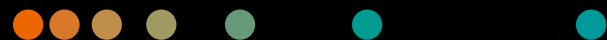


FTD SARS-CoV-2 Assay

Analytical & Clinical Performance Data (OUS)

Respiratory Testing

June 2020



FTD SARS-CoV-2 Assay*

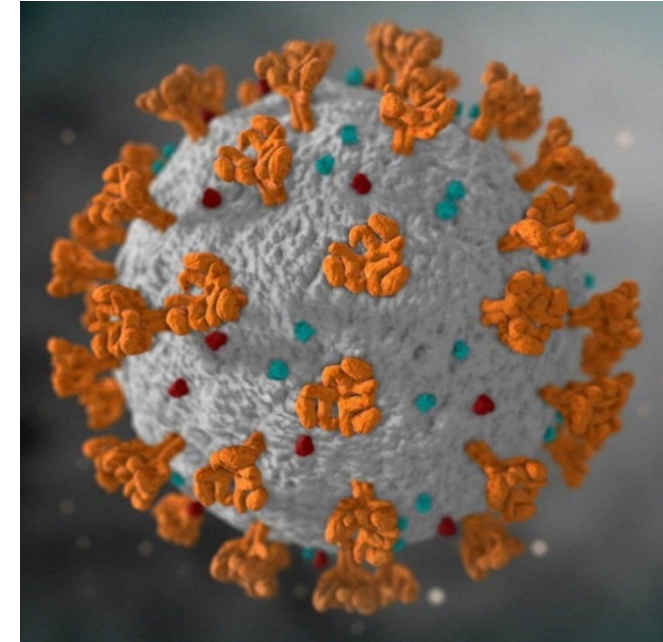
Instructions for Use

- The *Instructions for Use* provide general and technical information to inform the user of the intended purpose and proper use including contraindications, warnings, or precautions to be taken.
- This presentation is intended to provide an depth review specifically of the analytical and clinical performance of the FTD SARS-CoV-2 Assay.
- Users are encourage to please refer to the FTD SARS-CoV-2 Assay *Instructions for Use* for full details and information.
- The *Instructions for Use*, provided several languages, is downloaded directly from the [FTD website](#).

FTD SARS-CoV-2 Assay*

Intended Use

- FTD SARS-CoV-2 is a qualitative *in vitro* nucleic acid amplification test for the **detection of severe respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal and oropharyngeal swabs** of patients with signs and symptoms of SARS-CoV-2 infection in conjunction with clinical and epidemiological risk factors, who are suspected of Coronavirus Disease 2019 (COVID-19).
- The test is intended as an aid in the diagnosis of infections caused by the new human coronavirus SARS-CoV-2.



FTD SARS-CoV-2 Assay

Analytical Sensitivity (LoD)

- Extracted quantified RNA was diluted in TE* buffer and tested in the real-time PCR FTD SARS-CoV-2 Assay (24 replicates / conc. level).
- LoD was determine by Probit analysis (95% detectability).
- No extraction was performed (no extractable standard was available for BSL2 laboratories).

Human SARS-CoV-2 RNA BetaCoV/Germany/BavPat1/2020 p.1		
	LoD (cop/mL)	95% CI (cop/mL)
SARS-CoV-2	1155	854-1508

11.55 cop/reaction

LoD = limit of detection, cop/mL = copies per milliliter, CI = confidence interval

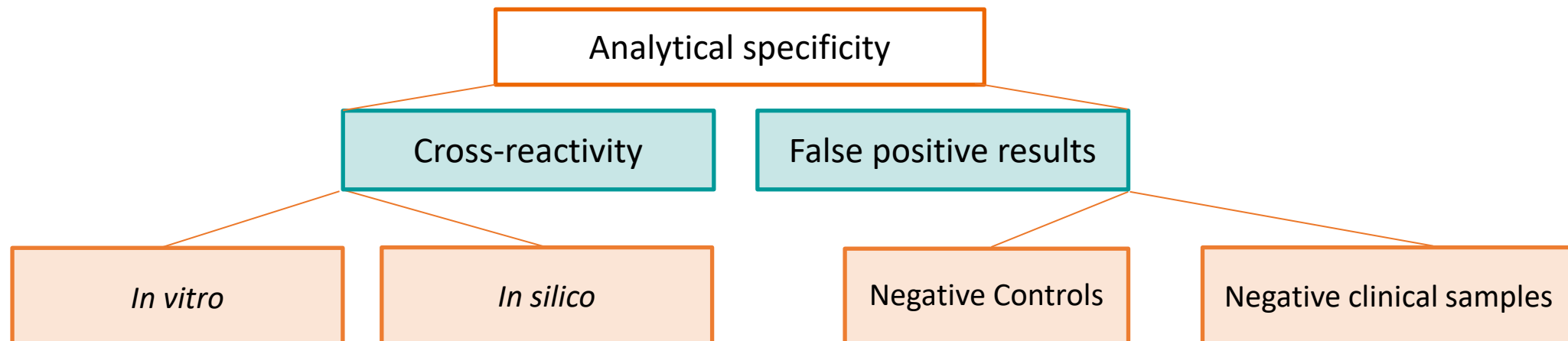
* Tris-EDTA, pH 8

FTD SARS-CoV-2 Assay

Analytical Specificity *in vitro* and *in silico* Analyses Overview

The analytical specificity of a PCR multiplex assay is the ability of a measurement to measure solely the target SARS-CoV-2.

- **Cross-reactivity:** The ability of the test to specifically detect the intended pathogens **but no other** organism in biological samples.
- **False positive results:** The assurance that the test will not report false positive results when testing negative samples/material (with human DNA/RNA background).



FTD SARS-CoV-2 Assay

Cross-reactivity *in vitro* Study

- The FTD SARS-CoV-2 Assay was tested *in vitro* for potential cross-reaction with **26 common human flora organisms and pathogenic organisms causing respiratory infections**.
- Cross-reactivity testing was performed in triplicate using several pools of contrived samples spiked with commercial pathogen sources or extracted pathogen DNA/RNA.
- Pools were **extracted** with bioMérieux easyMAG and tested on the ABI7500 thermal cycler.

List of pathogens tested for cross-reactivity purpose*

SARS-CoV-1 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	<i>Chlamydophila pneumoniae</i>
Parainfluenza virus 2	<i>Haemophilus influenzae</i>
Parainfluenza virus 3	<i>Legionella pneumophila</i>
Parainfluenza virus 4	<i>Bordetella pertussis</i>
Human metapneumovirus A	<i>Mycoplasma pneumoniae</i>
Human metapneumovirus B	<i>Streptococcus pneumonia</i>
Rhinovirus	<i>Mycobacterium tuberculosis</i>

* Highlighted in orange: All pathogens, including 4 related coronaviruses, that are part in FTD Respiratory pathogens 21 kit
 Highlighted in teal: Other related coronaviruses

No cross-reactivity observed

FTD SARS-CoV-2 Assay

Cross-reactivity *in silico* Analysis

- *in silico* analyses applying BLAST (basic local alignment search tool).
- Search regions of similarity for all primers and probes in the **NCBI Nucleotide collection database**.
- Exclude sequences belonging to SARS-CoV-2.
- Sequence similarity $\geq 90\%$ per primer or probe was used as criterion for potential cross-reactivity.
- Maximum of 4 mismatches allowed between primer/probe and target sequence.

No cross-reactivity observed
for the organisms of the normal human flora and any other pathogenic organisms causing infections of the human respiratory tract.

Pathogen	Potential cross-reactivity	Sequence homology to SARS-CoV-2 (NC_045512)
SARS-CoV-2	Bat coronavirus isolate RaTG13 (MN996532.1)	96.1%
	Pangolin coronavirus isolate MP789 (MT084071.1)	78.7%

FTD SARS-CoV-2 Assay

Specificity With Negative Samples

The FTD SARS-CoV-2 Assay was tested on extracted negative controls and negative clinical samples as well as non-template controls.

Pathogen	Negative control tested	Total reactions	Positive results	Analytical specificity %	Confidence interval %
SARS-CoV-2	Negative clinical sample	50	0	100	92.89-100.00
	Negative Control	35	0	100	90.00-100.00
	Non-Template Control	34	0	100	89.72-100.00
Total		119	0	100	96.95-100.00

100% Analytical Specificity

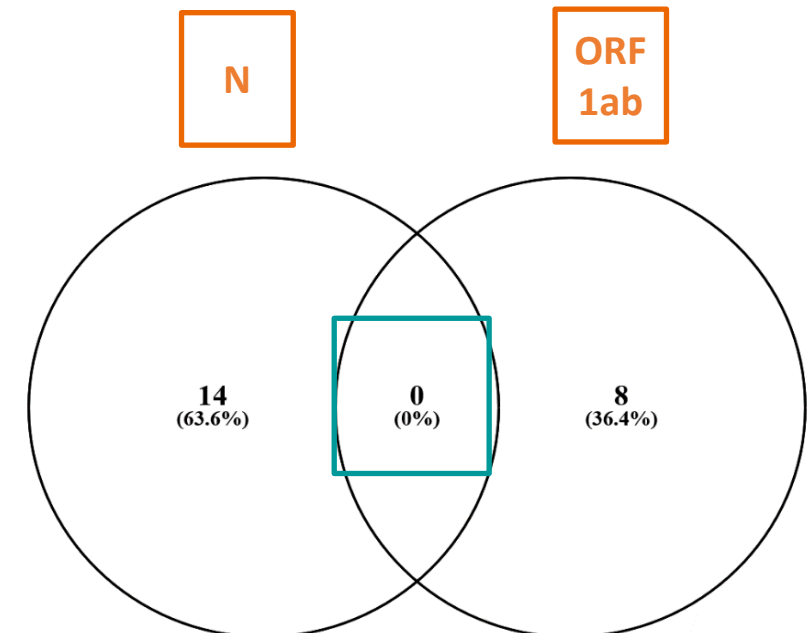
FTD SARS-CoV-2 Assay

Inclusivity (Analytical Reactivity) - *In silico* Analysis

- *In silico* analysis on 1048 full-length SARS-CoV-2 sequences downloaded from NCBI and GISAID database (ending up with **901 valid sequences**).
- Primers and probes were mapped to the sequences to check for potential matches producing amplicons (tolerance 4 mismatches).
 - **100% detection rate for SARS-CoV-2 N gene** assay: 887 sequences no mismatch, 14 sequences 1 mismatch.
 - **100% detection rate for SARS-CoV-2 ORF1ab** assay: 893 sequences no mismatch, 8 sequences 1 mismatch.
 - **No overlap between the sequences showing mismatch within the N gene and ORF1ab assay.**

→ Due to a dual-target approach, at least one of both assays binds without any mismatch to the above mentioned 22 sequences.

Assays	Database	complete genomes tested	complete genomes detected	% detection rate
SARS-CoV-2 (N gene)	GeneBank	96	96	100
	GISAID	805	805	100
SARS-CoV-2 (ORF1ab)	GeneBank	96	96	100
	GISAID	805	805	100



FTD SARS-CoV-2 Assay

Precision

FTD SARS-CoV-2 precision was assessed by repeatability and reproducibility studies with test material at a concentration of 5 x LoD and close to LoD.

- Repeatability evaluates measurements carried out under the same conditions (intra-assay variation).
- Reproducibility evaluates results of measurements under changed conditions (inter-assay variation*).

Concentration	n	Repeatability SD	Reproducibility SD	Repeatability CV (%)	Reproducibility CV (%)
5x LoD	24	0.43 (0.33-0.61)	0.49 (0.34-0.85)	1.2 (0.93-1.7)	1.35 (0.94-2.35)
close to LoD	23	0.8 (0.61-1.15)	0.95 (0.64-1.79)	2.1 (1.61-3.03)	2.49 (1.69-4.71)

CV < 5%

*E.g. Variation of time, operator and cyclor
CV = coefficient of variation

FTD SARS-CoV-2 Assay Interference Study

An interference study was conducted to evaluate the susceptibility of FTD SARS-CoV-2 Assay to provide wrong results in presence of potential interfering substances in the clinical sample.

- Artificial matrix spiked with interfering substance.
- Extraction in triplicates (easyMAG).
- Eluate spiked with SARS-CoV-2 RNA at 3x LoD concentration.
- RT-PCR with one lot of FTD SARS-CoV-2.

Substance	Tested concentration
Whole blood	10% (v/v)
Mucin (bovine)	60 µg/mL
Salbutamol	1.7 µmol/L
Nasal spray (Xylo.)	10% (v/v)
Nasal spray (Salts)	10% (v/v)
Guaifenesin	15.2 mmol/L
Acetylcystein	920 µmol/L
Nicotine	6.2 µmol/L
Benzocaine	0.63 mg/mL
Oseltamivir	1.5 mg/mL

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

**No interference
observed**

FTD SARS-CoV-2 Assay

Clinical performance: FTD SARS-CoV-2 vs. Seegene Allplex 2019-nCoV Assay

Comparison with 43 Seegene positive and 58 negative clinical samples.*

15 nasopharyngeal and 86 oropharyngeal swabs.*

Pathogen	Sample type	Diagnostic Sensitivity			Diagnostic Specificity		
		Percentage	Total number	95% Confidence Interval	Percentage	Total number	95% Confidence Interval
SARS-CoV-2	NPS	100%	12/12	73.5-100	100%	3/3	29.2-100
	OPS	100%	31/31	88.8-100	100%	55/55	93.5-100
	Overall	100%	43/43	91.8-100	100%	58/58	93.8-100

* Clinical samples were extracted with bioMérieux easyMAG for both assays.

FTD SARS-CoV-2 Assay

Summary of Performance Characteristics

Characteristics	Results
Limit of detection	11.55 copies/reaction (w/o extraction)
Cross reactivity	No <i>in vitro</i> cross reactivity with 26 common human flora organisms and the pathogenic organisms causing respiratory infections including 6 coronaviruses No <i>in silico</i> cross-reactivity to NCBI Nucleotide collection database
Analytical specificity	100% using 50 negative clinical samples, 35 negative controls and 34 non-template controls
Inclusivity	<i>In silico</i> analysis showed 100% detection rate on 901 full length sequences from NCBI and GISAID databases
Precision	Repeatability and reproducibility CV < 5%
Interference	No interference with 10 potential interfering substances in clinical samples
Clinical performance	100% diagnostic sensitivity and 100% diagnostic specificity against Seegene Allplex 2019-nCoV Assay

Thank you

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