

Table S1. Diagnostic Sensitivity and Specificity Testing- comparison between qPCR and tNGS assay for detection of feline respiratory pathogens from 31 clinical samples.

ACCESSION	SPECIMEN	qPCR Results			tNGS results
A13-4105	lung	FeHV-1 Ct	16.43	Other pathogens not tested	Results matched qPCR results
A14-7200	lung	FeHV-1 Ct	20.88	Other pathogens not tested	Results matched qPCR results
S12-1183	lung	FCV Ct	19.76	Other pathogens not tested	Results matched qPCR results
A18-14725	lung	FCV Ct	16.28	Other pathogens not tested	Results matched qPCR results
A19-8904	Nasal swab	FeHV-1 Ct	15.66	Other pathogens not tested	Results matched qPCR results
A12-603	lung	FeHV-1 Ct	17.36	Other pathogens not tested	Results matched qPCR results
A23-11429	Nasal Swab	<i>Mycoplasma</i> spp. Ct	28.33	Negative for <i>B. bronchiseptica</i> spp., FeHV-1	Negative for <i>B. bronchiseptica</i>, <i>Chlamydia felis</i>, FeHV-1, Positive for <i>Mycoplasma felis</i>, FCV
		<i>B. bronchiseptica</i> Ct	33.21		
		FCV Ct	27.54		
A23-13268	OP Swab	<i>Mycoplasma</i> spp. Ct	35.38	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1	Results matched qPCR results
A24-11806	Lung	<i>Mycoplasma</i> spp. Ct	20.02	Negative for <i>Chlamydia</i> spp., FCV, FeHV-1	Results matched qPCR results
		<i>B. bronchiseptica</i> Ct	29.62		
A24-14404	Endotracheal Wash	<i>Mycoplasma</i> spp. Ct	33.17	Other pathogens not tested	Result matched qPCR result
A24-14405	Nasal Swab	<i>Mycoplasma</i> spp. Ct	20.33	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1	Results matched qPCR results
A24-5450	Lung	<i>Mycoplasma</i> spp. Ct	31.42	Negative for <i>B. bronchiseptica</i>	Negative for <i>Chlamydia felis</i>, <i>B. bronchiseptica</i>, Positive for <i>Mycoplasma felis</i>, FCV, and FeHV-1
		<i>Chlamydia</i> spp. Ct	31.33		
		FCV Ct	35.58		
		FeHV-1 Ct	29.29		
A24-5450	Nasal Swab	<i>Mycoplasma</i> spp. Ct	20.54	Negative for <i>B. bronchiseptica</i>	Results matched qPCR results
		<i>Chlamydia</i> spp. Ct	23.44		
		FCV Ct	29.27		
		FeHV-1 Ct	14.91		

A24-6974	Ocular, OP, and Nasal Swabs	<i>Mycoplasma</i> spp. Ct	23.56	Negative for <i>Chlamydia</i> spp.	Results matched qPCR results
		<i>B. bronchiseptica</i> Ct	26.78		
		FCV Ct	25.44		
		FeHV-1 Ct	35.00		
A24-6974	OP and Ocular Swabs		*	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	Results matched qPCR results
A24-7717	OP Swab	<i>Mycoplasma</i> spp. Ct	22.76	Negative for <i>B. bronchiseptica</i> , FeHV-1	Positive for <i>B. bronchiseptica</i>, <i>Mycoplasma felis</i>, FCV, Negative for <i>Chlamydia felis</i>, FeHV-1
		<i>Chlamydia</i> spp. Ct	30.42		
		FCV Ct	30.26		
A24-8065	Endotracheal Wash	<i>Mycoplasma</i> spp. Ct	24.89	Other pathogens not tested	Results matched qPCR result
A24-8396	OP Swab	<i>Mycoplasma</i> spp. Ct	23.19	Negative for <i>B. bronchiseptica</i> , FCV, FeHV-1	Positive for <i>Mycoplasma felis</i> , Negative for <i>Chlamydia felis</i>, <i>B. bronchiseptica</i>, FCV, FeHV-1
		<i>Chlamydia</i> spp. Ct	27.69		
A23-13578	OP Swab	<i>B. bronchiseptica</i> Ct	34.16	Negative for <i>Mycoplasma</i> spp., <i>Chlamydia</i> spp., FCV, FeHV-1	Results matched qPCR results
A24-10580	Nasal Swab	<i>B. bronchiseptica</i> Ct	33.05	Negative for <i>Mycoplasma</i> spp.	Results matched qPCR results
		<i>Chlamydia</i> spp. Ct	31.64		
		FCV Ct	22.20		
		FeHV-1 Ct	16.02		
A24-5813	Lung pool	<i>B. bronchiseptica</i> Ct	32.02	Negative for <i>Chlamydia</i> spp., FeHV-1	Results matched qPCR results
		<i>Mycoplasma</i> spp. Ct	20.23		
		FCV Ct	19.08		
A24-8280	OP Swab	<i>B. bronchiseptica</i> Ct	35.00	Negative for <i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	Negative for <i>B. bronchiseptica</i>, <i>Chlamydia</i> spp., FCV, FeHV-1, Positive for <i>Mycoplasma felis</i>
A23-17385	OP Swab		*	Negative for <i>B. bronchiseptica</i> ,	Results matched qPCR results

				<i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	
A24-2384	Bronchoalveolar Lavage (BAL)		*	Negative for <i>Mycoplasma</i> spp.	Results matched qPCR results
A24-5509	Lung	FeHV-1 Ct	36.40	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, <i>Mycoplasma</i> spp.	Results matched qPCR results
A24-5650	OP Swab	<i>Chlamydia</i> spp. Ct	31.97	Negative for <i>B. bronchiseptica</i> and <i>Mycoplasma</i> spp.	Negative for <i>Chlamydia felis</i>, FCV, positive for FeHV-1, negative for <i>B. bronchiseptica</i> and <i>Mycoplasma felis</i>
		FCV Ct	25.57		
		FeHV-1 Ct	22.16		
A24-6304	OP Swab		*	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	Results matched qPCR results
A24-6455	Lung		*	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	Positive for <i>Mycoplasma felis</i>, Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1
A24-7609	Nasal Swab		*	Negative for <i>B. bronchiseptica</i> , <i>Chlamydia</i> spp., FCV, FeHV-1, <i>Mycoplasma</i> spp.	Results matched qPCR results
Cheerio	Ocular swab	<i>Chlamydia</i> spp. Ct	36.9	Other pathogens not tested	Results matched qPCR
Atticus	Ocular Swab	<i>Mycoplasma</i> spp. Ct	Positive, Ct unknown	Negative for FCV, FeHV-1	Results matched qPCR

Table S2. Comparison of MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and MagMAX CORE Nucleic Acid Purification Kit with silica bead beating

Sample 1	Viral/Pathogen Kit (avg number reads)	CORE (avg number reads)
SARS-CoV-2	3498	158
FeHV-1	152626	61152
FCV	1054	93
<i>C. felis</i>	259766	113676
<i>B. bronchiseptica</i>	141793	67118
Sample 2		
SARS-CoV-2	688	261
FeHV-1	55911	63182

FCV	222	94
<i>C. felis</i>	86056	112225
<i>B. bronchiseptica</i>	46264	77783
Sample 3		
SARS-CoV-2	655	211
FeHV-1	56761	56829
FCV	171	53
<i>C. felis</i>	79931	102064
<i>B. bronchiseptica</i>	46319	70982
Sample 4		
SARS-CoV-2	1404	238
FeHV-1	118188	55572
FCV	404	35
<i>C. felis</i>	162247	72399
<i>B. bronchiseptica</i>	46264	78088

Table S3. Comparison of MagMAX Viral/Pathogen Nucleic Acid Isolation Kit vs MagMAX Pathogen RNA/DNA Kit for extraction of SARS-CoV-2 from feline positive and negative clinical samples.

Sample- Ct value by qPCR	Viral/Pathogen Kit (avg number reads)	Pathogen RNA/DNA Kit (avg number reads)
1- 35.95, 38.49 (considered inconclusive by qPCR)	30	0
2- 31.08, 30.23	4123	3650
3- 33.04, 36.49	59	243
4- No detection	0	0
5- 32.02, 31.75	757	483
6- No detection	0	0