

# Clinical evaluation of a multiplex RT-PCR assay for detection of SARS-CoV-2 in individual and pooled upper respiratory tract samples

## Archives of Virology

Melissa Laverack<sup>a</sup>, Rebecca L. Tallmadge<sup>a</sup>, Roopa Venugopalan<sup>a</sup>, Brittany Cronk<sup>a</sup>, XiuLin Zhang<sup>a</sup>, Rolf Rauh<sup>b</sup>, Amy Saunders<sup>b</sup>, William M. Nelson<sup>b</sup>, Elizabeth Plocharczyk<sup>c</sup>, Diego G. Diel<sup>a#</sup>

<sup>a</sup>Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell COVID-19 Testing Laboratory, Cornell University, Ithaca, New York, USA

<sup>b</sup>Tetracore Inc., Rockville, Maryland, USA

<sup>c</sup>Cayuga Medical Center, Cayuga Health System, Ithaca, New York, USA

#Address correspondence to: Diego G. Diel, [dgdziel@cornell.edu](mailto:dgdziel@cornell.edu)

**Supplementary Table 1.** *In silico* cross-reactivity analysis of the of the EZ-SARS-CoV-2 Real-Time RT-PCR assay primers and probes.

Pathogen	Strain	GenBank Accession #
Adenovirus	Human adenovirus type 1, complete genome	AC_000017.1
Bordetella pertussis	Bordetella pertussis strain B3921, complete genome	CP011448.1
Candida albicans	Candida albicans strain L757 mitochondrion, complete genome	NC_018046.1
<i>Chlamydia pneumoniae</i>	Chlamydia pneumoniae genome assembly PB2, chromosome: 1	NZ_LN847241.1
Enterovirus	Human enterovirus 68 isolate EV68_NL_201013421 VP1 protein gene, partial cds	JF896312.1
Haemophilus influenzae	Haemophilus influenzae PittGG, complete genome	CP000672.1
Human coronavirus 229E	Human coronavirus 229E strain 229E/human/USA/932-72/1993, complete genome	KF514432.1

Human coronavirus 229E	Human coronavirus 229E strain 229E/human/USA/933-40/1993, complete genome	KF514433.1
Human coronavirus HKU1	Human coronavirus HKU1 isolate SI17244, complete genome	MH940245.1
Human coronavirus HKU1	Human coronavirus HKU1 strain HKU1/human/USA/HKU1-18/2010, complete genome	KF430201.1
Human coronavirus NL63	Human coronavirus NL63 strain NL63/human/USA/891-4/1989, complete genome	KF530114.1
Human coronavirus NL63	Human coronavirus NL63 strain NL63/human/USA/905-25/1990, complete genome	KF530113.1
Human coronavirus OC43	Human coronavirus OC43 isolate LRTI_238, complete genome	KX344031.1
Human coronavirus OC43	Human coronavirus OC43 strain OC43/human/USA/971-5/1997, complete genome	KF530099.1
Human Metapneumovirus (hMPV)	Human metapneumovirus strain HMPV/Homo sapiens/PER/FPP00726/2011/A, complete genome	KJ627437.1
Influenza A	Influenza A virus (A/New York/PV305/2017(H1N1)) segment 2 polymerase PB1 (PB1) gene, complete cds and functional PB1-F2 protein (PB1-F2) gene, complete sequence	MH798556.1
Influenza B	Influenza B virus (B/Nicaragua/8689_13/2017) segment 2 polymerase PB2 (PB2) gene, complete cds	MK969560.1
Legionella pneumophila	Legionella pneumophila strain Philadelphia 1 CDC, complete genome	CP015928.1
MERS-Coronavirus	Middle East respiratory syndrome-related coronavirus strain HcoV-EMC, complete genome	MH013216.1
Mycobacterium tuberculosis	Mycobacterium tuberculosis DNA, complete genome, strain: HN-506	AP018036.1
Mycoplasma pneumoniae	Mycoplasma pneumoniae strain 14-637 chromosome, complete genome	CP039772.1
Parainfluenza 1	Human parainfluenza virus 1 isolate NM001, complete genome	KX639498.1
Parainfluenza 2	Human parainfluenza virus 2 isolate VIROAF10, complete genome	KM190939.1
Parainfluenza 3	Human parainfluenza virus 3 strain HPIV3/AUS/3/2007, complete genome	KF530243.1
Parainfluenza 4	Human parainfluenza virus 4a isolate HPIV4_DK (459), complete genome	KF483663.1
Pneumocystis jirovecii	Pneumocystis jirovecii isolate SW7_full mitochondrion, complete genome	MH010446.1
Pseudomonas aeruginosa	Pseudomonas aeruginosa UCBPP-PA14, complete genome	CP000438.1

Respiratory syncytial virus	Respiratory syncytial virus strain B/WI/629-Q0190/10, complete genome	JN032120.1
Rhinovirus	Human rhinovirus 14, complete genome	NC_001490.1
SARS-coronavirus	SARS coronavirus A022, complete genome	AY686863.1
SARS-coronavirus	SARS coronavirus CUHK-AG01, complete genome	AY345986.1
SARS-CoV-2	Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1, complete genome	NC_045512.2
Staphylococcus epidermidis	Staphylococcus epidermidis strain SP3 16S ribosomal RNA gene, partial sequence	KY750253.1
Streptococcus pneumoniae	Streptococcus pneumoniae strain D39V chromosome, complete genome	CP027540.1
Streptococcus pyogenes	Streptococcus pyogenes MGAS8232, complete genome	AE009949.1
Streptococcus salivarius	Streptococcus salivarius strain LAB813 chromosome, complete genome	CP040804.1

**Supplementary Table 2.** Specificity of EZ-SARS-CoV-2 RT-PCR assay.

<b>Organism</b>	<b>Source</b>	<b>Isolate No.</b>	<b>Replicates Detected/Total</b>
Human coronavirus 229E	Zeptomatrix	0810229CF	0/3
Human coronavirus OC43	Zeptomatrix	0810024CF	0/3
Human coronavirus NL63	BEI Resources	NR-470	0/3
SARS coronavirus	BEI Resources	NR-9547	0/3
MERS coronavirus	BEI Resources	NR-45843	0/3
Adenovirus Type 7A	Zeptomatrix	0810021CF	0/3
Adenovirus Type 1	Zeptomatrix	0810050CF	0/3
Adenovirus Type 4	Zeptomatrix	0810070CF	0/3
Human metapneumovirus 16 Type A1	Zeptomatrix	0810161CF	0/3
Parainfluenza virus 1	BEI Resources	NR-48680	0/3
Parainfluenza virus 2	BEI Resources	NR-3229	0/3
Parainfluenza virus 3	BEI Resources	NR-3233	0/3
Parainfluenza virus 4A	BEI Resources	NR-3237	0/3
Parainfluenza virus 4B	BEI Resources	NR-3238	0/3
Influenza A H1N1	BEI Resources	NR-13663	0/3
Influenza A H3N2	BEI Resources	NR-41803	0/3
Influenza B	BEI Resources	NR-42006	0/3

Enterovirus Type 68	Zeptomatrix	0810237CF	0/3
Enterovirus 71	BEI Resources	NR-471	0/3
Enterovirus D68	BEI Resources	NR-49131	0/3
Respiratory syncytial virus A1998/3-2	BEI Resources	NR-28529	0/3
Respiratory syncytial virus B1	BEI Resources	NR-4052	0/3
Respiratory syncytial virus A1998/12-21	BEI Resources	NR-28528	0/3
Rhinovirus 20, 15-CV19	BEI Resources	NR-51439	0/3
Rhinovirus 60, 2268-CV37	BEI Resources	NR-51447	0/3
Rhinovirus 34, 137-3	BEI Resources	NR-51451	0/3
<i>Chlamydia pneumoniae</i>	ATCC	53592	0/3
<i>Haemophilus influenzae</i>	ATCC	33391	0/3
<i>Legionella pneumophila</i>	Zeptomatrix	0801645	0/3
<i>Mycobacterium tuberculosis</i>	Zeptomatrix	0801660	0/3
<i>Streptococcus pneumoniae</i>	ATCC	49619	0/3
<i>Streptococcus pyogenes</i>	ATCC	10782	0/3
<i>Bordetella pertussis</i>	BEI Resources	NR-42460	0/3
<i>Mycoplasma pneumoniae</i>	Zeptomatrix	0801579	0/3
<i>Pneumocystis jirovecii</i> (PJP)	ATCC	PRA-159	0/3
Pooled human nasal wash	In-House		0/3
<i>Candida albicans</i>	ATCC	18804	0/3
<i>Pseudomonas aeruginosa</i>	ATCC	27853	0/3
<i>Staphylococcus epidermis</i>	ATCC	14990	0/3
<i>Streptococcus salivarius</i>	ATCC	13419	0/3

**Supplementary Table 3.** Comparison of Tetracore EZ-SARS-CoV-2 Real-Time RT-PCR assay results with Xpert Xpress SARS-CoV-2 results for 60 NPS from testing laboratory W. Results determined on the ABI 7500 platform are on the left and results determined by the QuantStudio 5 platform are on the right.

<b>ABI 7500</b>	Xpert Xpress SARS-CoV-2 positive	Xpert Xpress SARS-CoV-2 negative
EZ positive	30	2
EZ negative	0	28
Diagnostic sensitivity		100
Diagnostic specificity		93.33
Positive predictive value		93.75
Negative predictive value		100
kappa (95% confidence interval)		0.95 (0.89-1.02)

<b>QuantStudio 5</b>	Xpert Xpress SARS-CoV-2 positive	Xpert Xpress SARS-CoV-2 negative
EZ positive	30	3
EZ negative	0	27
Diagnostic sensitivity		100
Diagnostic specificity		90.00
Positive predictive value		90.91
Negative predictive value		100
kappa (95% confidence interval)		0.93 (0.85-1.01)

**Supplementary Table 4.** Comparison of Tetracore EZ-SARS-CoV-2 Real-Time RT-PCR assay results with TaqPath COVID-19 results for 141 NPS from testing laboratory T. Results determined on the ABI 7500 platform are on the left and results determined by the QuantStudio 5 platform are on the right.

<b>ABI 7500</b>	TaqPath COVID-19 positive	TaqPath COVID-19 negative
EZ positive	66	4
EZ negative	4	67
Diagnostic sensitivity	94.29	
Diagnostic specificity	94.37	
Positive predictive value	94.29	
Negative predictive value	94.37	
kappa (95% confidence interval)	0.92 (0.87-0.98)	

<b>QuantStudio 5</b>	TaqPath COVID-19 positive	TaqPath COVID-19 negative
EZ positive	64	3
EZ negative	6	68
Diagnostic sensitivity	91.43	
Diagnostic specificity	95.77	
Positive predictive value	95.52	
Negative predictive value	91.89	
kappa (95% confidence interval)	0.92 (0.86-0.97)	