Notes from November 18 and 24 2011 Briefings on ISA testing results being conducted in the Molecular Genetics Laboratory

In attendance at both meetings were:

Kristi Miller
Karia Kaukinen (my biologist)
Mark Saunders
Mark Higgins
Kyle Garver

The initial meeting convened by Mark Saunders was to discuss the research we doing using various ISA TaqMan assays to determine if there is Orthomyxo viral sequence in salmon in BC. This research was undertaken as a follow-up to ISA screening we had performed a couple of years back when we were looking for a virus that might be causatively associated with our Mortality-related genomic signature. I had testified at the Cohen that we tested for ISA and did not find it. When I heard that Dr. Kibenge had disclosed that he had positive PCR tests for ISA virus on wild Pacific salmon from BC, I felt obliged to go back and look carefully at the testing we had done, and realized that the primers we had used at the time were no longer being used by other labs as they did not amplify all sequence variants. As a result, I felt obliged to re-test our tissues from wild-collected fish used in our Genomics program. As Kibenge had used primers in segment 7 and 8 of the virus (we had previously used primers in segment 6), we tried to find the primers and probes he was using. He did not respond positively when asked for his assays, so we ordered all Taqman assays available for segments 7 and 8. We started testing with the Plarre assays that Gary Marty pointed out in his document disclosed to the Cohen Inquiry.

I had told Mark Saunders that we were performing this testing the week of November 14, and on the 18th, he asked that I meet with him and fish health to let them know what we were finding. We told them we had tested >180 livers and >400 gill tissues from sockeye salmon smolts and identified a small number of PCR positives with a primerset from Plarre for ISA segment 8. 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. We had just obtained a 71 bp sequence that was a 100% match to ISA from one of these fish. We were still working to get both more individuals sequenced and trying to get a longer sequence by using other primer combinations. At the time, they (Fish Health) felt that there was not enough sequence information to make a judgement call on whether this was a viral sequence or not (despite the fact it was a published validated assay), as it had not been, to their knowledge, validated in sockeye salmon.

Hence, it was decided that Kyle Garver should try to validate the results we obtained using the CFIA validated assay that Nellie Gagne designed, as well as trying the primers/probe set we were using. We were to provide blinded positive and negative fish to Kyle, and primers and probe to the assay we used, and any technical details about how we ran the assay. 10 samples were provided to Kyle on Nov 21. On Nov 22, I asked that Kyle provide to me primers and probe and a positive control from the validated assay

so that we could perform reciprocal testing. These were not provided. I called Nellie Gagne to get permission to use her test on Nov 23 and she declined.

In the meanwhile, my group continued to try to get more sequence information from other primer combinations. The ISA7 primerset was amplifying product in about 20% of livers that we tested, and at the time, we felt this was too high and probably not picking up a virus. However, on the evening of Nov 23rd, Karia Kaukinen (a biologist in my group) called me to tell me that she got sequence data from this primer set and it carried a 95% identity with salmon Orthomyxo-viral sequences (all ISA). She had obtained this sequence from 2 sockeye salmon, one smolt and one pre-spawning adult mortality. We believe that the PSM was one of the individuals testing positive in the Kibenge lab. I had, early the previous week, disclosed to Mark Higgins that I thought we may have some of these Harrison Mills samples that were sent to us to test for Parvovirus and to look at in association with our Jaundice study (some were yellow in color). Species ID on these samples showed them to be sockeye. The ISA7 sequence we obtained was 81 bases, with 35 bases between the primers and 3 mutations separating our sequence and those of various strains of ISA (mostly isolated by Kibenge's lab and presented in a 2010 publication from his lab). We also identified a single mutation between the sequences from the two amplified fish. Based on one fixed base found in the North American strain isolates we could identify, the sequences we obtained appeared to be closer to the European-origin strains. This is the same finding that Kibenge had. Karia had also obtained two more sequences from the ISA8 primerset, both identical to the first sequence. These were obtained from a second sockeye salmon and a Chinook salmon.

This information was presented to the Fish Health Group and later summarized to Stephen Stephens in Ottawa on Nov 24 2011:

Present at the face to face meeting
Myself
Karia Kaukinen
Mark Saunders
Mark Higgins
Kyle Garver
Stewart Johnstone

At that time, Kyle Garver showed that he was unable to pick up any PCR positives from our samples using the CFIA validated primers/probe from Nellie Gagne's lab but when using our revised protocol and the ISA8 Plarre Taqman assay, he did pick up product. He concluded that it was not likely ISA because the CFIA assay did not work. Given the sequence information we had, we discussed whether this could be a distantly related Orthomyxovirus endemic to BC or an ISA virus that has been here for some time (to yet be determined—we had already shown that there was some divergence between our sequence and other known strains). We agreed that more information was needed to determine how long this virus had been here.