The following supplement accompanies the article

Diagnostic validation of three test methods for detection of cyprinid herpesvirus 3 (CyHV-3)

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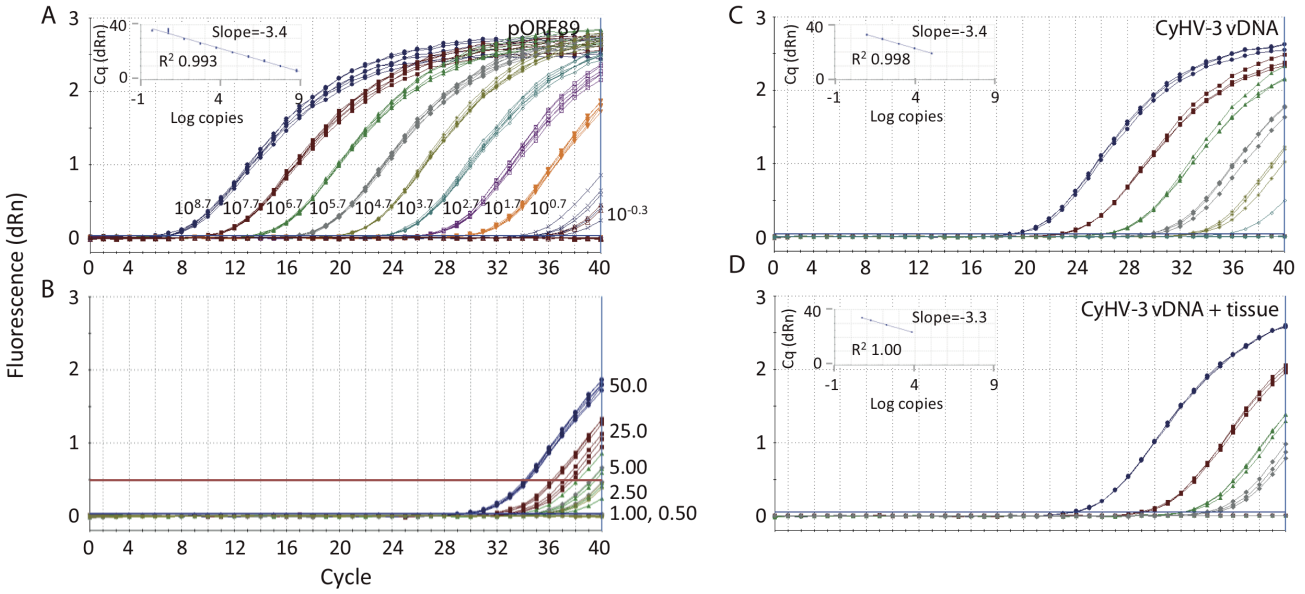


Fig. S1. Analytical sensitivity of the qORF89 test. Cq values for the FAM-labelled probe were generated using pORF89 DNA diluted from $10^{5.7}$ to 5 copies (A) or from 50 to 0.5 copies (B), partially purified CyHV-3 viral DNA 10-fold serially diluted and run by itself (C) or added as exogenous DNA to tissue (D). Linear regression for A, C and D are shown in the insets. The limit of detection is indicated by the horizontal red line in B.

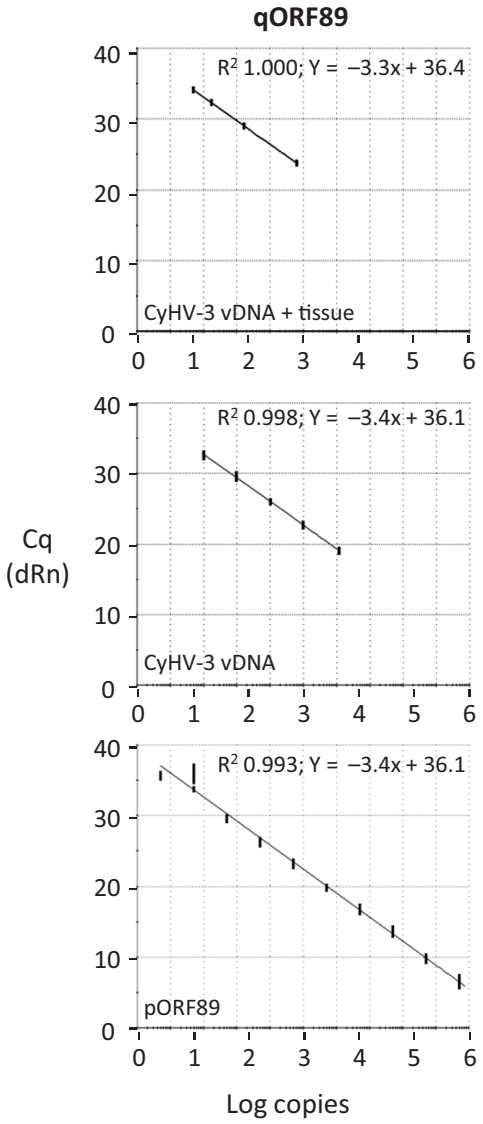
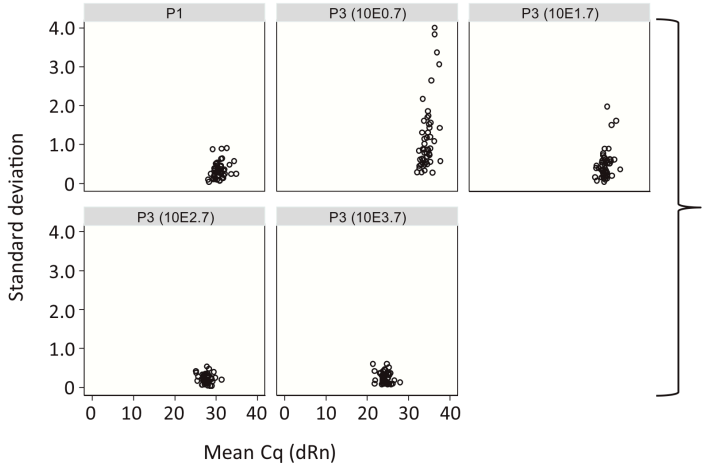


Fig. S2. Reaction efficiency of qORF89 test. Targets included DNA from tissue with exogenous CyHV-3 vDNA, CyHV-3 vDNA or plasmid encoding the CyHV-3 ORF89 protein (pORF89). Cq values for the FAM-labelled probe were generated using 10-fold serially diluted DNA. Standard curves were generated using linear regression.



Control sample	Number of Runs	Cq ± SD[a]	Coefficient of variation Intra-assay[b]	Inter-assay
P1	54	30.77±1.35	1.09	4.38
P3				
$5x10^0$	54	34.56±1.31	2.74	3.78
$5x10^1$	54	31.15±1.14	1.25	3.67
$5x10^2$	54	27.71±1.09	0.75	3.94
$5x10^3$	54	24.33±1.15	0.82	4.72

[a] Cq, mean of mean Cq/run; SD, mean of mean Cq standard deviation/run
[b] Average of within assay coefficient of variation for all runs

Fig. S3. Analytical repeatability of the qORF89 test with P1 and P3. Cq values for the FAM-labelled probe were generated with 25 ng of partially purified CyHV-3 DNA in 50 mg tissue (P1) or pORF89 DNA diluted from $10^{3.7}$ to 5 copies (P3). Measurements were performed in replicates of three in 54 independent runs. Intra- and inter-assay coefficient of variation for Cq values as well as the standard deviation and mean Cq values are presented for P1 and each dilution of P3 in the table.

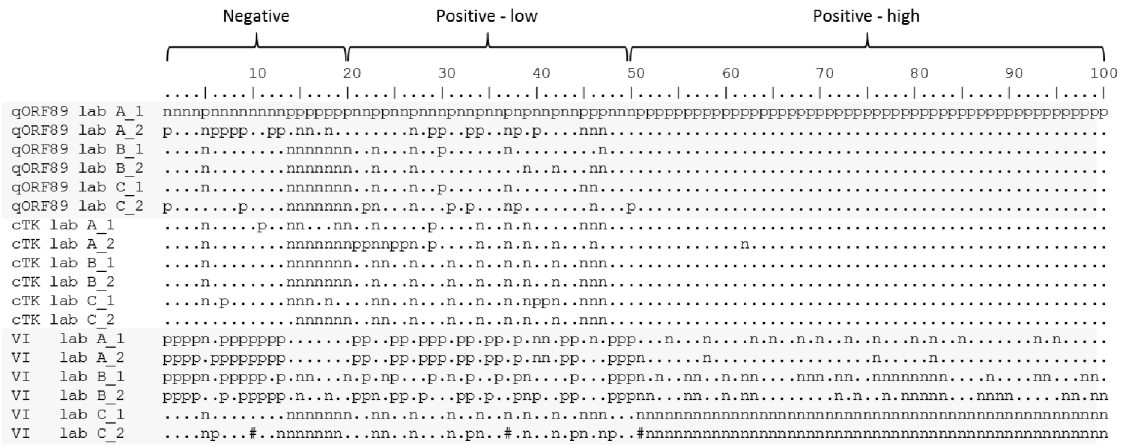


Fig. S4. Alignment of precision study test results for the qORF89, cTK and VI assays. Binary test results obtained for duplicate sets of 100 samples processed by labs A, B and C are reported as positive (p), negative (n) or contaminated (#). Test results concordant with those reported on the top line are indicated by dots. The three fish populations that make up the 100 fish are designated above the alignment.

A

Comparison	(+,+,+)	(+,+,-)	(+,-,+)	(+,-,-)	(-,+,+)	(-,+,-)	(-,-,+)	(-,-,-)	Total
qORF89, cTK, VI/qPCR	47	6	0	12	2	10	23	164	264

B

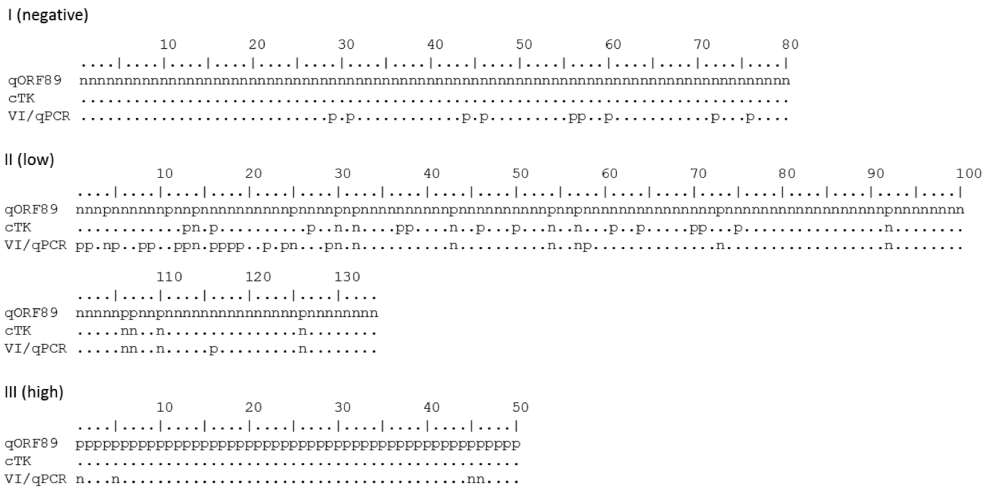


Fig. S5. Accuracy study test results for the qORF89, cTK and VI/qPCR assays. A) All combinations of positive or negative test results are presented for qORF89, cTK and VI/qPCR, respectively; B) Binary test results obtained for 264 samples processed by lab A are reported as positive (p) or negative (n). Test results concordant with those reported on the top line are indicated by dots. The three fish populations that make up the 264 fish are designated above the alignment.