

Supplementary Table 1. Primer and probe sequences used for detection of viruses and bacteria.

Pathogen	Target gene	Name	Sequence (5'-3')	Length (bp)	Reference
Bovine respiratory syncytial virus	F	BRSV-F-485F BRSV-F-569R BRSV F Taqman-546	AAGGGTCAAACATCTGCTTAACTAG TCTGCCTGWGGGAAAAAAG FAM-AGAGCCTGCATTTRTACAATACCACCCA-BHQ1	85	[1]
Bovine coronavirus	M	BCoV-F BCoV-R BCoV-P	GTTGGTGGAGTTTCAACCCAG GGTAGTCCTCAATTATCGGCC FAM-CATCCTTCCCTTCATATCTATACACATC-BHQ1	90	F, R and P (modified): [2]
<i>H. somni</i>	16S rRNA	HS-F HS-R HS-P	GAAGATACTGACGCTCGAGT TTCGGGCACCAAGTRTTCA FAM-TCCCCAAATCGACATCGTTTACAGCGTG-BHQ1	115	F and P: [3] R : [4]
Influenza D virus	PB1	Inf D-F Inf D-R Inf D-P	GCTGTTTGCAAGTTGATGGG TGAAAGCAGGTAAGTCAAGG FAM-TTCAGGCAAGCACCCGTAGGATT-BHQ1	136	[5]
<i>M. haemolytica</i>	<i>sodA</i>	M. hae-F M. hae-R M. hae-P	GCCGTTGTTTCAACCGCTAAC CGTGTTCCTCAACGTCTAAGAC FAM-TCGGATAGCCTGAAACGCCTGCCAC-BHQ1	100	[3]
<i>M. bovis</i>	<i>oppD</i>	PMB996-F PMB1066-R Mbovis1016	TCAAGGAACCCACCAGAT AGGCAAAGTCATTTCTAGGTGCAA FAM-TGGCAAACCTTACCTATCGGTGACCCT-TAMRA	71	[6]
<i>Mycoplasma</i> spp.	16S rRNA	Mycoplasma-F Mycoplasma-R Mycoplasma-P	GATCCTGGCTCAGGATGAAC CGTTGAGTACGTGTTACTCAC FAM-GGCTGTGTGCCTAATACATGCATGTCG-BHQ1	103	[3]
<i>P. multocida</i>	<i>kmt1</i>	PM-ny-F PM-ny-R PM-P	GACTACCGACAAGCCCACTC CTATCCGCTATTTACCCAGTGG FAM-GTGCGAATGAACCGATTGCCGCG- BHQ1	125	F and R: [3] P: [7]
<i>T. pyogenes</i>	plo-Pyolysin	T. pyogenes-F T. pyogenes-R T. pyogenes-P	CATCAACAATCCCACGAAGAG TTGCAGCATGGTCAGGATAC FAM-CCGTGACTCAAGGACTGAACGGCCT-BHQ1	98	F (modified) and R from [8] P: [3]
References: 1. Hakhverdyan, M. ; Häggglund, S; Larsen, L.E. ; Belák, S. Evaluation of a single-tube fluorogenic RT-PCR assay for detection of bovine respiratory syncytial virus in clinical samples. <i>J Virol Methods</i> . 2005,123(2):195-202. doi: 10.1016/j.jviromet.2004.09.016. 2. Decaro, N. ; Elia,G. ; Campolo, M. ; Desario, C. ; Mari, V. ; Radogna, A. ; Colaianni, M.L. ; Cirone, F. ; Tempesta, M. ; Buonavoglia, C. Detection of bovine coronavirus using a TaqMan-based real-time RT-PCR assay. <i>J Virol Methods</i> . 2008,151(2):167-171. doi: 10.1016/j.jviromet.2008.05.016. 3. Goecke, N.B.; Nielsen, B.H.; Petersen, M.B.; Larsen, L.E. Design of a High-Throughput Real-Time PCR System for Detection of Bovine Respiratory and Enteric Pathogens. <i>Front Vet Sci</i> 2021, 8, doi:10.3389/fvets.2021.677993.					

4. Angen, Ø.; Ahrens, P.; Tegtmeier, C. Development of a PCR test for identification of *Haemophilus somnus* in pure and mixed cultures. *Veterinary Microbiology*, 1998, 63(1):39-48, [https://doi.org/10.1016/S0378-1135\(98\)00222-3](https://doi.org/10.1016/S0378-1135(98)00222-3).
5. Hause, B.M. ; Ducatez, M. ; Collin, E.A.; Ran, Z.; Liu, R.; Sheng, Z.; Armien, A.; Kaplan, B.; Chakravarty, S.; Hoppe, A.D.; Webby, R.J.; Simonson, R.R.; Li, F. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. *PLoS Pathog.* 2013,9(2):e1003176. doi: 10.1371/journal.ppat.1003176.
6. Sachse, K.; Salam, H.S.; Diller, R.; Schubert, E.; Hoffmann, B.; Hotzel, H. Use of a novel real-time PCR technique to monitor and quantitate *Mycoplasma bovis* infection in cattle herds with mastitis and respiratory disease. *Vet J.* 2010, 186(3):299-303. doi: 10.1016/j.tvjl.2009.10.008.
7. Goecke, N.B.; Hjulsager, C.K.; Krog, J.S.; Skovgaard, K.; Larsen, L.E. Development of a high-throughput real-time PCR system for detection of enzootic pathogens in pigs. *J Vet Diagn Invest.* 2020, 32(1):51-64. doi: 10.1177/1040638719890863.
8. Kishimoto, M.; Tsuchiaka, S.; Rahpaya, S.S.; Hasebe, A.; Otsu, K.; Sugimura, S.; Kobayashi, S.; Komatsu, N.; Nagai, M.; Omatsu, T.; Naoi, Y.; Sano, K.; Okazaki-Terashima, S.; Oba, M.; Katayama, Y.; Sato, R.; Asai, T.; Mizutani, T. Development of a one-run real-time PCR detection system for pathogens associated with bovine respiratory disease complex. *J Vet Med Sci.* 2017, 18;79(3):517-523. doi: 10.1292/jvms.16-0489.