

Additional File 1:

Table: Primers and probes used to determine viral abundance through qRT-PCR and qPCR.

Target virus	Primer/Probe sequence 5'-3'
Bovine Nidovirus	F TCGCCCACGCTTAACGA R GGCATTGAGCAGCGAYTCA Pr CCAAATTTAAGGCGATGCTTGCTTCCG FAM-BHQ1
Bovine narmovirus-1 (Bulang virus)	F GGATTGTTACCAAGAGGATCATGAT R TATCCAGCATCCCTGCTATGC Pr CCATAATAATACCTCGCGCTGCACATGAGA Quasar 670 BHQ2
Influenza D virus	F CCCCRATAGGATCAATGTTCGAT R TTCTCCCCCTATGATTCCATTYTC Pr CAATTGAAGCCAAAATYCTTGACCGGG TAMARA BHQ2
Bovine Coronavirus	F GCGTCCAAAGGCTATATTGCTAA R CCCAACATTTGGATTCTGACATAA Pr TGCCTTTCAACAGGTATT FAM-MGB
Bovine Rhinitis A (adapted from Kishimoto et al., 2017) [1]	F CGGTGTAGCGACGACAACA R TGGGCATCCTTAGTCTCCCA Pr GGCCTTGGGACRCCCTCTCCA Quasar670/BHQ2
Bovine Rhinitis B (adapted from Kishmitot M et al., 2017) [1]	F GAACGCGATTGTGTYTAGGG R CGCMACTGAGGTTAGCTTCTCC Pr CTGTCCTTTGCACGGCGTGGC Fam/BHQ1
Bovine Viral Diarrhea From [2]	BVD190-F GRA GTC GTC ART GGT TCG AC F un_labelled_Primer R V326 TCA ACT CCA TGT GCC ATG TAC un_labelled_Primer Pr TQ-pesti TGC YAY GTG GAC GAG GGC ATG C Fam-BHQ1
Bovine alphaherpesvirus-1. From [3]	F gB-F TGT GGA CCT AAA CCT CAC GGT un_labelled_Primer R gB-R A GTC GAG CAG ACC CGT GTC un_labelled_Primer Pr BoHV1-P AGG ACC GCG AGT TCT TGC CGC FAM-BHQ1

Cycling parameters Reverse transcription step involving one cycle at 45°C for 10 min.

The PCR cycling conditions included an RT inactivation/initial denaturation of 95°C for 10 min followed by amplification with 45 cycles of 95°C for 15 sec and 60°C for 45 sec.

References:

1. Kishimoto M, Tsuchiaka S, Rahpaya SS, Hasebe A, Otsu K, Sugimura S, Kobayashi S, Komatsu N, Nagai M, Omatsu T *et al*: **Development of a one-run real-time PCR detection system for pathogens associated with bovine respiratory disease complex.** *J Vet Med Sci* 2017, **79**(3):517-523.
2. Hoffmann B, Depner K, Schirrmeier H, Beer M: **A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses.** *J Virol Methods* 2006, **136**(1-2):200-209.
3. Wang J, O'Keefe J, Orr D, Loth L, Banks M, Wakeley P, West D, Card R, Ibata G, Van Maanen K *et al*: **Validation of a real-time PCR assay for the detection of bovine herpesvirus 1 in bovine semen.** *J Virol Methods* 2007, **144**(1-2):103-108.