FastPlex Triplex SARS-CoV-2 detection kit （RT-PCR）

Performance Characteristics

Catalog # 02.01.1038
(96 Tests/kit)

For in vitro Diagnostic (IVD) Use
For Prescription Use Only
FDA's review of this EUA is pending

（Reports of results to healthcare providers should note that the test has been validated but FDA's independent review of this validation is pending）

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I. Limit of Detection (LoD) - Analytical Sensitivity

To determine the tentative LoD, negative clinical matrix was prepared by pooling oropharyngeal swabs specimens collected from person negative for SARS-CoV-2. The negative matrix was used to serially dilute quantified whole viral genomic SARS-CoV-2 RNA extracted from cells from a COVID-19 positive patient (NTHL-20200212) from 11.43 to 1.14 x10^5 copies/mL in replicates of three. Replicates were individually processed using the QIAamp Viral RNA Mini Kit according to the FastPlex Triplex SARS-CoV-2 detection kit (RT-PCR) instructions for use. The tentative LoD was determined to be 285.7 copies/mL.

The tentative LoD was confirmed by testing 20 additional replicates of clinical specimens diluted by negative clinical matrix at the preliminary LoD concentration of 285.7 copies/mL. The 20 replicates were prepared and extracted following the same procedure above. The LoD was confirmed to be 285.7 copies/mL as > 95% (20/20) of the replicates were positive. Data in shown in **Table 1**.

Table 1. Confirmatory LoD Study Results

RNA Input (copies/reaction)	Clinical Sample Input (copies/mL)	ORF1ab Ct	N Ct	RNase P Ct	Interpretation	#of Positive Samples	Detection Rate
4.86	285.70	34.27	34.23	25.39	Positive	20/20	100%
		34.95	34.55	25.43	Positive		
		33.39	35.11	25.41	Positive		
		34.43	34.83	25.54	Positive		
		33.97	34.48	25.38	Positive		
		34.69	35.23	25.42	Positive		
		33.94	35.35	25.41	Positive		
		34.44	34.40	25.35	Positive		
		36.57	34.93	25.49	Positive		
		34.34	34.27	25.33	Positive		
		34.16	34.94	25.42	Positive		
		33.62	36.55	25.34	Positive		
		34.96	35.74	25.22	Positive		
		35.03	34.54	25.39	Positive		
		34.11	35.83	25.28	Positive		
		33.39	35.91	25.34	Positive		
		39.03	34.33	25.38	Positive		
		34.65	33.85	25.18	Positive		
		34.05	34.15	25.27	Positive		
		35.67	34.77	25.26	Positive		

II. *Inclusivity (analytical sensitivity)*

Inclusivity was demonstrated by analyzing the sequence of each of the FastPlex Triplex SARS-CoV-2 detection kit (RT- PCR) primers and probes for homology with all complete genome sequence of SARS-CoV-2 sequences available in Betacoronavirus GenBank as of July 6, 2020, by in silico analysis using NCBI Nucleotide BLAST (BLASTn) alignment tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&DBSEARCH=true&QUERY=&SUBJECTS=). Among a total of 5042 complete SARS-CoV-2 genome sequences from 28 countries/regions (Australia, Bangladesh, Chile, China, Czech Republic, Egypt, Germany, Hong Kong , India, Iran, Italy, Jamaica, Japan, Korea, Netherlands, Nigeria, Pakistan, Poland, Russian Federation, Saudi Arabia, Serbia, Spain, Taiwan, Thailand, Timor-Leste, Tunisia, Turkey and United Sates), 4537 exhibited 100% identity to all FastPlex Triplex SARS-CoV-2 detection kit (RT- PCR) primers and probes sequences, while 94 contained a single mismatch in one of the two gene sequences that the assay targets, 2 contain 2 mismatch and 408 has 3 mismatch at the every 5' of the N forward primers.. The N forward primer (0.8uM primers, 50mM Na, 3mM Mg, and 0.8mM dNTP) gives a Tm of 65.2C and with the 3 5' mismatch the Tm is reduced to 58.4C, which is 1.6C lower than the 60C optimal annealing temperature. We predicted that the FastPlex Triplex SARS-CoV-2 detection kit should still detect the presence of N target. Furthermore, since the ORF1ab targeted region is 100% match with these SARS-CoV 2 genome sequences, we expect the second target, ORF1ab, should be detected.

Overall, these analyses predict the FastPlex Triplex SARS-CoV-2 detection kit should be able to detect all of these SARS-CoV-2 strains published in the NCBI Betacoronavirus GenBank.

Total complete genome analyzed						
5042						
Genome sequences excluded from analysis due to sequence issues						
33						
	N forward Primer	Percentage	N Probe	Percentage	N reverse Primer	Percentag
Primer/probe with 100% match	4566	90.56%	5039	99.94%	5021	99.58%
Primer/probe with 1 mutation	67	1.33%	3	0.06%	20	0.40%
Primer/Probe with 2 mutations	1	0.02%	0	0.00%	1	0.02%
Primer/Probe with 3 mutations	408	8.09%	0	0.00%	0	0.00%
Primer/Probe with 4 mutations	0	0.00%	0	0.00%	0	0.00%
	ORF1ab forward Prime	Percentage	ORF1ab Probe	Percentage	ORF1ab reverse Prime	Percentag
Primer/probe with 100% match	5040	99.96%	5042	100.00%	5040	99.96%
Primer/probe with 1 mutation	2	0.04%	0	0.00%	2	0.04%
Primer/Probe with 2 mutations	0	0.00%	0	0.00%	0	0.00%
Primer/Probe with 3 mutations	0	0.00%	0	0.00%	0	0.00%
Primer/Probe with 4 mutations	0	0.00%	0	0.00%	0	0.00%

III. Cross-reactivity (Analytical Specificity)

Cross-reactivity of the ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*** was evaluated using both *in silico* analysis and wet testing against normal and pathogenic organisms found in the respiratory tract.

a. *In silico* analysis:

In silico analysis for possible cross-reactivity with the organisms listed in **Table 2** was performed. With the exception of SARS-coronavirus, no organisms, including other related coronaviruses, were amplified by either the ORF1ab or N target PCR primers and probes. The results of the *in silico* analysis suggest that the ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*** is specific for SARS-CoV-2.

Table 2. Cross-Reactivity *In Silico* Study (Reference Sequences of Organisms)

Pathogen	GenBank Acc#	Pathogen	GenBank Acc#
Human coronavirus 229E	AF304460.1	A7 Respiratory syncytial virus	U39661.1
Human coronavirus OC43	NC_006213.1	Rhinovirus	NC_009996.1
Human coronavirus HKU1	NC_006577.2	*Chlamydia pneumoniae*	NC_000922.1
Human coronavirus NL63	NC_005831.2	*Haemophilus influenzae*	NC_000907.1
SARS-coronavirus	NC_004718.3	*Legionella pneumophila*	NC_002942.5
MERS-coronavirus	NC_019843.3	*Mycobacterium tuberculosis*	NC_018143.2
Adenovirus (e.g. C1 Ad. 71)	KF268207	*Streptococcus pneumoniae*	NC_003098.1
Human Metapneumovirus (hMPV)	MG431250.1	*Streptococcus pyogenes*	NC_002737.2
Parainfluenza virus 1	NC_003461.1	*Bordetella pertussis*	NC_002929.2
Parainfluenza virus 2	NC_003443.1	*Mycoplasma pneumoniae*	NC_000912.1
Parainfluenza virus 3	KF687319	*Pneumocystis jirovecii* (PJP)	NW_017264775.1
Parainfluenza virus 4	KF483663.1	*Candida albicans*	NC_032089.1
Influenza A	KT388699.1	*Pseudomonas aeruginosa*	NC_002516.2
Influenza B	AF101982.1	*Staphylococcus epidermis*	NC_004461.1
Enterovirus (e.g. EV68)	NC_038308.1	*Streptococcus salivarius*	CP013216.1

SARS-coronavirus, a closely related human SARS virus exhibited > 90% homology to the forward primer, probe of ORF1ab target, forward and reverse primer of N target and – based on the *in silico* analysis - would be expected to result in cross-reactivity

with the ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT- PCR).*** However, cross-reactivity was not observed in the wet testing (see Table 2 above). The impact of such cross-reactivity is further mitigated through the fact that SARS-CoV virus is not currently circulating.

b. *Wet-Testing*:

Wet testing against normal and pathogenic organisms of the respiratory tract, sourced from China National Institute of Food and Drug control and Fubio Biological Technology Co., was performed to confirm the results of the *in silico* analysis. The organism identified in **Table 3** below were tested with 3 lots of ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*** at the organism concentrations indicated (1 replicate per lot). Each replicate was tested with a different reagent lot. All results were negative with the ORF1ab and N genes (no cross-reactivity).

Table 3: Wet testing of organisms in VTM (Copan Collection, Transport and Processing Kit UTM 306) for cross reactivity with the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*

Pathogen	Source/Sample Type	Concentration	Fastplex Triplex SARS-CoV- 2 Detection Kit (RT-PCR)											
			LOT1 Ct				LOT2 Ct				LOT3 Ct			
			ORF1ab FAM	N HEX	RNase P Cy5	Interpretation	ORF1ab FAN	N HEX	RNase P Cy5	Interpretation	ORF1ab FAM	N HEX	RNase P Cy5	Interpretation
Influenza A virus (A/Aalborg/INS132/2009(H1N1 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
Influenza A virus (A/Addis Ababa/1514A07305892N/2013(H3N2 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
Influenza A virus (A/Anhui/1-DEWH730/2013(H7N9 (HA-NA))	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
Influenza B virus (B/Yamagata/222/2002 (HA-NA)	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
Influenza B virus (B/Victoria/1/2014 (HA-M1))	National Standard	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
Influenza A virus (A/goose/Guangdong/1/1996(H5N1))	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
FNV-SARS-ORF1a-N	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative
FNV-MERS-abEN	Pseudo-virus	≥ 1E+7 Copy/mL	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative	Undermined	Undermined	Undermined	Negative

1). Endogenous Interference Substances Studies:

Since this test uses the QIAamp Viral RNA Mini Kit, a well-established extraction method, an endogenous interference study for the EUA was not performed.

2). Clinical Evaluation:

Clinical Sample Testing

The performance of ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*** with oropharyngeal swab clinical samples was evaluated using 117 clinical specimens. Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were stored frozen at -80°C until use. All clinical samples were randomized, blinded and tested with ***FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*** and the FDA EUA authorized comparator (Sansure BioTech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (EUA authorized on 05-04-2020). The positive or negative is determined based on the manufacturers' instructions.

The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARSCoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, midturbinate swabs, nasal washes and nasal aspirates from individuals who are suspected of COVID19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Nucleic acid extraction was performed with the QIAamp Viral RNA Mini Kit. A total of 117 specimens were available for analysis when using the QIAamp Viral RNA Mini Kit. All 117 specimens were tested with ***FastPlex Triplex SARS-CoV-2 Detection kit (RT-PCR)*** and with Sansure BioTech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) on ABI 7500 Real Time System. The positive percent agreement (PPA) was 97.9%, 95% CI [88.9%-99.9%] and the negative percent agreement (NPA) was 95.7%, 95% CI [88.0%-99.1%] as shown in **Table 4**.

Table 4. Clinical Evaluation of the *FastPlex Triplex SARS-CoV-2 Detection Kit (RT-PCR)*.

	Comparator Test Positive	**Comparator Test Negative**	**Total**
FastPlex Triplex SARS-CoV-2 Positive	46	3	48
FastPlex Triplex SARS-CoV-2 Negative	1	67	69
Total	47	70	117

PPA = 46/47 x 100% = 97.9%, (95% CI: 88.7%-99.9%)
NPA = 67/70 x 100%= 95.7%, (95% CI: 88.0%- 99.1%)