

## Testing of candidate non-lethal sampling methods for detection of *Renibacterium salmoninarum* in juvenile Chinook salmon *Oncorhynchus tshawytscha*

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Table S1. Observed agreement (concordance) of positive and negative results between assays for detection of *Renibacterium salmoninarum* in kidney samples and candidate non-lethal samples from Chinook salmon *Oncorhynchus tshawytscha* that had been challenged with the bacterium by immersion (180 fish) or sham-challenged (180 fish) and sampled at 3 time points after challenge. The kappa ( $\kappa$ ) statistic is the ratio of the observed agreement beyond chance to the maximum possible agreement beyond chance

Assay and sample comparison	Observed % agreement	$\kappa$ value	Strength of agreement <sup>a</sup>
Kidney ELISA and gill nPCR	92	0.83	Almost perfect
Kidney ELISA and gill qPCR	85	0.71	Substantial
Kidney ELISA and fin nPCR	87	0.73	Substantial
Kidney ELISA and fin qPCR	75	0.51	Moderate
Kidney ELISA and mucus nPCR	93	0.87	Almost perfect
Kidney ELISA and mucus qPCR	95	0.90	Almost perfect
Kidney culture and gill nPCR	77	0.49	Moderate
Kidney culture and gill qPCR	76	0.40	Fair
Kidney culture and fin nPCR	78	0.49	Moderate
Kidney culture and fin qPCR	81	0.49	Moderate
Kidney culture and mucus nPCR	65	0.37	Fair
Kidney culture and mucus qPCR	74	0.47	Moderate
Kidney nPCR and gill nPCR	74	0.44	Moderate
Kidney nPCR and gill qPCR	76	0.45	Moderate
Kidney nPCR and fin nPCR	78	0.51	Moderate
Kidney nPCR and fin qPCR	80	0.47	Moderate
Kidney nPCR and mucus nPCR	69	0.42	Moderate
Kidney nPCR and mucus qPCR	74	0.47	Moderate
Kidney qPCR and gill nPCR	71	0.36	Fair
Kidney qPCR and gill qPCR	75	0.38	Fair
Kidney qPCR and fin nPCR	76	0.43	Moderate
Kidney qPCR and fin qPCR	82	0.46	Moderate
Kidney qPCR and mucus nPCR	63	0.29	Fair
Kidney qPCR and mucus qPCR	69	0.35	Fair

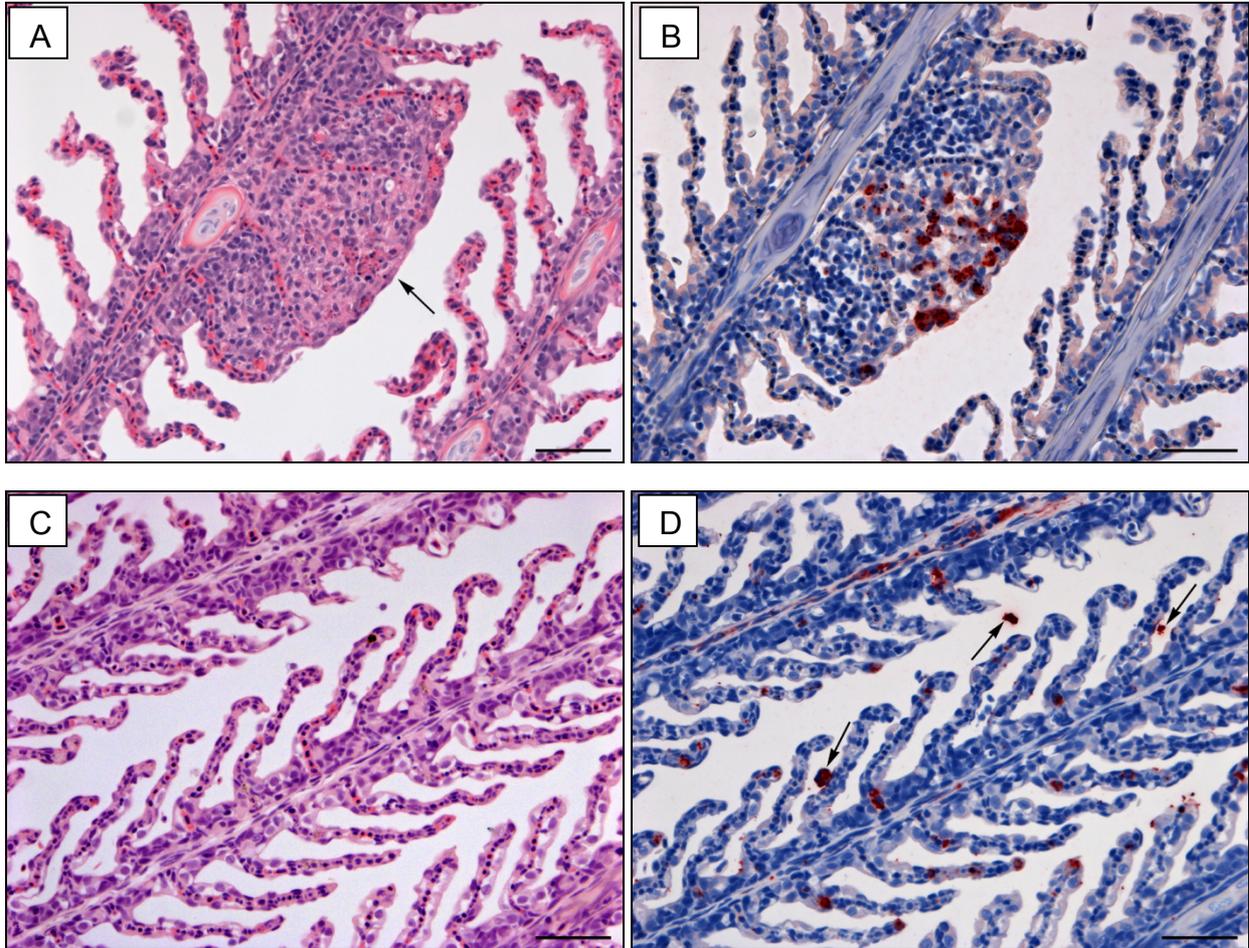
<sup>a</sup>Strength of agreement (Smith 2006):  $\kappa$  0 = no better than chance;  $\kappa$  0.01-0.20 = slight;  $\kappa$  0.21-0.40 = fair;  $\kappa$  0.41-0.60 = moderate;  $\kappa$  0.61-0.80 = substantial;  $\kappa$  0.81-0.99 = almost perfect;  $\kappa$  1.00 = perfect

Table S2. Correlation between *Renibacterium salmoninarum* infection or antigen level values from testing of Chinook salmon *Oncorhynchus tshawytscha* kidney tissue by individual quantitative and semi-quantitative assays, and quantity estimates ( $\log_{10}$  [*R. salmoninarum* cells  $g^{-1}$ ]) from testing of candidate non-lethal samples by qPCR. Values were obtained from fish that had been challenged with the bacterium by immersion 3–21 wk before sampling and had tested positive for *R. salmoninarum* by both assays in the comparison.  $r_s$ : Spearman's rank correlation coefficient

Sample and comparison <sup>a</sup>	No. of fish	$r_s$ (95% CI)	p
<b>All weeks (all fish)</b>			
Kidney ELISA and gill qPCR	130	0.23 (0.06, 0.39)	0.008
Kidney ELISA and fin qPCR	92	0.38 (0.19, 0.55)	0.0002
Kidney ELISA and mucus qPCR	166	0.25 (0.10, 0.39)	0.001
Kidney culture and gill qPCR	85	-0.03 (-0.25, 0.19)	0.75
Kidney culture and fin qPCR	68	0.40 (0.17, 0.59)	0.0007
Kidney culture and mucus qPCR	101	0.27 (0.07, 0.45)	0.006
Kidney qPCR and gill qPCR	51	0.16 (-0.13, 0.43)	0.18
Kidney qPCR and fin qPCR	44	0.56 (0.30, 0.74)	<0.0001
Kidney qPCR and mucus qPCR	59	0.41 (0.17, 0.61)	0.001
Kidney DFAT and gill qPCR	32	0.49 (0.16, 0.72)	0.004
Kidney DFAT and fin qPCR	25	0.59 (0.25, 0.80)	0.002
Kidney DFAT and mucus qPCR	35	0.42 (0.12, 0.68)	0.007
Kidney IHC and gill qPCR	17	0.73 (0.37, 0.90)	0.0008
Kidney IHC and fin qPCR	15	0.46 (-0.08, 0.79)	0.08
Kidney IHC and mucus qPCR	18	0.24 (-0.22, 0.68)	0.24
<b>All weeks (Wisconsin stock)</b>			
Kidney ELISA and gill qPCR	60	0.29 (0.03, 0.51)	0.03
Kidney ELISA and fin qPCR	48	0.43 (0.16, 0.64)	0.002
Kidney ELISA and mucus qPCR	82	0.08 (-0.14, 0.30)	0.45
Kidney culture and gill qPCR	39	-0.10 (-0.41, 0.22)	0.53
Kidney culture and fin qPCR	31	0.69 (0.44, 0.84)	<0.0001
Kidney culture and mucus qPCR	47	0.25 (-0.05, 0.50)	0.10
Kidney qPCR and gill qPCR	26	0.27 (-0.14, 0.61)	0.18
Kidney qPCR and fin qPCR	21	0.63 (0.27, 0.84)	0.002
Kidney qPCR and mucus qPCR	29	0.64 (0.35, 0.82)	0.0002
<b>All weeks (Washington stock)</b>			
Kidney ELISA and gill qPCR	70	0.20 (-0.04, 0.42)	0.09
Kidney ELISA and fin qPCR	44	0.20 (-0.11, 0.48)	0.18
Kidney ELISA and mucus qPCR	84	0.18 (-0.04, 0.38)	0.11
Kidney culture and gill qPCR	46	0.05 (-0.25, 0.35)	0.72
Kidney culture and fin qPCR	37	0.09 (-0.25, 0.41)	0.59
Kidney culture and mucus qPCR	54	0.26 (-0.02, 0.50)	0.06
Kidney qPCR and gill qPCR	25	0.10 (-0.32, 0.49)	0.62
Kidney qPCR and fin qPCR	23	0.36 (-0.07, 0.68)	0.09
Kidney qPCR and mucus qPCR	30	0.32 (-0.06, 0.61)	0.09

<sup>a</sup>Units used for comparisons: ELISA, optical density (OD)<sub>405 nm</sub>; culture,  $\log_{10}$  (colony forming units  $g^{-1}$ ); qPCR,  $\log_{10}$  (*R. salmoninarum* cells  $g^{-1}$ ); DFAT,  $\log_{10}$  (*R. salmoninarum* cells per 100 microscope fields) at 1000x magnification; IHC,  $\log_{10}$  (*R. salmoninarum* infection severity score) (distribution and intensity of IHC staining in areas of the anterior and posterior kidney; scoring procedure explained in article text)

Fig. S1. Histological appearance of gills of Chinook salmon *Oncorhynchus tshawytscha* sampled following an immersion challenge with *Renibacterium salmoninarum*. (A) Gill of a Washington stock fish 3 wk after challenge, showing a focal inflammatory lesion (arrow) with fusion of several adjacent lamellae (H&E stain). (B) Immunohistochemical (IHC) staining showing *R. salmoninarum* antigen (red color) in the focal gill lesion shown in (A). (C) Gill of a Wisconsin stock fish 12 wk after challenge showing minimal histological changes. (D) IHC staining showing *R. salmoninarum* antigen (red color) in exfoliated cells (arrows) and within cells and vascular spaces in filaments and lamellae of the gill shown in (C). Scale bars = 50  $\mu$ m



#### LITERATURE CITED

Smith RD (2006) Veterinary clinical epidemiology. Taylor and Francis, Boca Raton, FL