The following supplement accompanies the article

Development and validation of a reverse transcription quantitative PCR for universal detection of viral hemorrhagic septicemia virus

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Supplement 1. Optimisation and validation data for the universal VHSV RT-qPCR assay

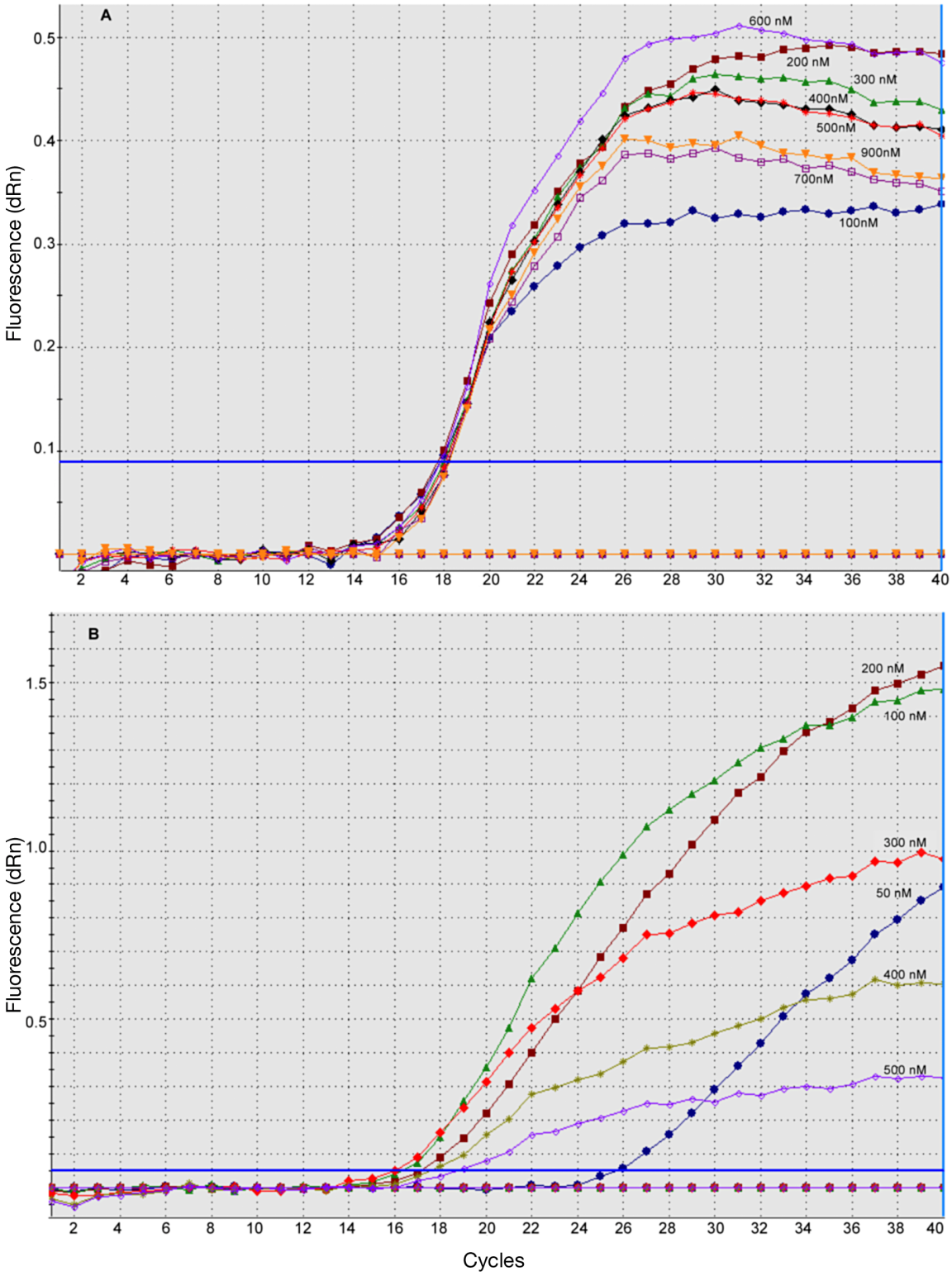

Fig. S1. MxPro amplification plots of primer/probe set 2 optimization. (A) Primer optimization ranging from 100 to 900 nM. (B) Probe optimization 50 to 500 nM

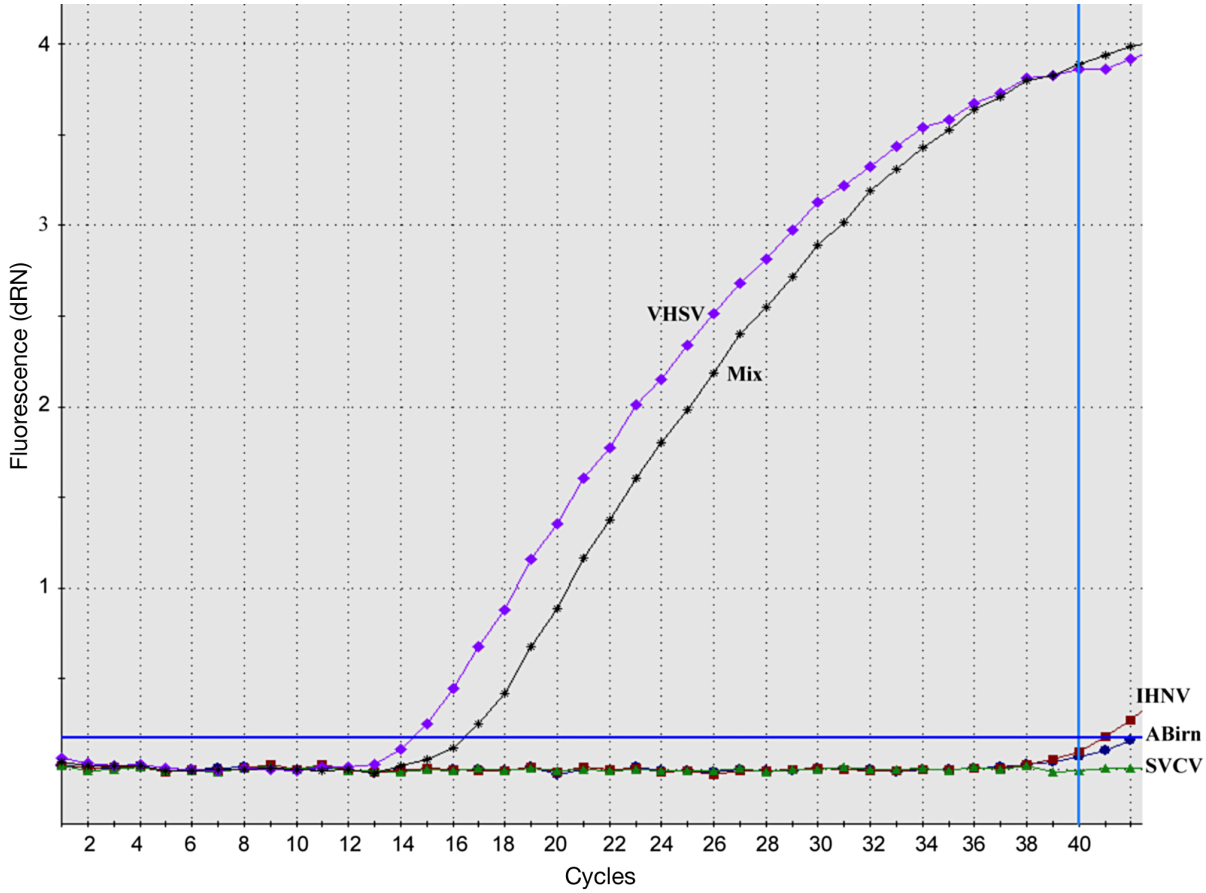

Fig. S2. MxPro amplification plot revealing viral hemorrhagic septicemia virus (VHSV) real-time reverse transcription quantitative PCR (RT-qPCR) primer/probe set 2 specificity. VHSV, infectious hematopoietic necrosis virus (IHNV), aquatic birnavirus (ABirn) and spring viremia of carp virus (SVCV) cell-culture-amplified RNA were tested individually and as an equal mix of all 4 isolates

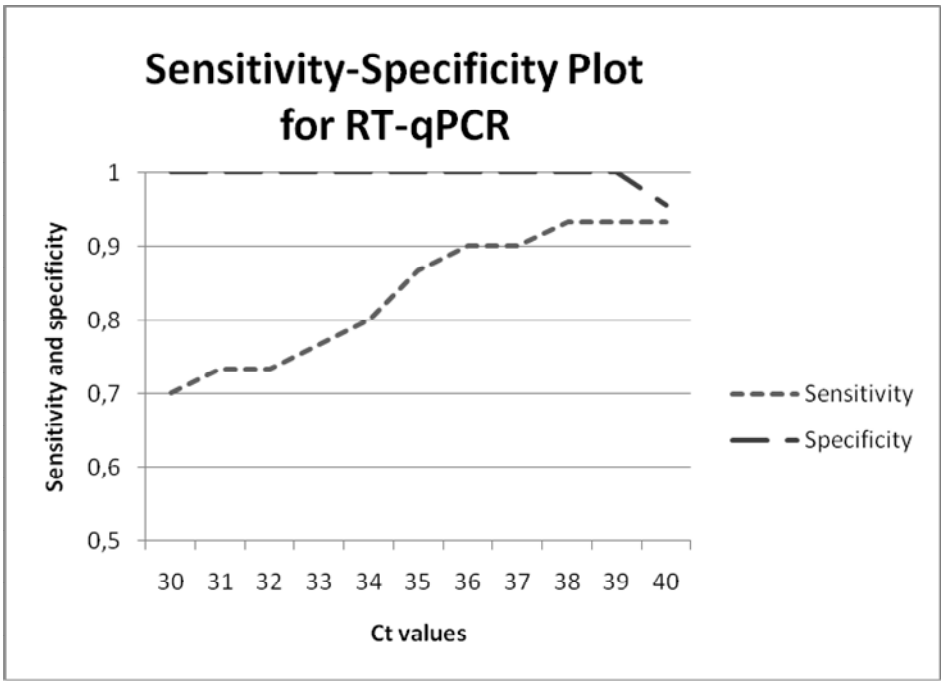

Fig. S3. Plot of the change in diagnostic sensitivity (DSe) and diagnostic specificity (DSp) as the cut-off value is moved from lower to higher cycle threshold (C_t) values

Table S1. VHSV RT-qPCR inter-assay variation. Mean ± SD cycle threshold (C_t) values were determined based on 5 independent runs

Transcript dilution	C_t	CV (%)
10^8	15.36 ± 1.34	8.81
10^7	18.71 ± 1.16	6.21
10^6	22.04 ± 0.71	3.21
10^5	25.08 ± 1.24	4.96
10^4	29.13 ± 0.85	2.90
10^3	32.38 ± 0.68	2.10
10^2	35.69 ± 0.88	2.46