

Report-I, 27th October 2011

Testing of gill samples from juvenile *Oncorhynchus nerka* (sockeye salmon) collected in Rivers Inlet on the central coast of British Columbia, 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). For each tissue sample a negative control sample was included. One positive control was included for testing of the three assays used. An assay targeting the housekeeping gene, elongation factor alpha, was used as an internal control to test the quality of the RNA. Two different assays targeting known ISA viruses were used: a) Assay **ISAV-S7** targeting segment seven from European ISA viruses (Plarre et al 2005), and b) assay **S8-ISAV-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.

Table 1. Preliminary results of the real time RT PCR screening.

	NanoDrop	Gill tissue	Gill tissue	Gill tissue	
Sample	ngr/μl	ELF	ISAV7	8-ISAV-uni	Neg control
1	522,3	19,4	Neg	Neg	Neg
2	373,7	22,0	Neg	Neg	Neg
3	460	22,9	Neg	Neg	Neg
4	162	21,3	Neg	Neg	Neg
5	137	23,0	Neg	Neg	Neg
6	298,2	22,0	Neg	Neg	Neg
7	272,3	23,7	Neg	Neg	Neg
26a	440,5	21,7	Neg	Neg	Neg
26b	330,1	22,9	Neg	Neg	Neg
36	531,8	21,9	Neg	Neg	Neg
Pos Control		14,8	21,7	26,3	

RNA was extracted twice from two different pieces of gill tissue in sample 26 (26a and 26b).

The quality of the RNA may not be optimal according to the internal control assay. All negative controls are negative for presence of ISA virus genome, while both ISA virus assays gave a positive result on the positive control. All gill samples from the juvenile sockeye salmon tested are negative in both Real time RT PCR tests for presence of ISA virus.

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 Aquat 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.

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