Report, 23rd November 2011

Testing of gill and heart samples from salmonids collected in British Colombia, Canada.

RNA from all gill and heart 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. An assay targeting the housekeeping gene, elongation factor alpha, was used as an internal control to test the quality of the RNA. This time we used the elf-alpha from rainbow trout/coho salmon (unpublished assay). 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 tissues are presented in tables 1 and 2. The positive samples of gills and heart were tested in five reruns. Only one rerun of the gill tissue was repeated. Both ISA viruses assay have the same sensitivity for detection of ISA virus genome ss can be seen from the positive controls.

Conclusion

The RNA from both tissue types (gills and heart) seem to be of reasonable quality for real time RT PCR, and the amount of RNA obtained after extraction was substantial. Hence, the amount and quality of the RNA should not have influenced on the results.

Under ideal conditions we should be able to repeat all positive results when the Ct values are below 37. The result obtained from sample H10 (gill tissues) was Ct = 34.5 and the result was repeated once (Ct = 35.4), while the other four reruns were negative. The heart tissue from the same individual was also negative. The gills from sample H14 was negative, but one of five runs from the heart tissue was positive. None of the samples were positive when using the Uni-ISAV8 assay. As can bee seen from the positive controls both assay have the same sensitivity for detection of ISA virus RNA from European ISA viruses. This fact raises the question: *What are we detecting with the ISA7 assay?* Based on my experience with both assays a reasonable answer to this question is that we are not detecting any of the known ISA viruses from Europe (or from eastern North America). A more exact answer requires that we are able to sequence the RNA that is target by the ISAV7 assay.

Table 1. Results from the testing of gill and heart tissues.

Sanple	Gills ngram/µl	Gills Omelf	Gills ISAV7	Gills Uni-ISAV8	Gills neg con	Repeat Gills ISAV7
H1	1734,2	16,6	Neg	Neg	Neg	
H2	2904,3	19,8	Neg	Neg	Neg	
H3	2455,0	19,8	Neg	Neg	Neg	
H4	2485,1	16,3	Neg	Neg	Neg	
H5	1458,8	16,0	Neg	Neg	Neg	
H6	1135,8	Neg	Neg	Neg	Neg	
H7	3051,4	16,2	Neg	Neg	Neg	
H8	2492,9	22,5	Neg	Neg	Neg	
H9	2441,7	Neg	Neg	Neg	Neg	Neg*
H10	1444,3	15,5	34,5	Neg	Neg	35,4*
H11	1491,0	18,7	Neg	Neg	Neg	
H12	1644,5	20,1	Neg	Neg	Neg	
H13	1365,1	23,1	Neg	Neg	Neg	
H14	1914,9	16,3	Neg	Neg	Neg	
15	295,0	19,7	Neg	Neg	Neg	
16	1621,4	16,3	Neg	Neg	Neg	
17	2210,9	15,9	Neg	Neg	Neg	
18	2242,6	14,9	Neg	Neg	Neg	
19	2383,5	10,2	Neg	Neg	Neg	
20	4138,8	10,1	Neg	Neg	Neg	
21	1321,8	17,0	Neg	Neg	Neg	
22	2049,1	15,6	Neg	Neg	Neg	
23	1088,0	14,3	Neg	Neg	Neg	
24	1392,9	15,0	Neg	Neg	Neg	
Pos control			20,4	19,2	Neg	

* = Sample H9 and H10 (gills) were tested in 5 reruns after H10 tested positive with the ISAV7 assay. Only one of the reruns of H10 was positive. All other reruns were negative.

Table 2. Results from the testing of heart tissues.

Sample	Heart ngram/µl	Heart Omelf	Heart ISAV7	Heart Uni-ISAV8	Heart neg con	Repeat Heart ISAV7
1 Hj	627,7	16,1	Neg	Neg	Neg	
2 Hj	884,8	19,7	Neg	Neg	Neg	
3 Hj	1066,1	18,7	Neg	Neg	Neg	
4 Hj	860,3	16,9	Neg	Neg	Neg	
5 Hj	1441,4	16,9	Neg	Neg	Neg	
6 Hj	605,2	10,1	Neg	Neg	Neg	
7 Hj	351,3	14,4	Neg	Neg	Neg	
8 Hj	1286,5	15,6	Neg	Neg	Neg	
9 Hj	484,7	21,5	Neg	Neg	Neg	
10 Hj	128,0	17,0	Neg	Neg	Neg	
11 Hj	403,5	16,0	Neg	Neg	Neg	
12 Hj	618,1	18,4	Neg	Neg	Neg	
13 Hj	321,3	16,2	Neg	Neg	Neg	
14 Hj	552,3	15,2	35,5	Neg	Neg	Neg*
15 Hj	189,0	15,3	Neg	Neg	Neg	
16 Hj	60,7	16,9	Neg	Neg	Neg	
17 Hj	147,8	15,8	Neg	Neg	Neg	
18 Hj	382,4	14,6	Neg	Neg	Neg	
No tissue	-	-	-	-	-	-
No tissue	-	-	-	-	-	-
21 Hj	403,7	16,7	Neg	Neg	Neg	
22 Hj	1182,2	16,1	Neg	Neg	Neg	
23 Hj	829,2	15,0	Neg	Neg	Neg	
24 Hj	603,4	16,2	Neg	Neg	Neg	
Pos control			19,5	20,1		
Pos control			21,2	19,8		

* = All five reruns of heart sample 14Hj were negative for presence of ISA virus genome.

Literature

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