# FTD SARS-CoV-2 Assay

## Analytical & Clinical Performance Data (OUS)

**Respiratory Testing**

February 2021

# FTD SARS-CoV-2 Assay*
# Instructions for Use

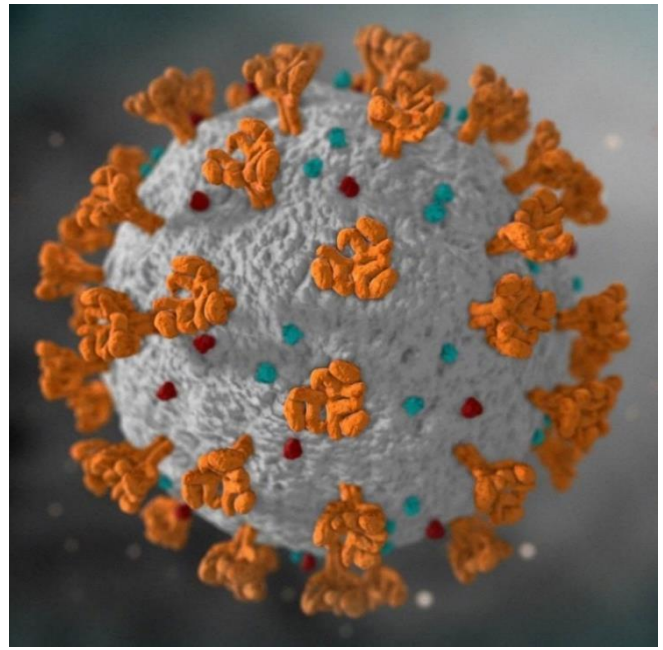
- The *Instructions for Use* provide general and technical information to inform the user of the intended purpose and proper use including contraindications, warnings, or precautions to be taken.
- This presentation is intended to provide an depth review specifically of the analytical and clinical performance 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.

*CE-IVD labelled for diagnostic use in the EU.

# 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.



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# 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

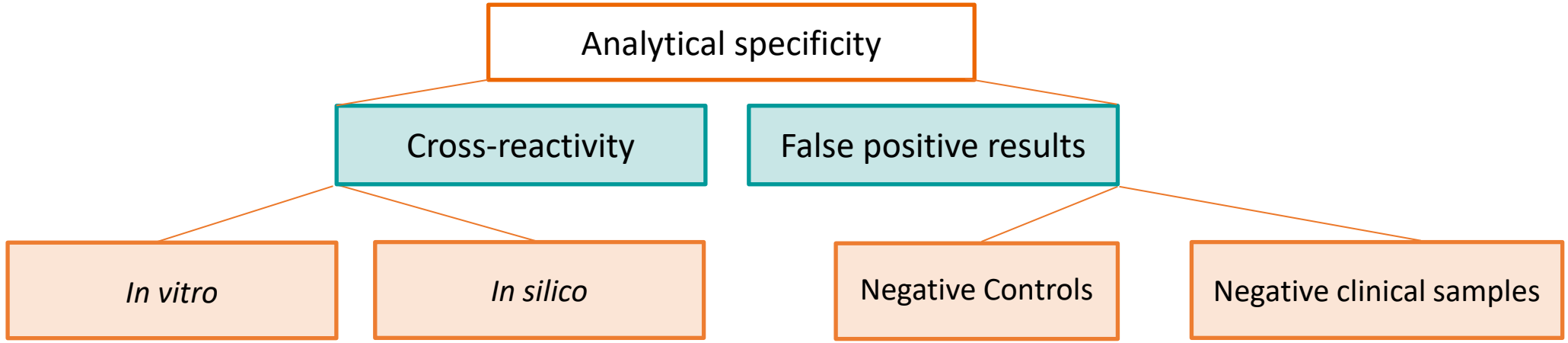
LoD = limit of detection, cop/mL = copies per milliliter, CI = confidence interval

* 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

SIEMENS Healthineers

- 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 | *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* |

**No cross-reactivity observed**

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

# 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 ≥ 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** |

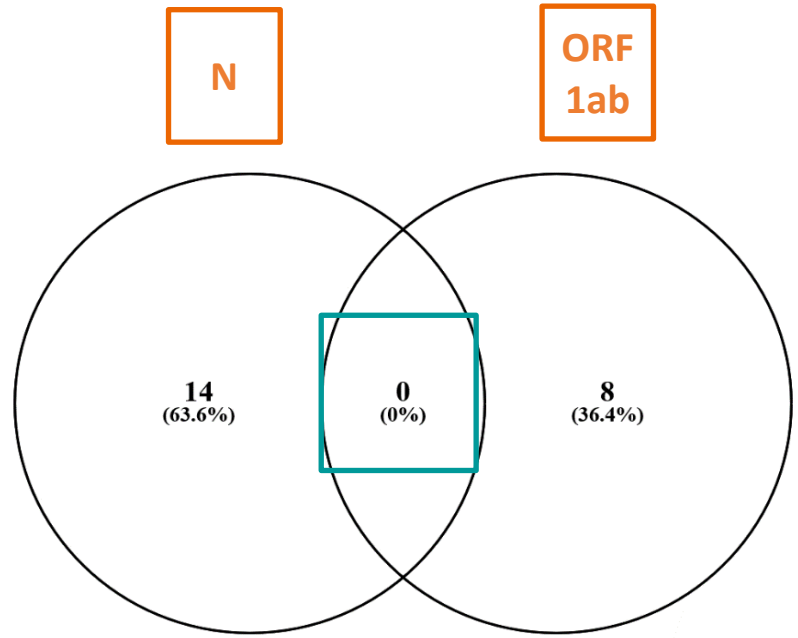
**100% Analytical Specificity**

# 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 cycler
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 | | 95% Confidence Interval | Diagnostic Specificity | | 95% Confidence Interval |
|---|---|---|---|---|---|---|---|
| | | Percentage | Total number | | Percentage | Total number | |
| **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 *in vitro* cross reactivity with 26 common human flora organisms and the pathogenic organisms causing respiratory infections including 6 coronaviruses<br>No *in silico* cross-reactivity to NCBI Nucleotide collection database |
| **Analytical specificity** | 100% using 50 negative clinical samples, 35 negative controls and 34 non-template controls |
| **Inclusivity** | *In silico* 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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