Report, 2nd November 2011

Testing of gill samples from juvenile *Oncorhynchus nerka* (sockeye salmon) collected in Rivers Inlet on the central coast of British Colombia, Canada.

RNA from all gill samples was extracted as described by Devold et al 2000. The amount of RNA in each extraction sample was measured by NanoDrop ND-1000 (Spectrophotometer) (Table 1). For each tissue sample a negative control sample was included. An assay targeting the housekeeping gene, elongation factor alpha, was used as an internal control to test the quality of the RNA. We used the elf-alpha from Atlantic salmon which is not optimal for use on *O. nerka*. Two different assays targeting know ISA viruses were used: a) Assay **ISAV7** targeting segment seven from European ISA viruses (Plarre et al 2005), and b) assay **ISAV8-Uni** targeting segment 8 from all known ISA viruses (Snow et al 2006). The results of the analysis of the first tissues are presented in table 1. All samples were negative for presence of ISA virus genome. To make sure that the assays were working we included one positive control (Norwegian ISA virus) (Table 2). In the second and third run of the positive control we used a dilution of the RNA used in the first run.

According to the report from Kibenge the heart tissues from individuals 26 and 36 were positive for ISA virus using the ISAV8-Uni assay. Hence, we performed reruns on the gill samples from these two individuals and, in addition to the two ISA virus assays already used, we added one assay targeting segment 8 (European ISA viruses) and an HPR0 ISA virus assay. The most sensitive of these assays, used on European ISA viruses, are the ISAV7 and ISAV8 assays. As can be seen from table 2 the ISAV8-Uni is less sensitive compared to the ISAV7 assay. The ISAV-HPR0 assay is targeting segment 6 and is three ct values less sensitive then the ISAV7 assay. The results of the rerun are presented in table 3. Sample 36 was positive for ISA virus genome in one of the replicas. The ct value of 36.3 is close to the detection limit for the ISAV7 assay which may explain why only one of the replicas was positive, ie. the amount of ISA virus genome is to low to give a reproducible result. It should be added that none of the negative controls were positive in the first run or in the reruns of samples 26 and 36.

To test our results we performed a new extraction of RNA from the remaining gill tissues from the two individuals 26 and 36. These samples were run as five replicas. All samples

were negative for presence of ISA virus (Table 4). The positive controls were positive and the negative controls were negative.

As additional controls we also tested for two other pathogens, the parasitic flagellate *Ichthyobodo* spp. and the gill chlamydia Candidatus Clavochlamydia salmonicola. The latter is associated with epithelicystis in fresh water (Karlsen et al 2008). The results are presented in table 1. All samples tested were positive for presence of these two pathogens. However, when we tried to sequences the SSU from both we were not able to obtain a PCR product when using the RNA after cDNA synthesis. This indicates that the quality of the RNA is poor. Since the target for all ISA virus assays used in this study is RNA the poor quality may have influenced on the results.

Conclusion

We were able to detect ISA virus genome in gill sample 36, but this result was not reproducible. The ct value of the positive sample was close to the detection limit for the assay. The results obtained by Kibenge (using heart tissue) could not be reproduced by us using gill tissues from the same individuals. This could be explained as a result of tissue tropism for ISA virus in *O. nerka,* or a combined result of tissues tropism and poor quality of the RNA in the gill tissues sent to us. To my knowledge nothing is known about the susceptibility of *O. nerka* to ISA viruses, and the tissue distribution of the virus in this species is unknown.

Table 1. Results of the first testing of gill tissues.

		Gills	Gills	Gills	Gills	Gills	Gills
	RNA	**Elongation f.**	**Negative**	**ISAV**	**ISAV**	***Ichthyobodo***	**Clavochlamydia**
Sample	**ngram/µl**	**alpha**	**Control**	**ISAV7**	**ISAV8-uni**	**spp.**	**salmonicola**
1	522,3	19,4	Neg	Neg	Neg	35,1	25,4
2	373,7	22,0	Neg	Neg	Neg	33,1	25,3
3	460	22,9	Neg	Neg	Neg	26,7	27,5
4	162,1	21,3	Neg	Neg	Neg	14,1	37,7
5	137,6	23,0	Neg	Neg	Neg	25,7	36,2
6	298,2	22,0	Neg	Neg	Neg	18,2	26,4
7	272,3	23,7	Neg	Neg	Neg	30,4	30,9
8	2,2	26,8	Neg	Neg	Neg		
9	223,8	25,6	Neg	Neg	Neg		
10	480,5	23,7	Neg	Neg	Neg		
11	702,4	25,4	Neg	Neg	Neg		
12	5,9	28,3	Neg	Neg	Neg		
13	447,9	23,3	Neg	Neg	Neg		
14	1752,6	29,3	Neg	Neg	Neg		
15	1067,7	22,3	Neg	Neg	Neg		
16	813,5	23,8	Neg	Neg	Neg		
17	813,3	24,0	Neg	Neg	Neg		
18	86,2	19,1	Neg	Neg	Neg		
19	446,7	20,2	Neg	Neg	Neg		
20	475,4	20,2	Neg	Neg	Neg		
21	485,4	20,2	Neg	Neg	Neg		
22	902,6	17,5	Neg	Neg	Neg		
23	779	21,4	Neg	Neg	Neg		
24	0	25,3	Neg	Neg	Neg		
25	693,6	17,1	Neg	Neg	Neg		
26a	440,5	21,7	Neg	Neg	Neg	32,7	18,4
26b	330,1	22,9	Neg	Neg	Neg	32	17,5
27	455	20,4	Neg	Neg	Neg		
28	1361,8	21,4	Neg	Neg	Neg	22,2	
29	498,7	19,5	Neg	Neg	Neg	27,0	
30	304,1	20,3	Neg	Neg	Neg	31,5	
31	507,8	18,7	Neg	Neg	Neg	35,0	
32	425,8	19,5	Neg	Neg	Neg	29,9	
33	457,6	19,1	Neg	Neg	Neg	25,1	
34	52,9	20,8	Neg	Neg	Neg	32,3	
35	776,5	19,6	Neg	Neg	Neg	33,5	
36	531,8	21,9	Neg	Neg	Neg	30,9	26,2
37	108,2	18,0	Neg	Neg	Neg	29,6	
38	986,9	18,9	Neg	Neg	Neg	20,2	
39	747,5	17,8	Neg	Neg	Neg	35,8	
40	835,7	19,6	Neg	Neg	Neg	29,4	
41	293,7	19,8	Neg	Neg	Neg	26,8	
42	924,6	19,0	Neg	Neg	Neg	30,8	
43	696	18,7	Neg	Neg	Neg	21,6	
44	567,6	18,5	Neg	Neg	Neg	24,7	
45	488	18,7	Neg	Neg	Neg	28,8	
46	87,9	19,4	Neg	Neg	Neg	30,1	
47	295,1	18,8	Neg	Neg	Neg	32,1	
48	537,4	16,9	Neg	Neg	Neg	31,4	

Table 2. Positive controls of the three assays (Norwegian isolate used).

Sample	ELF	ISAV7	ISAV8-uni	Date	Dilution
Pos control	14,8	21,7	26,3	26.10.2011	
Pos control	17,3	24,0	29,8	27.10.2011	1+ 9
Pos control	17,7	24,2	27,8	31.10.2011	1+ 9

Table 3. Rerun of samples 26 and 36.

	rerun	rerun	rerun	rerun
Sample	**ISAV7**	**ISAV7**	**ISAV7**	**ISAV7**
26a	Neg	Neg	Neg	Neg
26b	Neg	Neg	Neg	Neg
36	36,3	Neg	Neg	Neg

	rerun	rerun	rerun	rerun
Sample	**ISAV8**	**ISAV8**	**ISAV8**	**ISAV8**
26a	Neg	Neg	Neg	Neg
26b	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg

	rerun	rerun	rerun	rerun
Sample	**ISAV8-Uni**	**ISAV8-Uni**	**ISAV8-Uni**	**ISAV8-Uni**
26a	Neg	Neg	Neg	Neg
26b	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg

	rerun	rerun	rerun	rerun
Sample	**ISAV-HPR0**	**ISAV-HPR0**	**ISAV-HPR0**	**ISAV-HPR0**
26a	Neg	Neg	Neg	Neg
26b	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg

Table 4. New run of the second extraction of RNA from gill samples 26 and 36 (five replicas). The positive control was run as two replicas.

Sample	ISAV7	ISAV7	ISAV7	ISAV7	ISAV7
26	Neg	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg	Neg
Pos control	15,7	15,7			

Sample	ISAV8	ISAV8	ISAV8	ISAV8	ISAV8
26	Neg	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg	Neg
Pos control	15,0	14,9			

Sample	ISAV8-Uni	ISAV8-Uni	ISAV8-Uni	ISAV8-Uni	ISAV8-Uni
26	Neg	Neg	Neg	Neg	Neg
36	Neg	Neg	Neg	Neg	Neg
Pos control	16,3	16,9			

Sample	ELF
26	18,9
36	18,8
Pos control	24,9

Literature

Devold M, Krossay B, Aspehaug V, Nylund A (2000). Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout *Salmo trutta* after experimental infection. Dis Aqat Org 40: 9 – 18.

PlarreH, Devold M, Snow M, Nylund A (2005). Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway. Dis Aquat Org Vol. 66: 71–79.

Snow M, McKay P, McBeath AJ, Black J, Doig F, Kerr R, Cunningham CO, Nylund A, Devold M (2006). 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). Dev Biol (Basel) 126:133-45.

Karlsen M, Nylund A, Watanabe K, Helvik JV, Nylund S, Plarre H.(2008). Characterization of 'Candidatus Clavochlamydia salmonicola': an intracellular bacterium infecting salmonid fish. Environ Microbiol. Jan;10(1):208-18. Epub 2007 Sep 24.

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