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

Lab #	Sample ID		CHSE-214 P1 CPE	ISAV seg 8 Probe, Cts detects all ISAV	ASK-2 P2 CPE	ISAV seg 8 Probe, Cts detects all ISAV	SHK-1 P2 CPE	ISAV seg 8 Probe, Cts detects all ISAV	TO P2 CPE	ISAV seg 8 Probe, Cts detects all ISAV
VT 10042011-1	Sockeye heart	1	Neg	not done	not done					
VT 10042011-2	Sockeye heart	2	Neg	not done	not done					
VT 10042011-3	Sockeye heart	3	Neg	not done	not done					
VT 10042011-4	Sockeye heart	4	Neg	not done	not done					
VT 10042011-5	Sockeye heart	5	Neg	not done	not done					
VT 10042011-6	Sockeye heart	6	Neg	not done	not done					
VT 10042011-7	Sockeye heart	7	Neg	not done	not done					
VT 10042011-8	Sockeye heart	8	Neg	not done	not done					
VT 10042011-9	Sockeye heart	9	Neg	not done	not done					
VT 10042011-10	Sockeye heart	10	CPE*	0	not done					
VT 10042011-11	Sockeye heart	11	CPE*	0	not done					
VT 10042011-12	Sockeye heart	12	CPE*	0 (37.88**)	not done					
VT 10042011-13	Sockeye heart	13	CPE*	0	not done					
VT 10042011-14	Sockeye heart	14	Neg	not done	not done					
VT 10042011-15	Sockeye heart	15	CPE*	0	not done					
VT 10042011-16	Sockeye heart	16	Neg	not done	not done					
VT 10042011-17	Sockeye heart	17	Neg	not done	not done					
VT 10042011-18	Sockeye heart	18	Neg	not done	not done					
VT 10042011-19	Sockeye heart	19	Neg	not done	not done					
VT 10042011-20	Sockeye heart	20	Neg	not done	not done					
VT 10042011-21	Sockeye heart	21	CPE*	0	not done					
VT 10042011-22	Sockeye heart	22	CPE*	0	not done					
VT 10042011-23	Sockeye heart	23	Neg	not done	not done					
VT 10042011-24	Sockeye heart	24	Neg	not done	not done					
VT 10042011-25	Sockeye heart	25	Neg	not done	not done					
VT 10042011-26	Sockeye heart	26	Neg	not done	Neg	0	Neg	0	Neg	0
VT 10042011-27	Sockeye heart	27	Neg	not done	not done					
VT 10042011-28	Sockeye heart	28	Neg	not done	not done					
VT 10042011-29	Sockeye heart	29	Neg	not done	not done					
VT 10042011-30	Sockeye heart	30	CPE*	0	not done					
VT 10042011-31	Sockeye heart	31	Neg	not done	not done					
VT 10042011-32	Sockeye heart	32	Neg	not done	not done					
VT 10042011-33	Sockeye heart	33	Neg	not done	not done					
VT 10042011-34	Sockeye heart	34	Neg	not done	not done					
VT 10042011-35	Sockeye heart	35	Neg	not done	not done					
VT 10042011-36	Sockeye heart	36	CPE*	0	Neg	0 (40.92**)	Neg	0	Neg	0
VT 10042011-37	Sockeye heart	37	CPE*	30.68 (31.58)	not done					
VT 10042011-38	Sockeye heart	38	Neg	not done	not done					
VT 10042011-39	Sockeye heart	39	Neg	not done	not done					
VT 10042011-40	Sockeye heart	40	Neg	not done	not done					
VT 10042011-41	Sockeye heart	41	CPE*	0 (38.66)	not done					
VT 10042011-42	Sockeye heart	42	Neg	not done	not done					
VT 10042011-43	Sockeye heart	43	Neg	not done	not done					
VT 10042011-44	Sockeye heart	44	Neg	not done	not done					
VT 10042011-45	Sockeye heart	45	Neg	not done	not done					
VT 10042011-46	Sockeye heart	46	Neg	not done	not done					
VT 10042011-47	Sockeye heart	47	CPE*	0	not done					
VT 10042011-48	Sockeye heart	48	CPE*	0	not done					
ADL-ISAV (European genotype)				18.89 (18.34)		19.06 (18.78)		19.06 (18.78)		19.06 (18.78)
NBISAV01 (North American genotype)				19.04 (18.33)		19.21 (18.44)		19.21 (18.44)		19.21 (18.44)
NTC (water)										

EXPLANATORY NOTES:

1. Detailed description of how 600 ul of tissue homogenate of each sample were used is attached to this report.
2. All samples were inoculated on CHSE-214 cell monolayers in 24-well plates, and were incubated at 16C for 21 days. In 13 of the cultures, a cytopathic effect (CPE) was observed 14-17 days post-incoculation.
3. Samples #26 and #36 were also inoculated on ASK-2 cell monolayers in 24-well plates, and were incubated at 16C for 6 days. These cultures were CPE negative.
4. Samples #26 and #36 from P1 on ASK-2 cell line were blind-passaged on ASK-2, SHK-1 and TO cell lines in T-25cm flasks at 16C for 10 days. All the P2 cultures were CPE negative.
5. Cell lysates of the CHSE-214 cultures and the ASK-2, SHK-1, and TO cultures in T-25 cm flasks were were used to extract RNA for RT-PCR testing.
6. Using real-time RT-PCR with TaqMan probes for ISAV segment 8, some of the cultures had cycle threshold (or Ct) values (in one** in one of the replicates) with one or both of the algorithms.
7. All cell lysates were negative using conventional RT-PCR for segment 8. On this basis, all cultures were RT-PCR negative for ISAV.
8. Further passages in CHSE-214 and ASK-2, SHK-1 and T0 cell lines have either not yet been carried out or are awaiting RT-PCR testing. A sample will be considered negative for virus isolation after three blind passages without CPE.
9. The laboratory did not participate in the collection of the samples or in the custody of the samples prior to receipt of the samples. The laboratory therefore cannot guarantee the integrity of the samples.
10. For convenience, the samples are identified using the labels provided by the party who requested testing by the laboratory.
11. The samples were tested as received at the laboratory.
12. In accordance with the Health of Animals Act, the test results have been reported to representatives from the CFIA by the laboratory.

INTERPRETATION:

1. *CPE in CHSE-214 cell line consisted of cell rounding uniformly involving the whole cell monolayer. This is not characterictic of ISAV.
2. Ct up to 40 are positive. Ct between 40.1 and 45 are considered suspicious. Sample is negative if there is no Ct value.

Number in brackets is Ct using Fit Points algorithm

Detailed description of how the 600 µL of tissue homogenates of the 48 sockeye smolts samples (Case VT10042011) were used

All samples (hearts) were macerated and each was suspended in **600 µL** of L-15 medium.

For each sample, **300 µL** were used for RNA extraction and were eluted with **50 µL** of RNase/DNase free water [normally, there is some loss in volume during elution; therefore the amount recovered is NOT exactly the **50 µL** that is applied to the column].

For each sample, **100 µL** were used to inoculate CHSE-214 cell monolayer (in 24-well plate).

The extracted RNA was used as follows:

1. **16 µL** was used in ISAV segment 8 real-time RT-PCR (i.e., **8 µL** per reaction in duplicate).

2. For the 2 samples that were positive with ISAV segment 8 real-time RT-PCR (i.e., samples # 26 and # 36), conventional RT-PCR was set up to obtain PCR products for cloning and sequencing. Conventional RT-PCR was set up for ISAV segment 8, segment 6 (for HPR and full-length), and segment 5 (for 5' fragment and 3' fragment) for a total of 5 reactions each, using **2 µL** of RNA template each (total **10 µL**).

To obtain sufficient RNA to use in ISAV genotyping, the remaining **200 µL** for each of the 2 samples that were positive with ISAV segment 8 real-time RT-PCR (i.e., samples # 26 and # 36) were used for RNA extraction and were eluted with **30 µL** of RNase/DNase free water and pooled with the remaining volume from the first RNA extraction. This RNA was then used in ISAV segment 6 real-time RT-PCR genotyping [North American and European] using **8 µL** of RNA template per reaction, in duplicate, for each genotype reaction (total **32 µL** of RNA template per sample). There is some very small volume of RNA left; stored at -80^{o}C.