Analytical and Clinical Performance Characteristics of the FTD SARS-CoV-2 Assay

ECCVID 2020 ePoster

September 2020

HOOD05162003126921

Analytical and Clinical Performance Characteristics of the FTD SARS-CoV-2 Assay*

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Introduction

- Fast Track Diagnostics (FTD) responded to the COVID-19 pandemic with the development of the FTD SARS-CoV-2 Assay. This in vitro diagnostic test is intended as an aid in the diagnosis of COVID-19 disease in patients with signs and symptoms.
- A dual-target assay was developed targeting the ORF1ab and N gene of SARS-CoV-2.
- The same dye/detection channel is used for the two SARS-CoV-2 assays.
  - Increased signal/better sensitivity
  - Optimized detection of possible mutants

Excellent analytical sensitivity

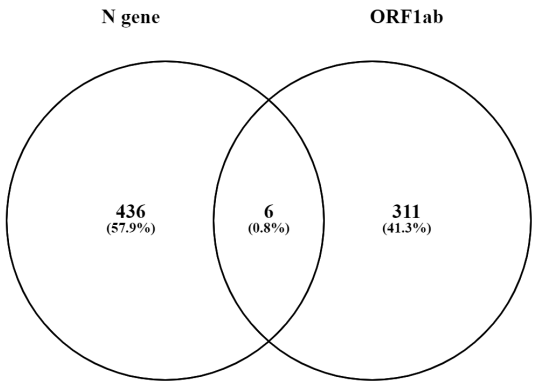
- Simulated respiratory matrix was spiked with SARS-CoV-2 heat-inactivated quantified culture (US-WA1/2020).
- Probit analysis was performed to calculate the LoD: 0.0023 TCID50/mL (CI: 0.0015–0.003).
- LoD was confirmed by testing 23 individual samples (SRM spiked with culture).

Pathogen	PPmix Lot	LoD (TCID50/mL)	Total Tested	Total Detected	Detection Rate (%)	Mean Ct Value	SD
SARS-CoV-2	1	0.0023	23	22	95.7	37.4	0.8
	2		23	22	95.7	38.0	0.9
	3		23	23	100.0	37.4	0.9

In silico inclusivity study shows 100% detection rate on more than 64,000 sequences from international database

Assay	Databases	Complete Genomes Tested/ Complete Genomes Detected	Detection Rate (%)
N gene	GeneBank + GISAID	64253/64253	100
ORF1ab	GeneBank + GISAID	65051/65051	100

- 442 sequences showed one mismatch with N gene assay.
- 317 sequences showed one mismatch with ORF1ab assay.
- 0.8% overlap between the sequences showing mismatch with the N gene and ORF1ab assay.
- Due to dual-target approach, at least one of the two assays binds without any mismatch to the remaining 747 sequences.



*CE-IVD–labeled for diagnostic use in the EU. This test has not been FDA-cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21. U.S.C. § 360bbb- 3(b)(1), unless the authorization is terminated or revoked sooner.

No cross-reactivity observed in vitro

List of pathogens tested for potential cross-reactivity

SARS coronavirus	Influenza A
MERS coronavirus	Influenza B
Human coronavirus 229E	Respiratory syncytial virus A
Human coronavirus HKU1	Respiratory syncytial virus B
Human coronavirus OC43	Adenovirus 71
Human coronavirus NL63	Enterovirus
Parainfluenza virus 1	Chlamydophila pneumoniae
Parainfluenza virus 2	Haemophilus influenzae
Parainfluenza virus 3	Legionella pneumophila
Parainfluenza virus 4	Bordetella pertussis
Human metapneumovirus A	Mycoplasma pneumoniae
Human metapneumovirus B	Streptococcus pneumonia
Rhinovirus	Mycobacterium tuberculosis
Streptococcus salivarius	Legionella spiritensis
Staphylococcus epidermidis	Streptococcus pyogenes
Legionella sainthelensi	Pneumocystis carinii (Pneumocystis jirovecii)
	Pool of nasal washes

No cross-reactivity observed in silico

- 32 bacterial/viral/fungal strains have been analyzed in silico with NCBI BLAST tool.
- Sequence similarity ≥80% per primer or probe used as criterion for potential cross-reactivity (six pathogens concerned).

Coverage (%)

	N Assay			ORF1ab Assay		
	Fw	**Pb**	**Rw**	**Fw**	**Pb**	**Rw**
Legionella non-pneumophila*	78	67	**83**	73	74	77
Staphylococcus epidermis	**83**	63	**83**	68	57	77
Staphylococcus salivarius	72	60	70	68	70	**91**
SARS coronavirus	67	**90**	78	68	57	**95**
Rhinovirus	**83**	50	65	59	57	64

*Among the Legionella non-pneumophila strains, Legionella sainthelensi and Legionella spiritensis revealed a homology >80% for given primers.

No effect from potential interfering organisms

- Microbial interference study performed on those six organisms.
- Cultures of pathogens extracted at high concentration and in presence of SARS-CoV-2 (3x LoD).

Sample	**ΔCt**
Interfering organism (IO)	**((SARS-CoV2 + IO) Ct – SARS-CoV-2 Ct)**
Streptococcus salivarius	0.57
Staphylococcus epidermidis	0.17
Legionella sainthelensi	0.23
Legionella spiritensis	0.27
Rhinovirus	0.43
SARS coronavirus (Frankfurt strain)	–0.3

No effect from potential interfering substances

10 interfering substances (IS) tested in presence of SARS-CoV-2 (3x LoD).

SARS-CoV-2 detected in presence of all IS with a maximum Ct shift of 1.0.

v/v: volume to volume, µg/mL: micrograms per milliliter, µmol/L: micromoles per liter, mmol/L: millimoles per liter, mg/mL: milligrams per milliliter, xylo: xylometazoline.

Substance	Tested Concentration	ΔCt ((IS+SARS-CoV-2) Ct – SARS-CoV-2 Ct)
Whole blood	10% (v/v)	0.8
Mucin (porcine)	60 µg/mL	0.0
Salbutamol	1.7 µmol/L	0.0
Nasal spray (xylo)	10% (v/v)	–0.3
Nasal spray (salts)	10% (v/v)	0.8
Guaifenesin	15.2 mmol/L	0.0
Acetylcystein	920 µmol/L	–0.2
Nicotine	6.2 µmol/L	0.8
Benzocaine	0.63 mg/mL	0.3
Oseltamivir	1.5 mg/mL	1.0

Precision study shows excellent repeatability and reproducibility

Study design: three lots and two instruments used, performed by two operators and over the course of 3 days.

Target	Test Concentration	n	Repeatability SD	Reproducibility SD	Repeatability CV (%)	Reproducibility CV (%)
SARS-CoV-2	ULoD	105	0.78 (0.68–0.9)	0.95 (0.73–1.37)	2.05 (1.8–2.38)	2.51 (1.92–3.61)
	5x LoD	108	0.48 (0.42–0.55)	0.72 (0.5–1.26)	1.33 (1.17–1.54)	2.01 (1.4–3.51)

Coefficient of variation <5%

Clinical studies show excellent diagnostic sensitivity and specificity

First study:

- Comparator 1: CE IVD NAAT kit
- Fresh specimens (15 NPS, 86 OPS)
- Study population: male and female children, teenagers, and adults

		CE IVD RT-PCR Test	
		Positive	Negative
FTD SARS-CoV-2	Positive	43	0
	Negative	0	58
Positive percent agreement	100% (43/43) (95% CI: 91.8, 100)		
Negative percent agreement	100% (58/58) (95% CI: 93.8, 100)		

Second study:

- Comparator 2: FDA EUA authorized NAAT kit
- Frozen specimens (NPS)
- Study population: male and female adults

		FDA EUA RT-PCR Test	
		Positive	Negative
FTD SARS-CoV-2	Positive	44	0
	Negative	0	30
Positive percent agreement	100% (44/44) (95% CI: 91.97, 100)		
Negative percent agreement	100% (30/30) (95% CI: 88.65, 100)		

Conclusion

The FTD SARS-CoV-2 Assay shows excellent analytical and clinical performance and is suitable for the specific detection of SARS-CoV-2.

- Comprehensive in silico inclusivity study shows 100% detection rate on more than 64,000 sequences from international database.
- Excellent analytical sensitivity, repeatability, and reproducibility.
- No cross-reactivity observed in vitro and in silico.
- No effect of potential interfering substances or microorganisms.
- 100% positive and negative agreement with two comparator methods on clinical samples.