

The following supplement accompanies the article

## Diagnostic test accuracy when screening for *Haliotid herpesvirus 1* (AbHV) in apparently healthy populations of Australian abalone *Haliotis* spp.

Charles G. B. Caraguel\*, Kevin Ellard, Nicholas J. G. Moody, Serge Corbeil, Lynette M. Williams, Peter G. Mohr, David M. Cummins, John Hoad, Joanne Slater, Mark St. J. Crane

\*Corresponding author: charles.caraguel@adelaide.edu.au

*Diseases of Aquatic Organisms* 136: 199–207 (2019)

Table S1. *Haliotid herpesvirus-1* (AbHV) real-time PCR assay primer and probe sequences and cycling conditions

qPCR set	Primer/Probe Name	Sequence (5'-3')	PCR Conditions	Amplicon	Reference
ORF49	AbHV ORF49F1	AAC-CCA-CAC-CCA-ATT-TTT-GA	95°C 59 seconds	126bp	Corbeil et al. (2010)
	AbHV ORF49R1	CCC-AAG-GCA-AGT-TTG-TTG-TT	95°C 3 seconds		
	AbHV 49Prb1	6FAM-CCG-CTT-TCA-ATC-TGA-TCC-GTG-G-TAMRA	62°C 30 seconds (50 cycles)		
ORF66	AbHV ORF66F1	TCC-CGG-ACA-CCA-GTA-AGA-AC	95°C 59 seconds	146bp	Corbeil et al. (2014)
	AbHV ORF66R1	CAA-GGC-TGC-TAT-GCG-TAT-GA	95°C 3 seconds		
	AbHV 66Prb1	6FAM-TGG-CCG-TCG-AGA-TGT-CCA-TG-TAMRA	60°C 30 seconds (50 cycles)		
ORF77	AbHV ORF77F1	CAA-CCA-CTT-GTT-CGG-GTT-CT	95°C 59 seconds	190bp	Corbeil et al. (2014)
	AbHV ORF77R1	CAG-GGT-GAT-TAA-TGC-GGA-GT	95°C 3 seconds		
	AbHV 77Prb1	6FAM-TCC-GTA-CGC-GGG-ATC-TTC-GT-TAMRA	60°C 30 seconds (50 cycles)		

Table S2. Repeatability of the *Haliotid herpesvirus-1* (AbHV) real-time PCR assays using the primers/probe sets ORF49, ORF66 or ORF77 with two separate plasmid controls each. SD – standard deviation, CV – coefficient of variation.

Operator	AbHV ORF49 qPCR POS Ctrl 1				AbHV ORF49 qPCR POS Ctrl 2			
	Assay runs	Mean C <sub>T</sub>	SD	CV	Assay runs	Mean C <sub>T</sub>	SD	CV
Operator 1	11	28.76	0.56	1.95%	13	32.49	0.72	2.22%
Operator 2	1	28.64	0.08	0.28%	2	31.85	0.52	1.63%
Operator 3	14	28.91	0.44	1.52%	20	31.78	0.99	3.12%
Operator 4	12	28.81	0.21	0.73%	9	31.96	0.89	2.78%
Total	33	28.83	0.44	1.53%	44	32.03	0.92	2.87%
Operator	AbHV ORF66 qPCR POS Ctrl 1				AbHV ORF66 qPCR POS Ctrl 2			
	Assay runs	Mean C <sub>T</sub>	SD	CV	Assay runs	Mean C <sub>T</sub>	SD	CV
Operator 1	15	26.83	0.24	0.89%	15	33.59	0.68	2.02%
Operator 2	11	26.67	0.23	0.86%	13	33.41	0.53	1.59%
Operator 3	3	26.75	0.06	0.22%	3	33.41	0.27	0.81%
Operator 4	46	26.80	0.44	1.64%	51	33.50	0.60	1.79%
Operator 5	26	26.81	0.33	1.23%	27	33.43	0.58	1.73%
Operator 6	2	26.29	0.14	0.53%	1	33.11	0.04	0.12%
Total	103	26.78	0.36	1.34%	110	33.48	0.59	1.76%
Operator	AbHV ORF77 qPCR POS Ctrl 1				AbHV ORF77 qPCR POS Ctrl 2			
	Assay runs	Mean C <sub>T</sub>	SD	CV	Assay runs	Mean C <sub>T</sub>	SD	CV
Operator 1	10	27.42	0.25	0.91%	9	33.90	0.58	1.71%
Operator 2	3	27.38	0.22	0.80%	3	34.41	0.50	1.45%
Operator 3	27	27.47	0.39	1.42%	32	34.25	0.67	1.96%
Operator 4	9	27.31	0.34	1.24%	10	33.94	0.81	2.39%
Total	49	27.43	0.35	1.28%	54	34.15	0.69	2.02%

Table S3. Cross-tabulation of observed counts for the 16 possible combinations of test results when detecting *Haliotid herpesvirus-1* (AbHV) when evaluating histopathology and three qPCR assays using ORF49, ORF66, and ORF77 primers/probe sets, respectively, for each of the four source populations of abalone.

Histopathology	ORF49	ORF66	ORF77	Source 1	Source 2	Source 3	Source 4	Total
Positive	Positive	Positive	Positive	0	1	0	0	1
Positive	Positive	Positive	Negative	0	0	0	0	0
Positive	Positive	Negative	Positive	0	0	0	0	0
Positive	Positive	Negative	Negative	0	4	0	0	4
Positive	Negative	Positive	Positive	0	0	0	0	0
Positive	Negative	Positive	Negative	0	0	0	0	0
Positive	Negative	Negative	Positive	0	0	0	0	0
Positive	Negative	Negative	Negative	0	0	0	0	0
Negative	Positive	Positive	Positive	11	8	0	1	20
Negative	Positive	Positive	Negative	18	7	0	0	25
Negative	Positive	Negative	Positive	6	0	0	0	6
Negative	Positive	Negative	Negative	16	8	0	0	24
Negative	Negative	Positive	Positive	1	5	7	0	13
Negative	Negative	Positive	Negative	4	15	1	0	20
Negative	Negative	Negative	Positive	3	2	3	0	8
Negative	Negative	Negative	Negative	644	390	127	170	1,331
Total				703	440	138	171	1,452

Table S4. Comparison of the deviance information criterion (DIC) across latent class analysis scenarios accounting for pairwise conditional dependence among histopathology, qPCR using the primers/probe sets ORF49, ORF66 or ORF77 when detecting *Haliotid herpesvirus-1* (AbHV). The dependence between two tests was evaluated by estimating the posterior median of the variance in infected and non-infected abalone and the corresponding fraction of the maximum possible covariance (between brackets). Posterior covariance term estimates were compared to zero using their 95% Posterior Credibility Interval, i.e. if zero was not included in the interval, the estimate was deemed significantly different from zero at the 5% level. Model VI (bold) was chosen as final model because its DIC did not differ much from Model VII and was more parsimonious (less parameters).

Model	Histopathology/ORF49		Histopathology /ORF66		Histopathology /ORF77		ORF49/ORF66		ORF49/ORF77		ORF66/ORF77		DIC
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	
0													178.4
I	0.0021 (38.2%)	0.0025* (85.2%)											170.1
II			-0.0201* (61.6%)	0.0002* (51.0%)									175.4
III					-0.0059 (33.8%)	0.0002* (51.2%)							179.1
IV							-0.0369 (34.7%)	0.0012* (56.3%)					178.9
V									-0.0238 (12.0%)	0.0006* (40.1%)			181.0
<b>VI</b>											<b>0.0555*</b> <b>(41.3%)</b>	<b>0.0079*</b> <b>(60.0%)</b>	<b>165.7</b>
VII	-0.000756 (7.9%)	0.002211* (85.2%)									0.0428 (33.0%)	0.0068* (60.0%)	162.7

\*significantly different from zero at the 5% level

Table S5. Diagnostic sensitivity and specificity estimates (median and 95% posterior credible interval, PCI) after removal of one of the four study population (1-4) at the time for the diagnostic sensitivity and specificity (median with 95% Posterior Credibility Interval, PCI) of histopathology or qPCR using the primers/probe sets ORF49, ORF66 or ORF77 in detecting *Haliotid herpesvirus-1* (AbHV).

Diagnostic tests	All four source populations			Without source population 1			Without source population 2			Without source population 3			Without source population 4		
	Median	PCI 2.5 <sup>th</sup> percentile	PCI 97.5 <sup>th</sup> percentile	Median	PCI 2.5 <sup>th</sup> percentile	PCI 97.5 <sup>th</sup> percentile	Median	PCI 2.5 <sup>th</sup> percentile	PCI 97.5 <sup>th</sup> percentile	Median	PCI 2.5 <sup>th</sup> percentile	PCI 97.5 <sup>th</sup> percentile	Median	PCI 2.5 <sup>th</sup> percentile	PCI 97.5 <sup>th</sup> percentile
Diagnostic Sensitivity															
Histology	6.3%	2.4%	13.1%	14.3%	4.9%	31.0%	1.3%	0.1%	6.8%	5.6%	2.1%	11.8%	7.0%	2.7%	14.23%
qPCR-ORF49	90.0%	59.2%	99.7%	76.2%	37.3%	99.0%	92.9%	73.2%	99.7%	74.3%	58.6%	96.6%	94.8%	73.9%	99.81%
qPCR-ORF66	59.1%	47.6%	71.5%	59.8%	41.1%	78.0%	60.4%	45.3%	79.7%	59.9%	48.1%	73.6%	59.4%	47.3%	73.57%
qPCR-ORF70	33.7%	23.5%	45.5%	32.6%	17.4%	52.5%	36.0%	23.1%	52.3%	33.8%	23.9%	46.1%	34.0%	23.7%	46.48%
Diagnostic Specificity															
Histology	100.0%	99.7%	100.0%	99.9%	99.5%	100.0%	99.9%	99.6%	100.0%	99.9%	99.7%	100.0%	99.9%	99.7%	100.00%
qPCR-ORF49	99.8%	99.0%	100.0%	99.8%	99.0%	100.0%	99.7%	98.4%	100.0%	99.7%	98.6%	100.0%	99.7%	98.5%	99.99%
qPCR-ORF66	97.9%	96.9%	99.7%	96.8%	94.9%	99.5%	98.8%	97.8%	99.56%	99.1%	97.7%	100.0%	97.4%	96.3%	98.53%
qPCR-ORF70	98.6%	97.8%	99.6%	97.9%	96.5%	99.3%	98.5%	97.6%	99.30%	99.6%	98.9%	100.0%	98.3%	97.4%	99.00%