

Final Report AHC Case: 09-4030

Last Updated: 01/28/10 4:22 PM

Pathologist: Gary D. Marty

Received Date: 10/15/09

Collected Date: 10/14/09

Client Ref No: 09-PCR/V

Veterinarian: **Dr. Ian Keith**

Clinic:

Phone:

Fax:

Case History

Submitted Atlantic salmon tissues for PCR validation. See data sheet provided. Attention: Dr. Robinson and Dr. Marty.

Submitted fresh tissues for Virology - PCR for *Piscirickettsia salmonis*. Culture any PCR positive samples (1 - 21). For PCR validation 09-PCR/V

Submitted formalized tissues for histopathology (1 - 21). 09-PCR/V

1. Liver, trunk kidney, and spleen: *Piscirickettsia salmonis* infection, mild to severe (slide #s 6, 13 - 21)

2. Liver, trunk kidney, and spleen: suspect *Piscirickettsia salmonis* infection (fish #12)

Final Comment: The small *P. salmonis* -positive piece of tissue included on the immunohistochemical - stained slide from fish #1 is probably a "float-on" artifact from one of the other fish. Therefore, fish #1 is considered a true negative. For all other fish, results of histopathology and conventional PCR are in complete agreement.

I suspect that differences in test results for fish #12 are because tissues containing *P. salmonis* were included in the real time PCR assay that were not in tissues analyzed by the other tests. In 5.5 years working at the Animal Health Centre, this is the first time I have had a case in which *P. salmonis* PCR and histopathology/ immunohistochemistry results did not agree. Alternatively, because the real time PCR test is about 100 × more sensitive than the conventional PCR test (at least on the single dilution comparison that was done), the real time test might have picked up a subclinical infection that was not detectable by other tests. The real time PCR test will be used on all future FHASP cases, so future cases will allow us to test how the real time PCR compares with histopathology and immunohistochemistry.

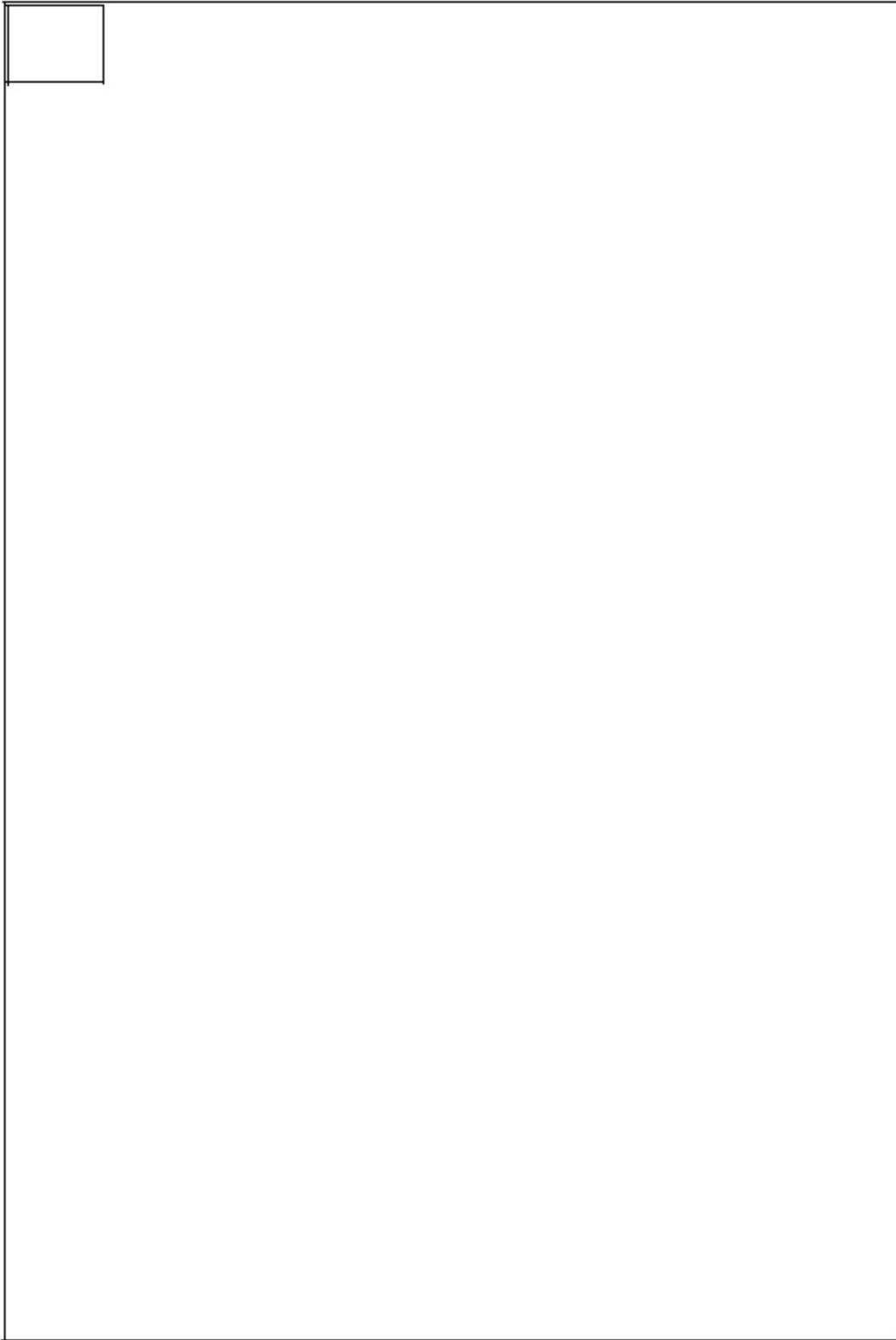
Formalin-fixed tissues from 21 fish were submitted in 21 cassettes for histopathology. Slide #s 1-21 are labeled in the same order as client #s 1 - 21.

Organs included on all slides - liver, spleen, trunk kidney.

Liver autolysis and severity of *Piscirickettsia salmonis* infection were scored as none (0), mild (1), moderate (2), or severe (3).

Conventional PCR - scored as positive (+) or negative (-) [Fish #1 was tested twice: positive on the first test, but negative on the second test.]

Real time PCR - positive (Ct value) or negative (-) [As the amount of *P. salmonis* DNA in the sample decreases, the Ct value increases.]



Slide 19 - liver has several organisms @ 15 × 13.3 (coordinates on the stage of my microscope)

Because fish #1 was PCR positive for *Piscirickettsia salmonis* but negative by histopathology, a section from the same paraffin block was also

stained with immunohistochemical reagents specific for *Piscirickettsia salmonis*. On this slide, a 1000 × 300 µm piece of liver--near the margin of the main 7 × 5 mm piece of liver--contains about 100 *P. salmonis*. This piece of tissue is not included in the H&E -stained slide.

Because fish #12 was positive for *Piscirickettsia salmonis* by real time PCR but not by conventional PCR or H&E histopathology, a section from the paraffin block was also stained with immunohistochemical reagents specific for *Piscirickettsia salmonis*. None of the tissues in the section contain *P. salmonis*.

Molecular Diagnostics

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/14/10 @ 11:53 AM

| Specimen | ID | Test | Result |
|----------|------------|--------------------------------|----------|
| Tissue | 09-PCRV-1 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-2 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-3 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-4 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-5 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-6 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-7 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-8 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-9 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-10 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-11 | PCR - Piscirickettsia salmonis | Negative |
| Tissue | 09-PCRV-12 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-13 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-14 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-15 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-16 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-17 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-18 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-19 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-20 | PCR - Piscirickettsia salmonis | Positive |
| Tissue | 09-PCRV-21 | PCR - Piscirickettsia salmonis | Positive |

History of Communication

| Date | To | Description |
|------------------|----------------------|---------------------|
| 01/28/10 4:23 PM | Keith, Dr. Ian - fax | bc report generated |



Gary D. Marty
D.V.M., Ph.D., Diplomate A.C.V.P.

These results relate only to the animals or items tested.

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