to it, and I think it's widely accepted that so far, it's been seen that Pacific Salmon are not -- they may be carriers, but they're not affected disease-wise by ISA. And this is an article in that regard. It's up on the screen. I'll ask that be the next exhibit, please.

MR. MARTLAND: It's already Exhibit 64.

MR. TAYLOR: Thank you.

MR. MARTLAND: It already has that number. Thank you.
MR. TAYLOR: Thank you.
Q Ms. Gagné, have you had a chance to look at the Molly Kibenge manuscripts that have been talked about?
MS. GAGNE: Yes.
Q And can you say whether any of the results that you found regarding Ms. Kibenge's work back in 2004 were ever put into the draft manuscript?
MS. GAGNE: No, I don't think they were.
Q Thank you. Dr. Kibenge, if, and you were speaking of this earlier, you thought that there was ISA being detected in 2004, is there a reason why you did not seek to publish on that before?
DR. KIBENGE: Well, that work was done at DFO PBS Nanaimo under the direction of Dr. Simon Jones.
Q Yeah?
DR. KIBENGE: And in my view, as the principal investigator, he has the overall authority on how that data is to be --
Q Fair enough, but why has it come up now and not before?
DR. KIBENGE: Actually, what I think was that the work had been done and I think a determination had been made that it was due to contamination and, therefore, it was not going to be published. And when we reported the --
Q Just bear in mind I've got the signal that I'm being yanked.
DR. KIBENGE: Yeah, but I hope I can finish this explanation.
Q Yes.
DR. KIBENGE: When we reported the two positives in the sockeye smolts, there was a very strong reaction from CFIA that this is a new finding, this has never been recorded in B.C. and so on. And it just occurred to me that, actually, there was some information to that effect that I was aware of, and my expectation was that if CFIA had this information, they'll be probably better informed and find they are dealing with this whole result. So my inclination was initially to ask Dr. Molly Kibenge if she could check with (indiscernible) to see if that work could be published. When the information came back that it would not be published, then I thought that at least we could make this information aware to CFIA.

Q All right.
DR. KIBENGE: So that they would use that information in their own understanding of the results and what we were finding.
Q All right. Thank you.
MR. TAYLOR: Two final documents, Tabs 49 and 51, which are two lab assessment reports, one on Moncton, one on Atlantic Vet College I seek to mark as exhibits, Canada's Tabs 49 and 51. I don't need them on the screen, I'm out of time.
MS. PANCHUK: 49 will be Exhibit 2074, 51, Exhibit 2075.

EXHIBIT 2074: Infectious Salmon Anaemia (ISA) Laboratory Assessment: NAAHLS Laboratory Global Fisheries Center Department of Fisheries and Oceans

EXHIBIT 2075: Infectious Salmon Anaemia (ISA) Laboratory Assessment: ISA OIE Reference Laboratory Atlantic Veterinary College

MR. TAYLOR: Thank you, panellists, for taking time to answer these, in particular, or as well, Dr. Nylund, I know it's late and you're far away so thank you very much.
MR. MARTLAND: Thank you. Mr. Commissioner, there's one -- just to narrate for the record, Canada's Tab 35, which we referred to, has the number 2011, Exhibit 2011. Counsel for the Province, for the remaining participants, we've divided time, Mr. Commissioner, between today and tomorrow for cross-examination to ensure that all participants have opportunity to ask questions of the two witnesses who can't return tomorrow. That will make this fast turnaround today and then again tomorrow morning, but it does give everyone that opportunity. I have next, counsel for the Province, 15 minutes.
MS. CALLAN: Mr. Commissioner, Tara Callan, appearing on behalf of Her Majesty the Queen in Right of the Province of British Columbia.

CROSS-EXAMINATION BY MS. CALLAN:

Q It's fair to say that there's a lot of chromosomal

DNA in the samples that have been tested?
DR. MILLER: Yes.
Q Okay. Now, Dr. Miller, you use a Fluidigm protocol where you run the PCR tests for 14 cycles with all of the primers for all of the various tests, then run it again with an individual primer set and probe for 40 cycles?
DR. MILLER: That's correct. The reason that Fluidigm requires that is because the volume of liquid in each well is only 10 nanolitres. In a typical PCR reaction, it's 10 to 15 microlitres so you have quite a large reduction in volume, and if you have a virus or another transcript of very low copy number, there's a very good chance that you will not have it in a 10-nanolitre volume.
Q If we could turn to Commission counsel document 118? Is that the standard operating procedure for your Fluidigm protocol?
DR. MILLER: Yes, it is.
MS. CALLAN: If we could mark that as the next exhibit?
MS. PANCHUK: Exhibit 2076.

EXHIBIT 2076: SOP FOR Fluidigm Real-Time PCR TaqMan Assay

MS. CALLAN:
Q You'd agree that this is not the standard diagnostic methodology used for virus research?
DR. MILLER: Again, I have to iterate, we were doing research, we were not a diagnostic testing lab. We were doing research to find out if there were Orthomyxo-like sequences in any of our wild migrating sockeye salmon. At the same time, we were looking at 20 other pathogens.
Q Dr. Nylund, would you agree that this isn't standard methodology for virus research?
DR. NYLUND: Yeah, like Dr. Miller said, this is not standard for -- I mean, I've never been acquainted with this method before and it's a bit worrying the way they're doing it, but as I said, it could lead to false positives.
Q Would you agree that it's the equivalent of running the test for 54 cycles and could increase the chances of non-specific amplification?
DR. NYLUND: I think, especially that first stage where she does the pre-amplification with only the primers, they could attach to more or less random

RNA or DNA, causing a segment that later could become positive in the real-time PCR.

DR. MILLER: I just need to add that the concentration of the primers in the pre-amp is 1/20th of the concentration that anyone would use to amplify the product in a normal reaction. And if we're able to amplify the product and gain a sequence that matches the sequence of what you're trying to amplify, I really do not understand, because we don't have ISA in our lab and we're not an ISA testing lab, how one would get four primer sets that give you the correct sequence that is ISA-like or matches ISA using some random primers to sockeye salmon.

Q Now, I understand in the pre-amplification, the first amplification, that you're putting primers from a number of different viruses, such as IHNV and VHSV at the same time as you're running the ISAV primers?

DR. MILLER: Yes. This is the protocol that is required for Fluidigm. I did not invent that protocol, but yes.

Q Would you agree that this contributes to non-specific replication and amplification, as well?

DR. MILLER: Not using TaqMan.

Q Dr. Nylund, what are your thoughts on this point?

DR. NYLUND: I think I already said that I think this could lead to unspecific amplification before you run the real-time PCR.

DR. MILLER: We have re-run samples that the Province has provided for other kinds of assays on the creative salmon fish and received exactly the same results that the Province had on those fish using the same system.

Q Now, when you actually went to sequence any of the samples that you tested, did you re-extract or did you use the same materials that were used in the first round of amplification in the Fluidigm system?

DR. MILLER: The samples used for sequencing had never been put into the Fluidigm system. They were fresh. They had never been used for TaqMan assays. But we did use the pre-amp material to do the regular PCR for sequencing.

Q Now, late last week, you received some test results from the Creative Salmon Jaundice Study on chinook. Now, these tests were interesting

because some of the samples from the study were healthy fish and some were jaundiced?
DR. MILLER: That's correct. And I mean, we weren't really testing for ISA exclusively in those fish. We were testing, again, a battery of different pathogens.
Q And these fish in particular were necropsied by Dr. Sonja Saksida, who's a veterinarian with experience in fish medicine, and histopathology was conducted by Dr. Gary Marty, who's a Board-certified veterinary pathologist on most of these fish so it's clear which fish were healthy and which ones were diseased?
DR. MILLER: Yes, it was.
Q Okay. If we could go to Provincial Tab 14? Would you agree that those are Dr. Marty's histopathology results?
DR. MILLER: Yes, they are.
MS. CALLAN: Okay. If we could mark those as the next exhibit?
MS. PANCHUK: Exhibit 2077.

EXHIBIT 2077: Histopathology results

MS. CALLAN:
Q Now, your results were interesting because you didn't only have unhealthy fish testing positive for ISAV?
DR. MILLER: Yes, and I never suggested that ISAV was anything to do with this jaundice disease.
Q Okay. And in fact, if we turn to provincial Tab 22, you'd agree that the positive ISAV PCR test results are as common in healthy fish as they are in sick fish?
DR. MILLER: Yes, I only saw this this morning, but yes, again, I never came forward and suggested there was any relationship.
MS. CALLAN: If we could mark that as the next exhibit?
MS. PANCHUK: Exhibit 2078.

EXHIBIT 2078: Evidence that Jaundice syndrome in farmed Chinook salmon is not associated with positive PCR test results for ISAV

MS. CALLAN:
Q Now, when you also looked at your PCR tests, they

didn't have consistent positive results between segment 7 and segment 8, they were usually either positive for one of the tests or the other, but not for both?

DR. MILLER: Yes, we do not see a high degree of consistency between the two segments. I mean, there are samples that can test positive for three of the four primer sets that we work with, but in general, segment 7 picks up a lot more positives than segment 8 does.

Q Okay. And these would be unexpected results if --

DR. MILLER: Not if you have sequence variation underneath the primers and probe in segment 8, I think it could be very easily explained.

Q Okay.

MS. CALLAN: If we could turn to Commission counsel Tab 56? And if we could highlight on case number 2011-0855.

MR. LUNN: Sorry, I'm not seeing it. Do you have a row number for that?

MS. CALLAN: I don't, but it's page 2 of the printed copy so just scroll down a little bit. Yeah, it's the section that's highlighted in pink.

Q Now, the Province also did ISAV testing on some of the same fish that you did and I put to you that the documents that are -- well, the entries that are in pink are the same fish that were submitted to you?

DR. MILLER: I'm assuming so, yes. I've never seen this document.

Q Okay.

MS. CALLAN: If we could mark this as the next exhibit?

MS. PANCHUK: Exhibit 2079.

EXHIBIT 2079: Excel spreadsheet entitled, "ISA testing January 2011 to present"

MS. CALLAN: Okay.

Q Now, when the Province tested them, they were negative on all of the OIE tests. Now, one test, conventional OIE reference 20 M1 gene Segment 8, yielded a band of similar size to the positive control?

DR. MILLER: I don't know. These aren't my data.

Q Okay. Okay. Well, I put it to you that -- what occurred. If we could go to the end of the document, that will explain it a little better.

And it's right in the middle of the page with the same case number on it.
DR. MILLER: I can't -- I mean, this isn't -- I have no idea what this document is trying to suggest, but it's not my data so I've never seen it before.
Q All right.
MS. CALLAN: If we could turn to provincial tab 18?
Q Now, sequencing was also done on these studies and if you could look to this document, would you agree that the result from the sequencing is that there's no significant match to ISAV?
DR. MILLER: Again, I have no familiarity with this. I don't know if this is something done in the provincial lab, but it says so, but I have no way of gauging that one way or the other.
MS. CALLAN: If we could mark this as the next exhibit?
MS. PANCHUK: Exhibit 2080.

EXHIBIT 2080: Molecular Diagnostics Sequence Identification Summary

MS. CALLAN:
Q Would you agree, however, that the document indicates that there is no significant match to ISAV?
DR. MILLER: I wouldn't agree to anything because I don't see any sequence data.
Q If we could go to the next page that will have some more results? Okay.
DR. MILLER: I don't see this going anywhere. I mean, all the sequences that we identified and that we have sequenced from the ISA positives that we have in our lab have come between 95 to 100-percent similar to known ISA isolates. If there is something that the provincial lab picked up that was non-ISA, I have no familiarity with that.
Q Okay. Now, I understand you've done some more infectability studies with respect to your Parvovirus in August?
DR. MILLER: Yeah. I'm not sure how that relates to ISA, but yes, we have.
Q Oh, I'll get there, don't worry. So this is a non-specific test and it would have caught ISAV if the ISAV was infectible, as well?
DR. MILLER: Dr. Kyle Garver did some injection challenges on sockeye salmon with tissues that we had shown to be positive for Parvovirus. We also

attempted to use tissues that we identified as negative for Parvovirus, and then he used a control that was a buffer control that had no tissue sample from sockeye salmon, period. But it was an injection challenge.

Q So would you agree, then, that it would have picked up ISAV if it were there?

DR. MILLER: We've never tested those for ISAV.

Q Now, I understand that the Plarre ISA test, you're getting hits, but it's a very short sequence. When you exclude the primers and the probe, you're only actually measuring four base pairs?

DR. MILLER: We have four regions of ISA-8, one of which doesn't overlap with the other one at all. The Plarre ISA-8 primer sets amplify a region of ISA-8 that does not overlap with the Snow or the Christensen primer. So the Christensen and the Snow primers are highly overlapping. One of them is a much longer sequence than the other one. I believe one is 104 bases, one is 70 bases, and the one on the other end is about 60 bases. So again, it's multiple primer sets. Each read, I would agree, is a relatively short segment because that's how TaqMan assays are designed, but every single one of them identifies in that short region as ISA.

Q But you're not getting consistent hits between the tests so when --

DR. MILLER: It doesn't matter because the fact of the matter is if there is sequence variation in ISA-8 that we don't know about, you don't know that you're always going to be able to amplify. So you really do need a full sequence of that segment in order to understand the dynamics of why some assays are working and other ones aren't. So in an ideal world, yes, if you develop an assay and you have a strain of ISA that you are trying to pick up, you should absolutely get the same results every time with each one of those assays. We don't have that situation here. We already show with ISA-7 that we have something that is divergent from any known strain. We don't know what the overall sequence is in ISA-8 because we're not picking it up as regularly as we are ISA-7. So yes, in an ideal world, if you have -- you know you're looking for Strain X and you have three different assays for it, you should be able

to pick it up consistently with all three of those.

We have an unknown sequence here and we don't know what the underlying variation is.

Q So when you are testing it in any particular fish, you're not getting the ISA-8 segment test consistent across any one particular fish?

DR. MILLER: We can. We can get all three of those tests to work on a single fish. More often, we get two of the three to work.

Q Okay. And Dr. Nylund, one last question. Earlier on in Commission counsel's evidence, you mentioned an issue about stop codons. I was very interested in hearing it and I was hoping you could answer what your concerns were with respect to the stop codon issue.

DR. NYLUND: Yeah, well, if you look at that presentation by Miller, she has an alignment of the ISA-7 showing three fixed differences. Actually, if you look at that alignment, and I meant alignment because I have a lot of sequences in my lab that hasn't been published yet, there are seven differences in the space between the two primers and those seven differences cannot be found in Canadian or European ISA virus. But unfortunately, those differences also introduces a stop codon into this sequence, which means that it's not a functional sequence, it can't be coding for an ISA virus or another virus protein because you don't have stop codons in there. A stop codon means that it's the end of the sequence, coding sequence and this is not the end of the coding sequence for an ISA virus.

Q Thank you for answering that question.

DR. NYLUND: So that means that I find it hard to believe that this could be a functional sequence. I think this could be due to unspecific annealing of the primers that are picking up something else than actually virus.

MS. CALLAN: Thank you for your questions (sic).

MR. MARTLAND: Mr. Commissioner, I have next counsel for the B.C. Salmon Farmers Association, with 15 minutes.

MR. BLAIR: Mr. Commissioner, Alan Blair appearing for the B.C. Salmon Farmers Association as counsel and with me is my associate, Mr. Shane Hopkins-Utter. Mr. Lunn, could we please have the B.C. Salmon

Farmers Association Tab number 1?

CROSS-EXAMINATION BY MR. BLAIR:

Q Members of the panel, on the screen you'll see a letter dated November the 25th this year. It's from my client's executive director, Mary Ellen Walling, and it's addressed to the Minister and it summarizes the meetings which occurred in recent times, in November, between the Canadian Aquaculture Alliance and DFO and others. And you'll see these questions are really for Ms. Gagné, and I suppose, Dr. Miller. You'll see here in this correspondence that there's an offer by my client to provide ongoing samples in real time, as well as pointing out that there's about 5,000 samples of farmed salmon which have already been tested for ISA. Ms. Gagné, do you see that reference in the letter to the Minister?

MS. GAGNE: Sorry, I didn't hear that?

Q You see these? I've accurately summarized the letter to the Minister, have I?

MS. GAGNE: Yes.

MR. BLAIR: I wonder if we could have this marked as the next exhibit, please?

MS. PANCHUK: Exhibit 2081.

EXHIBIT 2081: Letter to DFO from BCSFA dated November 25, 2011

MR. BLAIR: Thank you.

Q Dr. Miller, earlier in your evidence, certainly this morning and somewhat less this afternoon, I had the impression from your responses to some of the questions put to you that my client was not cooperative in terms of providing samples to you. Firstly, it is true to say that if an industrial client like my own, or indeed, anybody, provide samples to DFO, DFO can sample them as they wish, correct? There's no control exerted over a sample once DFO has it?

DR. MILLER: Well, I mean, DFO is new to having these samples. I mean, it was under provincial authority before this year, but I would imagine that DFO does have authority over those samples once they collect them, yes.

Q So if you were not able to sample fish from farmed

operations, it's not because the salmon farmers stopped you, any samples that were within DFO's present control would be able to be sampled by DFO, including yourself, for anything, correct?

DR. MILLER: We had an agreement, a verbal agreement before I testified at the Cohen last time that they would work with me and provide samples from the farms that were not anything to do with the audit program, but that we would sample from their farms, basically, of their healthy fish.

Q And a series of meetings occurred both before you gave your evidence last time and as well as since you've been on the stand, correct?

DR. MILLER: We had one face-to-face meeting after the aquaculture wrapped up with the Cohen and it was my understanding, walking away from that meeting, that we were going to be working together and they would be providing samples. I wrote a proposal based on that meeting. I sent that proposal back to Mary Ellen Walling two days before it was due and said, "We need to go back and forth and iron some of this out." One of the things that had come up in our meeting was that they really wanted to know how long the virus had been here, this is the Parvovirus.

Q You know, Dr. Miller, I'm going to ask questions that are specific and I'm going to ask you to confine your answers because we only have a very short period of time. I'm going to propose to you that the industry proposed funding as part of the dialogue, correct?

DR. MILLER: They proposed funding only to look at sockeye salmon, not to look at industry samples, and said after we had that information and knew how long this had been here, then we could sit down again and talk about testing of their fish, if it had been here long enough that it predated the industry.

Q They commented to you that you had access to thousands of samples within DFO and proposed that a stepped approach occur where sampling for farmed salmon, wild salmon and spring and hatchery salmon could all be sampled next spring, and that was their proposal, and you rejected that proposal, correct?

DR. MILLER: There was a funding opportunity in DFO and we were going after that funding, as far as I was

concerned, to test aquaculture fish. And they proposed that I use that funding to test sockeye salmon instead. And so no, I did not feel that what they proposed was what we originally had talked about and what I had said that we were going to do in the Cohen Inquiry and I did feel that there was no need to move forward. I didn't need them to run sockeye salmon, I needed them to provide Atlantic salmon to test.

Q And they said they would in a stepped approach, correct?

DR. MILLER: They wanted a level of control over the data and the information that we have in sockeye salmon and I was not willing to give that level of control on our sockeye salmon when we have the samples, there are samples in our lab.

Q Which you could have tested?

DR. MILLER: We have tested.

Q And they were proposing that you test the samples that you have before they produce additional samples for farmed salmon, proposed funding for that, and proposed that hatchery salmon, wild salmon and farmed salmon all be tested and sampled next spring, and you rejected that proposal, yes or no?

DR. MILLER: I rejected the specific proposal to test sockeye salmon and have them as a collaborator on the testing of sockeye salmon.

Q Thank you. Dr. Nylund, thank you for staying awake. By my count, it's very late, or perhaps very early, I think. I have some questions for you, sir. Recognizing you've made a career out of studying ISA, and in particular, from a distance, you've been undoubtedly watching and listening and reading the sampling and testing methodology that's been used by these various separate Canadian labs, and I wonder if you could take a few moments, sir, and comment on your own gene bank that you have as a result of your extensive career in sampling in ISA and a critique, if you could, with respect to the some of the testing methodologies and sequencing work that have been done by any or all of the other panel members, please?

DR. NYLUND: Well, that's quite a large task to do and I would say that the methods used by most of these laboratories are well known and very reliable and

they will pick out most of the known ISA virus that you can find in the Atlantic and in Chile. But of course, they may not pick out any natural-occurring viruses in the Pacific. And if you look at all the viruses, for instance, the HSV virus, the Paramyxovirus and so on that you find in fish, you will find one type of strain in the Pacific and then another in the North Atlantic. And there may very well be a Pacific ISA virus that we have not yet detected and it could be very different from the North Atlantic ISA virus. But I think the method that they are using are quite good, except the one that has been used by Miller. I think that can more easily be picking out things that are not ISA virus, but that are more random RNA DNA in the sample. I think there's a danger of that, but then again, I have to say that I don't have any experience with that method, but intuitively, it sounds like it could be a problem the way it was designed.

Q There was reference to a term, "pre-amplification," I believe. Could you describe what pre-amplification is and where errors may creep in to using that, as Dr. Miller has described in her work?

DR. NYLUND: Well, ordinarily, in real-time PCR, you have three different primers or probes. And the chances that all of them should match at the same time, unless you have the target gene you're looking for, is very small. But if you remove the probe, then you only have two primers like an ordinary PCR, and you may have a match or a partial match with those two and they could produce products with different lengths, even though they may not be specifically ISA virus targets. And that could create a lot of strands where the primers match. So when you are using this product in the real-time PCR, you already have a match for the primers. You only need a partial match for the probe and then you will get the positive result. And that increases the chance that you may get the false positive, in my opinion.

And when she have sequenced the products, they are, of course, 100-percent similar to the primers and probes that have been used, except for the sequence of Segment 7, which is a sequence

with a stop code on it, which couldn't be correct if it was coding for a protein. So I think it's a bit worrying, that method, but then again, I don't have a lot of experience with it. But the other methods used by Kibenge and by Gagné seems quite okay and should be doing the job very well.

Q Dr. Nylund, in the process of getting some of these samples from Canada and testing for ISA, I think it's fair to say that you received the samples through Alex Morton and, in a sense, she was your client; is that correct?

DR. NYLUND: Well, I think she put the report on the Internet so she probably would admit it. And I'm going to say I have communicated with Alexander Morton and she said she was very happy when we had the results come out negative.

Q Well, I'm glad you're --

DR. NYLUND: So it's not, in my opinion, that she's looking for ISA virus and want to find ISA virus, but she want to find, in my opinion, the cause for the mortality, and she was afraid that the ISA virus could be the cause. But in my opinion, we haven't been able to document that the ISA virus has been cause for any mortality in natural population, not with the samples we looked at so far. So I think it was a good thing to send the samples to more than one lab, because then you have larger chances of controlling each other and you have a control between the laboratories.

Q And Dr. Nylund, it's fair to say that some of the samples that were sent to you through Alex Morton tested positive for some other important diseases, and those would be reportable diseases?

DR. NYLUND: Well, according to the list from Western Canada, of course, VHS and IHL, we are reportable diseases and we did find IHNV virus in some of the samples.

Q So that --

DR. NYLUND: But of course, then, it's a very common virus in the Pacific side of Canada.

Q Yes, but technically, the IHN found in the samples provided to you would have been reportable by a Canadian citizen who was aware of that, correct?

DR. NYLUND: Yes, as far as I can see, yes.

Q Just to be clear, because you're from Norway, you don't have any obligation to report, correct?

DR. NYLUND: No, in Norway, we can't be -- as a

scientist, we can't be forced to report anything because you have the secrecy for scientists in Norway so we don't have to report anything.

Q I actually meant you --

DR. NYLUND: As long as it's science.

Q I actually meant you don't have any obligation to report into Canada because you're a scientist from a foreign country, correct?

DR. NYLUND: And certainly not to Canada.

Q Thank you. But Dr. Nylund, Alex Morton, being aware of those positive results from her samples, would have an obligation to report and you're unaware of any report being made, correct?

DR. NYLUND: Well, as far as I understood, I had a deal with Alexandra Morton that she would report these results to the authorities or I should just send the report directly to the authorities.

Q Did she indicate --

DR. NYLUND: But as far as I've seen, she made the reports available on the Internet so they have been, in a way, reported.

Q I suppose --

DR. NYLUND: I don't know if that's good enough for the Canadian government, but they certainly have been reported, as far as I can see.

Q I suppose it depends who reads the *New York Times*.

MR. BLAIR: Thank you, those are my questions.

MR. MARTLAND: Thank you. Mr. Commissioner, I have counsel for the Aquaculture Coalition with 15 minutes today. Thank you.

MR. McDADE: Thank you, Mr. Commissioner. My name is Gregory McDade. I'm counsel for Dr. Morton and the Aquaculture Coalition.

CROSS-EXAMINATION BY MR. McDADE:

Q Dr. Miller, I want to move from the highly technical to the cover-up or the DFO reaction to some of these issues. You spoke in your evidence about the reaction from your superiors when you first discovered ISA, and in particular, a conversation you had with Stephen Stephen. Can you tell us about that conversation?

DR. MILLER: Well, up and to that point, I do not believe that Stephen Stephen was aware that we were conducting this research and so I think, you know, this was news to him on the 24th of

November, when we put that data forward. And I believe that he was not happen that Ottawa was unaware that we were doing this research and wanted to know, you know, who had advised us that we could be doing work on ISA.

Q He was angry at you?

DR. MILLER: Well, he's the head of the NAAHP program, and I'm not a NAAHP lab so those are the National Aquatic Animal Health labs, and I'm a molecular genetics lab and I have worked in the area of disease and host response for a number of years. I guess I have not -- well, I worked on IHN so I have worked on reportable diseases, but probably before CFIA was involved in reportable diseases. And I think unhappy with not being aware that I was doing this, yes.

Q Did he raise his voice at you?

DR. MILLER: I can't specifically recall if he raised his voice, but I think there were basically questions on whether random DFO scientists should be working on disease issues when they're not in the NAAHP problem.

Q Random DFO scientists, meaning the Pacific Biological Station Lab in Nanaimo?

DR. MILLER: I guess I would be within that, yes.

Q Did he tell you you weren't to do any more with ISA?

DR. MILLER: Not specifically. Basically, it was recognized that what I was doing was research, I was not trying to do testing and validation, I was simply doing research on a number of different pathogens, ISA or Orthomyxo virus being one of them.

Q Did he tell you you weren't to talk about ISA?

DR. MILLER: That I was not to talk? Well, I mean, I'm not really supposed to be talking publicly about much of this, anyway, but I don't recall a specific statement, you know, not to discuss ISA, but I think it's a given that I don't go and speak publicly about this.

Q You're under restrictions from speaking publicly about this?

DR. MILLER: Well, I don't think anyone in DFO is speaking publicly about this at the time.

Q Did you have a discussion with him at the time about whether it should even be called ISA? Did he want you to call it by something else?

DR. MILLER: Well, very definitely that discussion happened, and it happened before Stephen Stephen was there, as well, with the fish health group. You know, and you can -- and there's this discussion going on here, right? Is it just an Orthomyxo virus and can you really call it ISA? And so we discussed how ISA, as a disease, is defined by CFIA and under that definition, that it needs to be culturable and it needs to be validated with the assay used in the CFIA lab. This would not fit that definition of ISA. I contended that based on the sequence information, it does appear to be an ISA-like virus. Whether it causes ISA disease, or not, is a totally separate issue. So we had that conversation.

Q Can I suggest to you he told you you were not to report it to CFIA?

DR. MILLER: It's not my job to report to CFIA. I was told that it is his job to report to CFIA.

Q So you did have that discussion, he told you not to report it to CFIA?

DR. MILLER: He -- well, I was told that scientists do not report to CFIA, Stephen Stephen is the contact with CFIA.

Q Did you have any discussion about aquaculture and any implications for your findings around fish farms?

DR. MILLER: I didn't have any samples run from aquaculture at the time.

Q No, I'm asking about the conversation with Stephen Stephen. Did he raise that question with you?

DR. MILLER: He did not say anything specifically about aquaculture. He did say something about the repercussions of new diseases on wild fish and their price and exchange between countries, et cetera. There was no mention of aquaculture.

Q Are you under any other restrictions, or did he put you under any new restrictions as a result of your findings?

DR. MILLER: Not really directly. It was recognized that I was going to continue doing the research that I was doing, but I think that he wanted there to be some broader discussions about boundaries and about what kinds of pathogens we would be looking at.

Q Well, you say not really directly. What indirectly?

DR. MILLER: Nobody said that I could not continue on with my research, but I think that there was the recognition that this needs to be something that's discussed in the department in the future.
Q You're very concerned about the funding for your lab overall and your lab workers, I would think. Was there any discussion about your funding?
DR. MILLER: At that time, I don't believe there was a discussion about my funding. He is the head of the GRDI, which is one of the places that I do get funding out of from DFO. At the time that we had that conversation, I didn't have notification that I had funding from GRDI.
Q Are you under any restrictions around, say, sending out emails?
DR. MILLER: I think it's fairly recognized in the department that we weren't talking about ISA over email.
Q You weren't to talk about ISA over email?
DR. MILLER: Largely, no.
Q Let me ask you more generally, as a result of these findings of ISA, have you felt any pressure or adverse reaction from your other superiors?
DR. MILLER: I'm pretty alienated in the department at the moment so the end result of all of this is I'm not included in any conversations about any of this so once I reported this information on the 24th, nobody in the department talked to me about disease or ISA after that.
Q Let me ask you about the 2004 paper that Molly Kibenge was involved in, that's been discussed. You had some involvement, as I understand it, in sequencing some of it?
DR. MILLER: Unfortunately, I personally didn't, and I didn't --
Q Your lab did?
DR. MILLER: -- I did not know that Molly -- once we talked about it, I did remember her being there, but I didn't know that we sequenced for her. It was only when my technical staff went back to our computers and found our archives because they recognized her name and found some sequences on our computer that we realized that we had worked with her.
Q And so as a result of doing that, you confirmed that her findings in 2004 had been sequenced as ISA?

DR. MILLER: The difficulty is we had sequences on our computer that came from her, but we did not know what she was sequencing at the time, whether those were sockeye salmon, whether those were sequences from East Coast fish, because I know she was doing work on ISA on the East Coast, as well. So all I could really say was we did sequencing for her and what we sequenced appeared to be ISA, but it didn't match directly exactly the sequences that we'd been obtaining.

Q But it does appear now that the DFO knew in 2004 that ISA was present in the Pacific?

DR. MILLER: I don't know how much I want to weigh into that because I really had no involvement back then. So apparently, according to what I've heard, but I know no more than you do.

Q Well, yes, but in your discussions with your superiors, say, Stewart Johnson or Simon Jones, or anyone else at PBS, did they confirm they were aware of these earlier findings?

DR. MILLER: Well, first of all, Stewart Johnson and Simon Jones are not my superiors, but --

A I'm sorry.

DR. MILLER: -- but yes, Simon Jones was not involved in the meetings that I was in, but Stewart Johnson was there and he confirmed on the 24th that he knew about Molly Kibenge's work and wasn't sure whether there had ever been any sequencing, which is what spurred us to go back and look at our computers, to find out if there had been.

MR. McDADE: Can I have Commission document number 58, Mr. Lunn?

DR. MILLER: The 24th was the first time I'd ever heard of Molly Kibenge's work.

MR. McDADE:

Q And but as of the 24th, senior people in DFO were aware that the Pacific Biological Station in Nanaimo was finding ISA?

DR. MILLER: By the 24th, they were aware of my work, yes.

Q And so when statements were coming out from DFO after November 24th, and in particular, the statement from the Minister on December 2nd, saying they were not aware of any ISA, that would have been a surprise to you, wasn't it?

DR. MILLER: Yes, it was, but nobody was speaking to me at that point.

Q So those statements --
MR. TAYLOR: It's also not an accurate quote of what the Minister said.
MR. McDADE: Well, we'll come back to that with the next panel.
Q Sorry, can I just -- Dr. Miller, you're aware of the nature of Dr. Marty's testing for ISA over the past eight years, or so?
DR. MILLER: I'm aware he's conducting testing and I'm aware that it's his own in-house test.
Q Yes. And that's what the document on the screen is is a reference, you'll see in the email at the bottom of the page, as I understand it, it's a test that was designed by his Masters student?
DR. MILLER: That's what he said.
Q And it's not the OIE standard test, is it, Ms. Gagné?
MS. GAGNE: It's not, no. I remember myself having to answer the question, my opinion of all the tests that were running.
Q So it's quite clear it's a different test than you've been running?
MS. GAGNE: It's a different test.
Q And it's not verified by any of the standard literature?
MS. GAGNE: I don't know if they have in-house validation data.
Q Right. So what we have is the 4,700 tests that we heard so much about in the last hearing have all been under a process that is not an approved process by the OIE; is that right, or by your organization?
MS. GAGNE: We're not approving assays for other labs. That's not our business.
Q Well, Dr. Miller, what's your opinion about that test? Is that going to have picked up ISA? It's simply the wrong test, isn't it?
DR. MILLER: I don't know, I've never used this test so I really wouldn't know. I don't believe that it is published.
Q So it's a completely unverified -- to the best of any of the knowledge of the three participants here, there's no verification of that test at all?
DR. MILLER: I'm not aware of any.
Q Dr. Kibenge?
DR. KIBENGE: Yeah, I'm not familiar with this test, but I notice here that the target is

(indiscernible) PB1 gene, which is probably one of the largest genes in the virus and my thinking is that probably the copy numbers for this gene may not be as high as we see in segments 7 and 8. And just on that basis, I would expect this not to be as sensitive as segments 7 and 8.

Q We don't know today, though, whether this test that's been conducted in a B.C.-only version would have been picking up the ISA even if it had been there for the last seven or eight years; isn't that right, Dr. Miller?

DR. MILLER: I wouldn't know, no.

Q Dr. Miller, let me ask you a little bit about the Clayoquot Sound test from Creative Salmon. You found -- I understand that Creative Salmon was the one fish farm company that would cooperate with you?

DR. MILLER: Yes, they were, which is unfortunate, that the only result I have is from Creative Salmon because I think they are a very forward-thinking, cooperative and responsible company.

Q Yes. The fact that they were prepared to let you test their fish shows a certain amount of cooperation?

DR. MILLER: I actually -- I tested for general pathogens. I did not discuss with them ahead of time exactly what I was testing for there, but the project was about trying to find out if there was a virus that might be causing the jaundice disease, and so I felt that doing the general pathogen testing would at least screen out possibilities of known viruses and known other pathogens.

Q And you found over 20 percent of the fish you tested had ISA?

DR. MILLER: That was the same rate that we find in wild migrating sockeye salmon, as well.

Q But that was what percentage?

DR. MILLER: It was 25 percent.

Q 25 percent of the fish in that fish farm are testing positive for ISA under your test?

DR. MILLER: Yes, with similar CT values of what we see in wild migrating fish so they're high CTs so low copy number.

Q Did you find other viruses of note in their fish?

DR. MILLER: Yes, we did.

Q What?

DR. MILLER: We're still doing sequence confirmation of some of this and this is ongoing research and I'd rather not go into a lot of detail in what we did find in those fish, but ISA was not the one I was most interested in.
Q Did you find HSMI?
DR. MILLER: We did find fish positive for the pasendrial (phonetic) virus, which is thought to be causing HSMI.
Q Dr. Nylund, you know about HSMI in Norway, do you?
DR. NYLUND: Yes, I know quite a lot about it.
Q Were you aware that it had been found in Canada, on the West Coast?
DR. NYLUND: Not in Canada, but I know it has been found in Chile, who has been importing embryos from Europe.
Q And that's a significant disease of concern in fish farms in Norway?
DR. NYLUND: Yes, it gives up to 10 percent losses in detected farms and up to 100-percent morbidity. And it effects the muscle of the fish so it may reduce the quality of the fish.
Q And that's not been found, as far as I know, Dr. Miller, in any place to date?
DR. MILLER: We see positives for that in our sockeye salmon, as well.
Q You're beginning to see positives for HSMI in sockeye?
DR. MILLER: Not for HSMI, the disease, we see pasendrial virus in our wild migrating sockeye salmon.
Q And has that finding been disclosed publicly before today?
DR. MILLER: No, this is research in progress.
Q One last question for you, Dr. Miller. When you first became aware of these findings of ISA, did you go back and reassess the '07 and '08 smolts that had been the subject of your testimony around the MRS? Did you do any further testing on those fish?
DR. MILLER: Yes. Yes, we did, and we are -- one of the things that we're doing is looking for a differential that might explain the difference between 2007 and other years. The three differentials that we can see, and this is, again, based on a very small sample size, we have to be very careful with these data, but 2007 fish left

the Fraser River with the high incidence of a flava bacterium and it's pseudochromis, or something. It is a pathogenic strain of a flava bacterium that we haven't seen in other years. And when we sampled them in the marine environment, they had quite a high positive rate for the pasendrial virus that is possibly causative of HSMI. And they had, I believe -- I can't remember the exact percentage, but a relatively high percentage of ISA, as well.

Q In comparison to the '08s?

DR. MILLER: In comparison to other years, including '08s. '08s, there was -- in '08, I believe, if I'm correct, that -- I don't have the data in front of me right now, but that there were a fair number of Harrison fish that were positive in the fall for ISA, as well.

Q So it does appear that the '07 smolts, which became the '09s, had a number of diseased-based factors that distinguished them from the later year?

DR. MILLER: Yes, but again, we did have very small sample sizes, but yes, we -- I think that the biggest findings with those were the flava bacterium and the pasendrial virus.

Q All right. And are these going to be reported to the Commission, these findings?

DR. MILLER: They haven't been reported.

Q All right. I just have a couple of more minutes and I've got another participant who's agreed to reduce its time.

MR. MARTLAND: Yes, Mr. Commissioner, we have encouraged and allowed participants to trade time. There's some hand signals across the room. So Mr. McDade has received two five-minute contributions that I'm aware of. I've calibrated the time according to that.

MR. McDADE: Okay. I don't think I should take the whole of that.

Q Let me just, Dr. Nylund, you've been very patient through the day. A couple of questions for you. In the first set of samples you received, the set of 48 that came from the SFU testing, you tested a positive for fish number 36, I think you testified? Can you hear me, Dr. Nylund?

MR. MARTLAND: In fact, I think the video link may be frozen because we have different images of him

doing different things so I wonder if I can suggest this. Mr. Commissioner, this may be a technical matter that requires a few minutes. Mr. McDade, I think, is in the home stretch, but perhaps this is an opportune time to move to the afternoon break and hopefully resolve that issue?

MS. PANCHUK: The hearings will recess for 15 minutes. Please remain standing in place while the Commissioner exits the room. Thank you.

(PROCEEDINGS ADJOURNED FOR AFTERNOON RECESS)
(PROCEEDINGS RECONVENED)

THE REGISTRAR: The hearing is now resumed.

MR. McDADE: Thank you, Mr. Commissioner. I've been reminded I should mark the document on the screen as the next exhibit.

THE REGISTRAR: Exhibit 2082.

MR. McDADE: Thank you.

EXHIBIT 2082: Email from Gary Marty dated August 12, 2011 re ISAV PCR tests

CROSS-EXAMINATION BY MR. McDADE, continuing:

MR. McDADE:

Q Dr. Nylund, you can hear me now?

DR. NYLUND: Yes.

Q The original 48 fish that you got from the Simon Fraser University batch, you found a positive in fish 36. That's correct, isn't it?

DR. NYLUND: That's correct, yeah.

Q And I understand that Dr. Kibenge also found a positive at fish 36.

DR. NYLUND: Yes.

Q And my question to either of you, really, but I'll address it to you first is what are the odds of finding a false positive in the very same fish out of a batch of 48 by two different labs?

DR. NYLUND: If I may answer that first, I would say the chances are very small, to tell the truth, but then again, the reason for finding this was that I repeated the real-time PCR on this sample several times, and so what I believe is when you look at Kibenge's result from fish 26 and 36, he gets different Ct values on the different assays. The difference between those Ct values suggests that

his findings are correct, because you find exactly the difference you would expect with the two assays he's been using. So, to be honest, I think that Kibenge's results on this are correct.

Unfortunately the material I looked at were so degenerated and so destroyed that it was impossible to reproduce any results at all, but we got one positive. But I -- since it's only one we -- positive and it was not possible to repeat, I wouldn't put too much into that. But I think that Kibenge's results are reliable, yeah.

Q But, Dr. Nylund, is it fair to say that it would be absolutely incorrect to refer to your finding in fish 36 as a negative?

DR. NYLUND: No, it's not a negative, it's a positive.

Q And the other question I have for you draws on your extensive experience in Norway, and that is with ISA. It's really -- can you explain to us how an avirulent strain or non-virulent strain present in the wild can mutate or evolve as a result of having concentrated populations that you see in fish farms?

DR. NYLUND: Well, I mean, if you look at evolutionary biology, in a fish farm where you have a large population density, many hosts, if you have mutation it has the opportunity to spread and multiply. In a wild population, which is very small, few individuals, such a mutation will be very fast lost in a wild population, while in a farm population it can multiply for several -- yeah, for years, actually, depending on how the farms are run.

Q So if in fact ISA is present in a non-virulent strain in the wild in British Columbia, this adds to the risks that fish farms present?

DR. NYLUND: Well, we don't know exactly the virus in the wild, if it's a Pacific ISA virus or if it's a North Atlantic ISA virus, because I'm not yet convinced by the sequences we've seen so far.

But if it is a North Atlantic ISA virus, of course it can mutate into a pathogenic strain.

Q All right. Thank you, Dr. Nylund.

I'll just finish with one question to you, Dr. Miller. You talked about the difficulties you had in getting the provincial audit samples in order to be able to test those, and the degraded condition they came to you. Am I correct in

understanding that there were conditions put on your ability to get those audited fish and, in particular what I want to know, was one of those conditions that the province made you promise not to test for ISA if they gave you the fish?

DR. MILLER: ISA wasn't on the radar screen at that point, but we had decided -- material transfer agreement that specifically stipulated that these were only for testing for parvovirus and that those data and results were to be shared with the province and if published, would also be shared with the province.

MR. McDADE: All right. Those are my questions and my client just wants me to say again for the record, Dr. Miller, thank you for your courage in having done the testing you've done. Thank you.

MR. MARTLAND: Thank you. We have next counsel for the Conservation Coalition with 15 minutes.

MS. CAMPBELL: Good afternoon. My name is Karen Campbell and I am counsel for the Conservation Coalition. I'm hear with my colleague Judah Harrison. The Conservation Coalition is a group of six non-governmental organizations and one individual who are concerned about the conservation of the species.

CROSS-EXAMINATION BY MS. CAMPBELL:

MS. CAMPBELL:

Q Dr. Kibenge, we talked earlier today about the strains of ISA and how there are two major strains and that there is uncertainty about whether there may be more. Is that, simply put, a fair statement? If you had your microphone.

DR. KIBENGE: Yes, in general there are two genotypes of ISA that are known. There's the North American genotype and the European genotype. Within those two genotypes, there are several different strains of ISA.

MS. CAMPBELL: Okay. I'd like to turn to Conservation Coalition document number 1, and I'd like to ask that it be marked as an exhibit. The document is an email. I'll just see if I can work off the screen with people.

Q So the document is an email that is from Stephen Stephen to Brian Evans and it's a letter. In the second paragraph, it talks about -- and I'll just

read this to you.

> Based on molecular strain typing data from two "third party" laboratories --

And they're referenced, the Charlottetown and the New Brunswick laboratories.

> -- it would appear that this ISAV is a new strain having a 9 amino acid deletion in the hemagglutinin protein.

My question to you, Dr. Kibenge, is does this to you constitute a new strain, an emerging potential strain of ISA?

DR. KIBENGE: The strain referred to in that email is a true --

Q If I could just --

DR. KIBENGE: It's a true new strain. I sequenced it in my lab and we would verify that it's a North American isolate that had never been found anywhere in New Brunswick, the Bay of Fundy, where we had ISA for a few years.

Q Great.

MS. GAGNE: And may I add that we confirmed that strain.

Q And you have confirmed that strain. Thank you, Dr. Gagné.

There's also been some conversation about strains becoming virulent, and the notion that they mutate. Dr. Miller, you've indicated that the strain of ISA that you're detecting in B.C. may be avirulent; is that accurate?

DR. MILLER: We really have no data on that, but it is recognized that HPR0, which is a non-culturable ISA virus, is avirulent, but the lack of culture doesn't make something -- the lack of culturability doesn't actually, in itself, make something virulent or non-virulent. There's many viruses out there that are not culturable.

But the thought was that if this was something that wasn't culturable, maybe it is an HPR0-like, but we have no direct evidence of that.

Q Would it be correct to say that this is still a cause for concern and that there is a worry that it could, at some point, mutate into a more virulent form?

DR. MILLER: Well, you should probably ask the three virologists here, but I would say this is not something we should just simply drop.
Q Dr. Kibenge, can I ask for your thoughts on that?
DR. KIBENGE: In my opinion, I think we need a little bit more information on the sequence of this virus to be able to be definitive about what it could do or what could happen to it in terms of mutation, so (indiscernible).
Right now the only information we have, at least in terms of sequence, is the bits and pieces from Dr. Miller and also from Molly Kibenge as well. So I don't think there's enough information there for me to speculate on what would happen.
MS. CAMPBELL: Thank you. And I've just been reminded that Conservation Commission -- or counsel document number 1 be marked as an exhibit.
THE REGISTRAR: Exhibit 2083.

EXHIBIT 2083: Email from Stephen Stephen to Brian Evans dated November 27, 2009 re positive finding of ISAV

MS. CAMPBELL: I'd like to turn to Commission counsel document number 42 which I believe is now an exhibit. I'm not sure that I got the exhibit number, so if it isn't an exhibit, I'd ask that it be marked as an exhibit. This document is the notes -- the Dr. Miller notes from the meetings that had occurred on November 18th and 24th.
MR. MARTLAND: Our note is 2055.
MS. CAMPBELL: Thank you.
Q In the middle of the second paragraph of that note, there is a reference to Gary Marty and some of the work that had been done by the province. If you just pull up the middle of that, you can see there it says [as read]:

Gary Marty had previously provided this TaqMan assay to the Cohen Commission, but this is not the assay that he is applying in his lab.

I'm wondering if I might ask Dr. Miller to comment on what that means?
DR. MILLER: Well, he had provided a document to the Commission on the assays that people use to detect

ISA, and the assay that we were applying was among the ones listed, but that was not the one that his lab was currently using, so basically as it says. That's about as much as I know about that.

Q And can you tell us how or why -- and we've talked about the fact that the Province has not detected ISA. Can you give us any indication as to how or why the Province has not detected ISA to date? For example, might it have to do with the fact that they would be using different primers?

DR. MILLER: Well, as you can see from our discussion here today that there's many different assays out there and we are getting some divergent results between labs with different assays, so it is possible that what he's using is not -- may have mutations or is not a direct match to the ISA-like virus that we have here.

MS. CAMPBELL: I'm just going to keep flipping on documents. This is a public document but I'm going to ask that we turn to it anyway. It is Conservation Coalition document number 15. It is a statement from the federal minister of Fisheries and Oceans on negative infectious salmon anaemia test results in British Columbia.

Q I'd like us to go down about halfway down the document. There's a paragraph that says [as read]:

> This reinforces --

And it is the Minister's statement, and one of the statements is:

> This reinforces the regular testing conducted by federal and provincial officials. In recent years over 5,000 fresh properly collected and stored samples have been tested and there has never been a confirmed case of ISA.

Dr. Miller, would you agree that the number of tests that have -- and in light of today's testimony, would you agree that the number of tests that have been conducted is meaningless if we don't know what the primer or the methodology was used for those tests?

DR. MILLER: One of the issues that I potentially have

with the way that the Province tests for various diseases is that they combine homogenates of multiple fish and multiple tissues in their testing. So if you had a single fish that was positive in one tissue and then you combined all of the RNA from six different fish and multiple different tissues into the same slurry and then tested that slurry, you're reducing your copy number of the pathogen considerably if you only have one tissue from one fish that was positive.

So I think that that method is somewhat flawed. It certainly decreases the cost of running the analysis, but -- so 5,000 samples, I believe - and you'd have to ask Gary Marty if this is correct - I believe is 5,000 different homogenates from farm fresh silver fish. That's not 5,000 fish, in other words.

Q So if we weren't testing for the right thing, we're not going to get the accurate result.

DR. MILLER: It is possible that what they really mean is 5,000 fish and that each of those homogenates counts for five fish. I don't know the answer to that.

But it seems like a lot of assays, but if you don't know that your assay picks up whatever variant is here, it's sort of meaningless. In my view, if you really wanted to do this properly, you would look at more than one segment of a virus to make sure that you were picking up -- that you weren't picking up false negatives.

Q I'd like to keep moving because time's tight.

MR. MARTLAND: That's fine, and this document is Exhibit 2004 that's on the screen, number 28 from Commission's list.

MS. CAMPBELL: And I'd like to go to Commission counsel document number 45, which I don't think has yet been entered as an exhibit. So this is an email from Laura Richards to Mark Saunders, and it describes a chain of emails between Kristi Miller and Mary-Ellen Walling, and I'd like to have that marked as an exhibit, please.

THE REGISTRAR: Exhibit 2084.

EXHIBIT 2084: Email from Laura Richards to Mark Saunders dated October 4, 2011

MS. CAMPBELL:
Q So this email references a disagreement between you and Mary-Ellen Walling. One of the points that's in here is -- and I think you've spoken about this earlier -- is where you say you wanted samples from industry to test for parvovirus and industry removed that part of the project proposal; is that accurate?
DR. MILLER: Yes.
Q Do you often get samples from industry to do any testing you might do?
DR. MILLER: I probably will again, but I have worked with industry in the past on various issues. I've been involved in the development of molecular assays for pathogens. I worked on the development of the IHN quantitative PCR assay. I've worked on the development and quantitative assay for kudoa thyrsites, so I have worked with industry in terms of developing diagnostic assays for disease in the past.
Q And have they provided you samples of fish in addition to those that were provided by Creative Salmon?
DR. MILLER: In the past?
Q Yeah.
DR. MILLER: Yes.
Q And were the samples of consistent quality or did the quality vary over time and over the -- over companies?
DR. MILLER: I don't think that there are quality issues with the samples that we run. I should also say that my lab does a lot of genetic work with the various aquaculture companies. That's led by Ruth Withler in my lab, and we have a very good relationship with them in terms of tracking genetic brood stocks. So we do -- we do work with industry in my lab, but more recently with the disease stuff, it hasn't been in fruition (sic).
Q And how about getting samples from the province? Have you had any challenges in getting samples from the province at all?
DR. MILLER: Well, the only challenge was that the samples were too degraded by the time we got them, so we went through a series of discussions with the province and we -- they said that they would only transfer the samples with this material transfer agreement which we did sign, but did note

that it was very limited in what we were able to do with those samples.

But the biggest problem really was that the way that those samples were shipped and the way they came, the condition they came to my lab in.

Q And I'd actually like to ask, just a bit more broadly to take it up a notch, do you think that in terms of sample collection in the future, that it would be good for one entity to collect samples? I know that recommendations like this have been made elsewhere so, for example, an independent entity or DFO would be collecting samples directly.

DR. MILLER: I don't know that I have a huge comment on that, but I do think that it's very important, something that the province didn't do when they were in charge of the audit program, was keep the samples that were collected as an archive, so that anyone -- so that one could go back, and if new pathogens are discovered, one can go back and find out how long those things have been here. So for all of the years that the province has been involved, they do not keep tissue samples. They keep the histology slides, but not the tissue samples.

So I think DFO is taking a different view on that, that they do need to have archives of these tissue samples over time. But in terms of an independent body, I mean, I do trust DFO to collect the samples and if they're archived properly, they should be made available to look at in terms of emerging diseases in the future.

MS. CAMPBELL: In terms of the detection of the ISA virus, there's been a number of developments. And I'd like to turn to Conservation Coalition document number 18.

I changed my mind. I'd like to turn to document number 19, so it's the one right after document number 18. What this is, is a result of the detection of ISA in B.C. Two U.S. senators have submitted a bill to Congress that would address concerns about ISA on the west coast. This bill was submitted by -- I believe they were Washington and Alaska State senators. I'd like to scroll down to the research objectives which are quite far down, and I'd like to ask her if you -- and I'd like to ask the panel if you agree or

disagree with these recommendations.
Q So some of these recommendation are that the bill is calling for coordinated research for a variety of named subject matters including the prevalence of ISA in farmed and wild salmon, the susceptibility by population or species, the management strategies to respond to an outbreak, and the role that fish farms might have played.
Dr. Kibenge, would you agree that research into these matters would be prudent?
DR. KIBENGE: Yeah, in my view I think absolutely necessary.
Q Thank you. Dr. Gagné (sic), would you agree?
MS. GAGNE: I agree.
Q Thank you. Dr. Miller?
DR. MILLER: Yes.
Q And Dr. Nylund in Norway, would you agree as well, sir?
DR. NYLUND: I would agree that you need a lot of knowledge about diseases in wild populations and, of course, if you look at Norway and Canada, we know too little about natural occurring disease agents in wild populations.
But before you start looking at ISA virus in wild population, maybe you should find it in farm populations. If you want to find it in farm populations, you should probably start looking at the brood fish at stripping time, because in our experience, you will have 80 to 90 percent of a brood fish positive for ISA virus if you look at Atlantic salmon.
So if you have North Atlantic ISA virus in Western Canada, you should look at the brood fish.
MS. CAMPBELL: That is all of my time and all of my questions. Thank you.
MR. MARTLAND: I don't know if this document has been marked.
MS. CAMPBELL: Oh, my apologies.
MR. MARTLAND: Perhaps if that happens, and then I have Mr. Rosenbloom for Areas D and B with ten minutes next. Sorry, the exhibit number on that...?
MS. CAMPBELL: The exhibit number.
THE REGISTRAR: Exhibit 2085.
MR. MARTLAND: Thank you.
MS. CAMPBELL: Thank you.

EXHIBIT 2085: Document describing U.S.

Congressional amendment with recommendations

MR. ROSENBLOOM: Good afternoon, witnesses. My name is Don Rosenbloom. I appear on behalf of Area B and Area D, and for those that are foreigners, those are -- that's a portion of the commercial fleet here on the west coast. The time right now is 3:21. I have only ten minutes. The two witnesses that won't be here tomorrow that obviously I'm concentrating on -- and in fact my questions are solely directed at Dr. Miller.

CROSS-EXAMINATION BY MR. ROSENBLOOM:

Q Firstly, Dr. Miller, I want to feed on something that came out during Mr. McDade's cross-examination of you. You spoke of being alienated, you spoke of not being on speaking terms with members of your Department and superiors of your Department. I wonder if you could tell us what is your perception of the cause of this situation, of this rift? Is it because you took the initiative to carry out an investigation of ISA in your lab, or is it hat you received or obtained positive results?

DR. MILLER: I think it more speaks to that I'm working in the area of disease and fish health, and there's a -- that we are obtaining data quite quickly on wild populations on diseases that they may carry, and we're not the Fish Health Lab.

Q Have you sensed that your superiors are angry about the fact that you have obtained some positive results about ISA?

DR. MILLER: I have to be clear. I don't believe that all of my superiors are angry about this. I think that there's some issues about the Molecular Genetics Lab, which is my lab, and the Fish Health Lab's ability to work together. But, yeah, I don't really want to paint all managers as being angry about these results.

I don't think managers like surprises, and the one thing that I have been told is that we get data too fast, and just when they're trying to catch up with one thing that they're told that our data are showing, we come up with a whole bunch of more information. So the speed at with which -- and this is just genomics. We have some very high

through-put technology and we can learn. I mean, we can run 30 pathogens in 200 fish in a day, quantitatively. And so there's a lot of power in the level of information one can get very quickly, and I'm learning that for managers, having new information all the time is not necessarily a good thing because they don't have time to adapt to that.

Q Would you not agree with me that some of your superiors would be unhappy that positive results would lead to an internationally bad reputation for Canada?

DR. MILLER: Oh, I think that there's some underlying issues with that, yes.

Q Yes. And to that point, tomorrow Mr. Stephen Stephen will be testifying, and in a will-say document that was provided to us that's not in evidence right now, the document says that [as read]:

> He may answer questions about what he told Dr. Miller about her testing fish samples for ISAV and what the consequences of her making a positive report of ISAV findings would be.

My question to you is - Mr. McDade flirted with this issue and you gave a bit of a response - what did Mr. Stephen say to you were the consequences of you having come up with a positive finding of ISA?

DR. MILLER: Just to understand this, specifically what he talked to me about was that there was a policy in place about ISA that was developed between DFO and CFIA. Policy cannot be a moving target, so research could come up with new results of new orthomyxoviruses, but that the sentiment that I got was that research should not fog policy, so -- but my take, as a scientist, is that research should inform policy, and if policy has to change based on new findings, then that's what it has to do. But I don't come from a manager's standpoint, I come from a scientist's standpoint.

Q Did you interpret his comments to you in any way that he was attempting to intimidate you, Dr. Miller?

DR. MILLER: I personally took a level of intimidation at the idea of my samples perhaps being taken

away. I don't know that he meant -- you know, I mean, it was said to me by a number of different individuals over again, and of course I did read about what happened to Rick Routledge's samples in his freezer in his graduate students' program when CFIA took away all those samples and they weren't able to continue with the research that they were doing.

Of course, I look at my own program and I think I have a lot to lose here if CFIA decided to sweep in and take all my samples. I've got thousands of samples and a very big program in jeopardy, so whether Stephen Stephens (sic) meant that or not, I certainly have been very concerned about that.

Q Did he say anything in terms of how positive findings might be consequential in terms of our relations with the Americans?

DR. MILLER: I think he just intimated that I, as a scientist, would not understand the complexities of these issues and that, as a scientist, I should not be undertaking research on something if I didn't understand the ramifications of what the results could do.

Q And you took that as being intimidation, did you not?

DR. MILLER: Some level of intimidation.

Q Thank you. My last area of examination relates to this. How is the public to take what we are hearing today in terms on -- on a non-scientific level. We have heard that there are some positive findings of ISA. We hear from Dr. Kibenge, we hear from yourself, and we hear from Norway. Assuming the worst scenario here for a moment, where does all this take us?

If indeed it is determined that the virus in question can be isolated, can be sequenced and cultured, and if indeed it is determined that it can mutate into a pathogenic strain, what are the consequences here in British Columbia if indeed that is the situation? My question to you, Dr. Miller?

DR. MILLER: Well, that's a lot of if's. I don't even know where to go with that. Personally, I think we do have to be concerned and we certainly have to look at this. I don't think ISA is the only pathogen out there that we need to be concerned

with.

But it really would depend if it mutates to something that causes mass mortality in wild fish, because that's my main focus, and I don't believe that we have any indications to date that show that. But yet we haven't done enough research to know.

Q Well, I have two or three minutes left. Basically, the reasons why you feel this is important work, the reasons why obviously Dr. Morton has sent samples off for testing to laboratories, and I would like to have explained to this Commission and to the public, why is there this great interest in isolating this virus through lab analysis and, indeed, why this could take us to the next stages of culturing and indeed the possibility of pathogenic consequences?

DR. MILLER: Well, again, I don't think that this virus is the only one that we need to be focused on. However, I think that it is recognized by a lot of experts that viruses do carry the potential, some viruses, to cause the level of devastation that we're seeing on our wild stocks, and we just need to know whether or not that could be a mechanism that is undermining the performance and lowering the productivity of our wild stocks.

I came away from the last round with the Fish Health really quite dismayed by the thought that we simply can't study wild fish, and the lack of culturability of so many of the pathogens - this particular strain of this one possibly being one - and by the sort of flippant dismissal of pathogens that we don't know exist in our wild salmon yet. So I think we really do need to get a fundamental baseline of what viruses and what other pathogens these fish carry and what their potential to cause epidemic levels of disease are.

So there's a lot of speculation about ISA here, and again, we don't have those data but if ISA have a virulence that they see in Norway were to come here and be virulent in our wild salmon, that would be a disaster.

MR. ROSENBLOOM: Thank you very much. I have no further questions.

MR. MARTLAND: Mr. Commissioner, next I have counsel for the First Nations Coalition with 15 minutes.

MS. REEVES: Good afternoon, Mr. Commissioner. Crystal

Reeves with the First Nations Coalition and, with me, my co-counsel, Leah Pence. Just for the witnesses, the First Nations Coalition is a broad range of First Nations in British Columbia, including the First Nations Fisheries Council, the Council of Haida Nation, First Nations up and down the Fraser River as well as some First Nations on Vancouver Island.

CROSS-EXAMINATION BY MS. REEVES:

Q My first set of questions will go to you, Dr. Miller, and if we could have Exhibit 2052 brought up, please, and to page 5.

Earlier today you spoke, Dr. Miller, about the work of your post-doctoral student, Dr. Brad Davis. We won't go into the details again of that, but at the last page, just the very last paragraph is -- he says that:

> What we can take [away] from these analyses is that salmon infected with the BC...ISAV oxymythoxin (sic) virus are [is] responding quite strongly...in a manner that is [remains] similar to responses to influenza viruses in mammals. Therefore, we cannot at this point assume that this virus does not cause disease in these fish. Follow up controlled laboratory challenge work is warranted.

I'm just wondering if you could comment on what further work you're hoping to do in the lab to confirm some of these results?

DR. MILLER: Well, again, I'm not a virologist so I don't do the follow-up laboratory challenges. That would be the virologists in the lab to do that.

But clearly one needs to try to isolate this. I mean, culture might be very difficult. I think something that didn't come up previously is ISA took something like eight years to culture out of Norway. I mean it wasn't culturable in the beginning either, the strains that are in Europe, and it took the development of a special cell line to be able to culture it.

So maybe some efforts to try to culture what

we have here are warranted, because once you have a culture, it's much easier to do very controlled laboratory challenges. Otherwise you are stuck with having to just take positive tissue and that tissue could have other viruses or other pathogens in it, and it's not the ideal source of challenge material.

But, really, these guys are better people to speak to about what kind of challenge work one would design in association with this. I'm really the molecular biologist who does the molecular part of things.

Q Right. But do you have concerns, given what we've heard today, that this challenge work might not actually go forward or might take place given sort of the -- I guess you could say the transparency issues that we've heard about today?

DR. MILLER: Well, I can say there's been reluctance to do the challenge work on the parvovirus that I've discovered. But I think with these proceedings, I think that there will be enough inertia (sic) behind this, that this will be done.

Q Okay. Thank you. My next set of questions is for Dr. Gagné -- or Ms. Gagné, sorry. I heard you say this morning that your diagnostic lab only tests for known viruses and not the unknowns. Did I get that correct? Is that a proper characterization of what you said?

MS. GAGNE: Unknown viruses in the sense that the way the real-time assays work, you target something, you look for something. We're not able to have an assay that looks for any other -- any known or unknown viruses out there.

Q And so as you're moving forward, I guess my question is there was a differentiation made between research labs and diagnostic labs and you are being a combination of a research lab and a diagnostic lab. If a research lab is to, I guess, find novel viruses in the future, at what point does you (sic), as a diagnostic lab, engage with those tests if you're not working with unknown assays?

MS. GAGNE: It's written in our documents that we adapt, we evolve. Some years ago we didn't know about some of the strains that we know now, and assays have changed over time to adapt when new knowledge appear, so if there's a new knowledge

that warrants that we have to revisit the assays that are in use, we will. There's no question about that.

Q And so is the new information that Dr. Miller and Dr. Kibenge, both of them, and Dr. Nylund, is something that would, I guess, move that forward then? Would you reconsider?

MS. GAGNE: At this stage, I have not yet seen anything that shows this assay we're using is not able to pick ISA as we know it, and I think it was said earlier today we have shown on the sequences that have been found in PBS lab that the reversing probe are matching, and we're probably going soon enough to know if there's a match also on the fourth primer which is the last piece missing of this information, but at this stage, there is, up to now, no indication that it's not working.

Q Thank you. My next question is given the explanations we heard today about the possibility of viruses mutating, particularly perhaps in wild fish, how will a diagnostic lab such as yours meet the challenges of mutations and viruses? Do you have protocols in place for that?

MS. GAGNE: There's a list of viruses or diseases that are regulated in the sense that we look for them because they are of a concern for import and export, for example, so the decisions for the virus tests that we have to do, doesn't rely solely on my shoulders. It's based on, like I said, import/export, presence of viruses or absence of viruses in other regions, zones or other countries, so it's a more complex question to answer that just there.

Q Okay. Thank you. I'd like to move now to Exhibit 2004. This was a statement from the federal Ministry of Fisheries and Oceans Canada, and in the middle of this statement -- I'll maybe just read it out:

> After Canada's reputation has needlessly been put at risk over the past several week[s] because of speculation and unfounded science, additional in-depth, conclusive tests, using proper and internationally recognized procedures, are now complete and we can confirm that there has never been a confirmed case of ISA in BC salmon, wild or farmed.

Dr. Kibenge, do you -- how did you interpret this statement? Are you aware of this statement?
DR. KIBENGE: Yeah, I'm aware of (indiscernible - no microphone).
Q Could you --
DR. KIBENGE: Sorry. Yes, I'm aware of this statement and I've read it several times. My thinking here is that I don't feel it was directed to my work, because I -- this is not the way I see what we do.
Q Mm-hmm.
DR. KIBENGE: So I couldn't identify with it.
Q And so you feel that your science is both valid and founded on proper techniques?
DR. KIBENGE: Oh, of course. Definitely.
Q Dr. Miller, do you have any thoughts about this statement?
DR. MILLER: Well, if you notice, it says "no confirmed case of ISA", not ISAV. So again, it could be a play on words here. There's no confirmed case of ISA as a disease in B.C. and I would say that that's still true. But if one were to read it as ISA virus, it may not be completely accurate.
Q Thank you. Did you feel that this statement was a criticism at all towards your work that you had been doing?
DR. MILLER: I guess the short answer would be yes, but I was a bit surprised when I saw this. Again, I was not really in the loop so...
MS. REEVES: Thank you. If I could go to First Nations Coalition Tab 13. I don't believe it's an exhibit. I'm not sure if it's up on the screen. Sorry, I have the wrong -- it's actually Exhibit 2011, sorry. If we could go to page 5? I believe it's a multi-page -- that's all right. I'll move on to my next question, thanks. Perhaps it's Exhibit 2000. Yeah, that's the right exhibit. If we could go to page 5.
Q Doctor -- or, sorry, Ms. Gagné, just at the bottom of that page, it talks about the accreditation or certification status of the laboratory and this is referencing your laboratory. I'm just wondering what is the OIE quality standard? Is that a standard that your lab could reach?
MS. GAGNE: I'm not sure about this, and I'm not sure that ISO 17025 is inclusive of the OIE standards in the sense that if you have reached 17025 you

are equally as competent as the OIE quality standards. I prefer to differ (sic), I don't know.

Q Okay. And then the other point there is that it says that preparation for ISO 17025 started in 2005, and no accreditation as of November 2011, the tentative date for accreditation for your lab is early 2017; is that correct?

MS. GAGNE: This date has moved a lot -- has changed a lot of times. It takes -- our group, itself, we are probably in a better position than the other sections regarding accreditation, but there's also right now implementation of a laboratory information management system, like a computer system to manage everything, so all this work is going to delay the final implementation date.

Q Right. So you're saying, though, it's not going to take till 2017, or...?

MS. GAGNE: It's not a date set in stone. There's no date right now set in stone. If we're ready before that, we will go for it before that. It depends.

Q Right. And so if you haven't reached accreditation, what does that mean for your lab in terms of what you're allowed to do or can do?

MS. GAGNE: I don't think it allows us -- it doesn't allow us to do things, it's just that we are running as if we are ISO accreditated. We have internal audits, for example, but we didn't have the external auditor coming in the lab and telling us -- like doing the audits himself and telling us, yes, you are ready to receive the certificate that is going to tell you are accreditated.

Q Okay. And if you could just turn to page 19, I understand that this validation that was done, you did in concert with several other labs in Canada; is that right?

MS. GAGNE: Yes.

Q And how were these labs chosen?

MS. GAGNE: They were -- RPC, we had already collaborations with them. We had worked with this lab on different projects in the past, and we knew they were -- they are a private lab and they are running their own assays, so we were confident in their capacity of running real-time PCRs and PCR assays in general.

The Department of Aquaculture, they were

interested in running assays themselves, so we included them in the -- they did it for free. We included them in the process.

Q Did you ever approach Dr. Kibenge's lab to be a part of this study?

MS. GAGNE: We -- no, and I must admit that I kind of didn't think about that. This project was run in collaboration with people at AVC, and somehow it -- it was never brought up.

Q Is that something that you would consider for the future, given Dr. Kibenge's expertise in ISA in Canada?

MS. GAGNE: Yes, probably. It's just at the time the group at AVC running -- we were working with the epidemiology group, and now that you mention it, they never themselves -- it's not that we excluded anyone. They never suggested to include the lab of Dr. Kibenge.

Q Now, I understand that CFIA, in putting together the national Aquatic Animal Health program is looking at other labs to participate. Do you know if -- you may not know this, but do you know if Dr. Kibenge's lab or other labs have been approached to be part of that network of labs?

MS. GAGNE: This is not discussions that are done at my level and I'm not privy to all the details, but I think that that's the intent at some point, that other labs will be able to run assays and -- yes, so I don't see at the moment that any lab is excluded from the process if they're interested. But how it's going to be rolled out, this program, I don't know, and we're still a bit far from that.

MS. REEVES: I think those are all my questions. Thank you.

MR. MARTLAND: Mr. Commissioner, counsel for the Sto:lo and Cheam is the last participant setting aside, I think, Canada had asked a few minutes of time for re-examination in relation to the witnesses - the one witness of Canada's, that is - who can't return after today. So counsel for the Sto:lo and Cheam at ten minutes, now.

MS. SCHABUS: Mr. Commissioner, Nicole Schabus.

CROSS-EXAMINATION BY MS. SCHABUS:

Q Panellists, if I may ask you some questions, and

starting off, I'm going to focus on the two panellists who are only here today, I believe mainly Dr. Miller.

I think, Dr. Miller, it's fair to say that in your research, you are generally taking a different approach from the fish-off (sic) people who will test when told so or engage in testing. Your approach, the way I've seen it, is you start off with a research question or an observation of a certain phenomenon. Initially was early entry of the late-run sockeye.

I think more recently you've been looking at an overall fish health issue that you've been noticing on the basis of your genomics research, and we've already heard from you in the disease hearings regarding that, and you set out and try to find the cause and then gather as much information as possible so to make an informed decision on the basis of it. Is that a fair description of the approach you follow in your research?

DR. MILLER: Yes, I believe that is fair.

Q And one of the things that you found when, let's say, the ISA virus issue became infectious (sic), is your sequencing showed that there is also effects and you're seeing a response in the host, so that is a significant issue that should be taken into account when you're looking at overall fish health issues.

DR. MILLER: My view is that I recognize that we can't -- it's very difficult to study disease 'cause we don't see wild fish die. If we can not only understand what pathogens that wild fish are exposed to and are carrying as they're migrating, look at the loads of those pathogens, and then look at the degree of the host response that they have to those pathogens, it's one way that one might be able to rank which, among the various pathogens, they carry might be causing harm. So that is the approach we're taking.

I only show the data for ISA. We've done this with other pathogens as well. It is a novel approach. We do a lot of development of novel approaches. Really, it's just a way -- if we survey 30 pathogens and we find that there are 12 that we're seeing repeatedly and seven of those we see with very high loads, we go to the genomics

and we say, okay, among those seven, which ones are the salmon responding to the most strongly? That is a way to suggest which ones we should be following up with more work on.

Q And you're also seeing it in the overall context of stresses that these fish are encountering. We were talking about temperatures, but obviously pathogens are something you actually didn't start out studying, but your genomics research led you there.

DR. MILLER: Yes, and we have now a very valuable resource in over 3,000 arrays that have been run on migrating fish over multiple years and multiple species that we can go back to and start asking these kind of questions. We call it retrospective genomics. So we didn't start out with this as an idea, but we gain new information about those same very fish that we ran, and retrospectively we can go back and analyze our microarray data.

Q And looking at it from my clients and from a First Nations perspective, it's actually a similar approach, that they look at overall issues that are going with the fish. They're seeing the -- they start off with an observation, they are realizing there's something wrong with the stocks; for example, Cultus stocks are not recovering, although we are doing a lot of work on this and they want to get to the bottom of it.

I understand First Nations have been collaborating with you in your research, right, and you found that to be very valuable, correct?

DR. MILLER: Yes. They've collaborated in some of social sciences.

Q And they would also be ready to share samples with you and have them tested for all the diseases. You've never been told they wouldn't, right?

DR. MILLER: We work a lot with First Nations in our lab in terms of stock ID, and I would imagine that that would carry through with some of the disease work as well.

Q But currently you would not be able to share any of your findings with them if you had disease-related findings?

DR. MILLER: I don't know that that's the case. I would certainly -- when I work with people, I do certainly try to have a relationship where information is shares both ways.

Q But are you currently in a position, with the directions that you're under, to actually share those research results?
DR. MILLER: Apparently not if it's a reportable disease. The reportable disease would have to go to the CFIA before it could be reported to other people.
Q But you agree with the importance of working with First Nations doing research with them and sharing information as a basis of decision-making.
DR. MILLER: I absolutely do, because they are actually on the ground and they are seeking the salmon in their natural environment. If we -- like, for instance, with the jaundice work that we're doing with Creative Salmon, when some of this came out in the Cohen, there was a surge of people finding these yellow fish out in streams throughout British Columbia. Having those people on the ground making those observations is extraordinarily value.
Q And, for example, if you got an email now from an aboriginal fisheries manager saying, look, in light of everything that we are hearing about ISA virus, should we get samples to you, you wouldn't actively discourage them from sending samples and saying, you know, this is not really an issue?
DR. MILLER: I've been pretty open about receiving those kind of samples.
Q Now, you sat through the Cohen Commission disease hearings, not just the day you were a witness, or the days, but also when, let's call it, the traditional pathologist, fish health people were testifying before you.
DR. MILLER: I'm sorry, what was your question?
Q Sorry, you sat through the Cohen Commission hearings on disease for the full set of hearings. There was a first panel that I would call the more traditional fish health people or fish pathologists, and you heard their testimony, right?
DR. MILLER: Yes, I did. I sat in on --
Q And that's what you were referring to when you were saying it was a little -- it was really concerning to see that they actually don't have a real good grip on assessing disease and dealing with disease when it comes to wild stocks, right?
DR. MILLER: That's what I came away with, yes.

Q And everybody in that panel actually agreed that it was important to do integrated fish health research in the first panel that came before you.
DR. MILLER: Yes. Yes, absolutely.
Q Yet they didn't really have anything much to suggest how they would do it with their traditional methods, right?
DR. MILLER: That was again my feeling, and yeah, very much so, yes.
Q And we had that conversation at the end. That's actually what you're trying to do with your genomics research. And while you're being modest in saying that you can obtain data quickly, but actually the reason why you are able to do that now is because you actually already collected a lot of data and you have so many datasets in your genomic research going back decades by now.
DR. MILLER: We have a very good resource available to us now, certainly, in our lab with our genomics.
Q So your lab is probably in the best position, just has a head start, because of that basis -- that information base when it comes to wild fish and disease, right?
DR. MILLER: I believe that we can add a layer to our knowledge of fish disease and wild fish by using the genomic, and by using the microarray data that we already have, yes.
Q And you have a head start on everybody else because they actually don't have that information in hand.
DR. MILLER: That is correct.
Q And actually haven't ever really focused, as we heard from those panels on wild stocks.
DR. MILLER: No, they haven't. I've been working on wild sockeye salmon for about seven years now.
Q And you're probably the only -- the Fish Health people haven't been doing that really.
DR. MILLER: No, the --
Q It's your lab that's been doing that.
DR. MILLER: Their interest is fairly recent, although Kyle Garver has studied IHN in a couple of wild sockeye salmon stocks for a number of years, and Garth tracks or did before that, so there's like a 35-year database on IHN.
Q Yeah, and it's also indicative that in the research that you had done before this whole ISA virus issue became infectious (sic), that you

already had gone to a number of those areas where you're now positive samples. You had other samples from that same area, the Harrison River, Harrison Creek, Beaver Creek. You were already looking into the issue of pre-spawn mortality because you were seeing increased pre-spawn mortality in the area, right?

DR. MILLER: Yes, and we also have an added advantage that I have a lot of students at UBC who are doing controlled laboratory studies looking at temperature responsiveness, et cetera, and so I can go back to the samples that they have, and we've run microarrays on those sorts of things too.

So I have a very large program on genomics on wild fish and it's quite a valuable resource to look at sort of whole organismal health and pathogens being one aspect of that.

Q And a lot of the research has actually come to you when they have fish health concerns, right, from SFU, from UBC. They actually come to your lab and ask you to also check run arrays on it.

DR. MILLER: I'm usually really clear with people. I'm not the disease lab. I only do the molecular analysis and they really do need to, if they want to do cultures and other things, they need to contacting the Fish Health Lab. But I've done a lot of microarray work with the universities, yes.

Q And you describe, and I think rightfully so, kind of as a threat when you're now in a position where you could actually get a head start and look into those issues related to ISA and other disease, that you're in a position where you're looking at a potential seizing of your samples, correct?

DR. MILLER: It hasn't happened yet, but it is a concern.

Q But you're a DFO research lab, you follow procedures for keeping samples isolated, you avoid cross-contamination and you avoid -- and have everything in place to avoid escape into the natural environment, right?

DR. MILLER: Absolutely.

Q And just as a final question, if we could bring up Tab 23 from Canada's list, Exhibit 2065, 2065.

MS. SCHABUS: It's the suggested survey, Mr. Lunn. Do we have it on? Sorry, I didn't see it. I was looking at the wrong screen, sorry.

Q Dr. Miller, I'd just like you to look at the very beginning where it says -- where basically the research parameters are set out. I'd just like you to comment, in the light of your findings that you've already made, and noting that it says to confirm that ISA with a V, so virus, is not present in B.C. waters.

Can you just comment on that and how that makes you feel as a scientist when what you're trying to study is to get to the bottom of these issues, and yet the suggestion that the intent of the research project is to confirm ISAV is not present in B.C. waters? What would you suggest to do?

DR. MILLER: I think you've picked up on a very important philosophical approach, and the difference between what my lab does and what people studying fish health do. At least, again, this is my view.

Their approach is to make sure it's not there. My approach is to ask if there's any way that it is there. So I might take a different approach to it than they would on that basis.

Q And you'd agree that it's important to continue research into the field and into dealing with ISAV potentially being present in B.C. waters.

DR. MILLER: I would say so, yes.

MS. SCHABUS: Thank you.

MR. MARTLAND: Thank you. Mr. Commissioner, we have only till four o'clock today. I know that's just a few minutes. An hour or two ago Mr. Taylor told me he thought he had only a few minutes of questions on re-examining Dr. Miller specifically, and otherwise re-examining tomorrow. I'm hoping that estimate is still true. If so, that can proceed now.

MR. TAYLOR: I will ask redirect questions of Dr. Miller only, not Dr. Gagné -- Ms. Gagné, those two being the only witnesses that I am entitled to redirect. Ms. Gagné will be here tomorrow and I'll ask her then.

CROSS-EXAMINATION BY MR. TAYLOR, continuing:

Q Dr. Miller, you testified earlier, I think in response to questions from Mr. McDade, that you haven't spoken with anyone after the 24th about

ISA, that is, after November 24th. Am I right, though, that there have been some conversations and emails between yourself and Ms. Gagné about ISA since then?

DR. MILLER: I was talking in Pacific Region, but yes, 'cause I sent samples to her, so there has been some conversation back and forth. But in terms of speaking with managers about their approach to the Cohen and what DFO's approach and response was going to be, no one from DFO could elaborate on that with me.

Q All right. And am I right that you've also had some conversations with local officials, and by that, I mean British Columbia officials of CFIA?

DR. MILLER: Yes, I had a teleconference with CFIA about the Creative Salmon results yesterday.

Q All right. And in terms of not speaking publicly, is it your understanding that the reason for that is so that the evidence, information and facts about matters pertaining to the Cohen Commission are given in this room and not through the media, at least in terms of DFO?

DR. MILLER: Yes, but until this most recent session was called, as far as I was aware, the hearings were over and that was --

Q We all thought so.

DR. MILLER: -- not lifted after the hearings were over.

Q All right. I think coincidentally, the ISA reared up about the same time that the lawyers' arguments were finishing.

With that, is it correct that not speaking publicly is a general requirement and not specifically directed at you.

DR. MILLER: Oh, yeah, I've said that before.

Q Now, Ms. Gagné, as I understand it, said this afternoon that there's nothing to indicate that her assay is not working. So a question of you, Dr. Miller, do you have any comment on whether Ms. Gagné's assay should pick up the -- whatever it is that you've been partially sequencing?

DR. MILLER: I don't have sequence in the region of her forward primer, so I don't know if there are any mismatches in that region. We have, as I've said before, tried to amplify with her primers only, not using a real-time assay, but just using conventional PCR, but with the pre-amplification

step and we were not able to obtain any products from her PCR primers.
Q All right. Do you know why that would be or --
DR. MILLER: Again, I would speculate that there's mismatch underneath the forward primer.
Q All right. Now, my final question, which is a big one, but I want to give you this opportunity as I think it's important. Earlier, Dr. Nylund was commenting on the methodology that you're using and, in particular as I recall, he spoke of pre-amplification and non-specific annealing. I want to give you the opportunity, if you choose to say anything, to reply to anything that you were hearing there.
DR. MILLER: With the parvovirus and some of the other markers that we use, we've run them on the 7900 and we've run them -- with no pre-amp, and we've run them on the Fluidigm system and we get very highly corroborative results. We have sequenced many other pathogens that were pre-amped or not pre-amped and we get the same sequences back. I do not believe that that pre-amp is any issue in terms of getting false sequences.
MR. TAYLOR: All right. Thank you, and I've started a bit late and gone a bit over. I thank you for your indulgence, Mr. Commissioner.
MR. MARTLAND: Mr. Commissioner, it's been a dense and a long day of hearings. We're at the end of today's session. From our point of view, we want to thank all the participants, indeed, everyone in facilitating this, especially Mr. Lunn for linking us to Norway. It's 1:00 a.m. I know for a fact that Dr. Nylund's day at the office in University of Bergen started at 7:00 a.m., so that's a truly heroic effort and we're grateful to him and Dr. Miller for their work, and I wanted to express that gratitude.

I suggest now that we're in a position to adjourn till 9:00 a.m. tomorrow when we continue with Dr. Kibenge and Ms. Gagné.

THE COMMISSIONER: Thank you, Mr. Brock (sic). Is Dr. Nyland still there?
DR. NYLUND: Yes.
THE COMMISSIONER: Good. Doctor, we have heard for years and decades about the superior strength of Norwegians over Canadians, and you today have proven that once again. Thank you very much for

staying up with us. I know it's very late -- very early in your part of the world. We're most grateful that you made yourself available and for sticking with us during the course of the day.

And of course to Dr. Miller for returning and testifying here today, thank you very much, Dr. Miller.

We're then adjourned, is it, until nine o'clock tomorrow morning? Yes. Thank you very much to everyone.

(PROCEEDINGS ADJOURNED AT 4:05 P.M. TO DECEMBER 16, 2011 AT 9:00 A.M.)

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

_____

Pat Neumann

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

_____

Karen Hefferland

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

Irene Lim

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

Diane Rochfort