

Report, 8th November 2011

Testing of gill and heart samples from smolt and herring collected in British Columbia, 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. We used the elf-alpha from Atlantic salmon which is not optimal for use on *Oncorhynchus spp.* Two different assays targeting known 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 tables 1 and 2. All samples, both heart and gill tissues, were negative for presence of ISA virus genome.

Conclusion

Using the method described above we were not able to detect any ISA virus genome in the samples. (NB: The quality of the RNA has not yet been tested).

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

| Smolt | Gills RNA | Gills | Gills | Gills | Gills Control |
|--------------------|----------------|-------|-------|-----------|------------------|
| Sample | ngram/ μ l | Elf | ISAV7 | ISAV8-uni | Negative |
| 12 | 408,8 | 25,0 | Neg | Neg | Neg |
| 13 | 202,2 | 19,7 | Neg | Neg | Neg |
| 14 | 621,9 | 25,8 | Neg | Neg | Neg |
| 15 | 103,9 | 20,3 | Neg | Neg | Neg |
| 16 | 91,2 | 24,8 | Neg | Neg | Neg |
| 17 | 413,8 | 27,3 | Neg | Neg | Neg |
| 18 | 430,2 | 28,5 | Neg | Neg | Neg |
| 19 | 750,4 | 26,2 | Neg | Neg | Neg |
| 20 | 254 | 24,4 | Neg | Neg | Neg |
| 21 | 413,4 | 24,3 | Neg | Neg | Neg |
| 22 | 546,2 | 24,2 | Neg | Neg | Neg |
| 23 | 978,9 | 26,9 | Neg | Neg | Neg |
| 24 | 712,3 | 25,8 | Neg | Neg | Neg |
| 25 | 376 | 26,4 | Neg | Neg | Neg |
| 26 | 1109,8 | 26,6 | Neg | Neg | Neg |
| 27 | 999,6 | 25,0 | Neg | Neg | Neg |
| Pos control | | 23,3 | 16,0 | 16,8 | |

| Smolt | Heart RNA | Heart | Heart | Heart | Control |
|--------------------|----------------|-------|-------|-----------|----------|
| Sample | ngram/ μ l | Elf | ISAV7 | ISAV8-uni | Negative |
| 12 | 256,6 | 23,4 | Neg | Neg | Neg |
| 13 | 396,8 | 19,6 | Neg | Neg | Neg |
| 14 | 629,6 | 24,3 | Neg | Neg | Neg |
| 15 | 194,5 | 19,5 | Neg | Neg | Neg |
| 16 | 585,6 | 27,3 | Neg | Neg | Neg |
| 17 | 1105 | 23,9 | Neg | Neg | Neg |
| 18 | 153,1 | 27,7 | Neg | Neg | Neg |
| 19 | 877 | 24,0 | Neg | Neg | Neg |
| 20 | 1532,5 | 22,9 | Neg | Neg | Neg |
| 21 | 674,9 | 23,8 | Neg | Neg | Neg |
| 22 | 780,6 | 28,1 | Neg | Neg | Neg |
| 23 | 953 | 25,4 | Neg | Neg | Neg |
| 24 | 827,9 | 34,8 | Neg | Neg | Neg |
| 25 | 732,8 | 23,4 | Neg | Neg | Neg |
| 26 | 467,9 | 23,1 | Neg | Neg | Neg |
| 27 | 758,7 | 25,5 | Neg | Neg | Neg |
| Pos control | | 26,9 | 21,9 | 23,0 | |

Table 2. Results from the testing of heart tissues from herring.

| Herring Sample | Heart RNA ngram/μl | Heart ISAV7 | Heart ISAV8-uni | Control Negative |
|---------------------------|--|------------------------|----------------------------|-----------------------------|
| 12 Herring | 496 | Neg | Neg | Neg |
| 13 Herring | 896,6 | Neg | Neg | Neg |
| 14 Herring | 574,8 | Neg | Neg | Neg |
| 15 Herring | 261,8 | Neg | Neg | Neg |
| 16 Herring | 27,1 | Neg | Neg | Neg |

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.

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