EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

CentoFast-SARS-CoV-2 RT-PCR Assay

(SARS-CoV-2 detection based on E and RdRp genes)

CENTOGENE US, LLC.

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

The CentoFast-SARS-CoV-2 RT-PCR Assay will be performed at CENTOGENE US, LLC or other laboratories designated by CENTOGENE US, LLC that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests as per Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE

The CentoFast-SARS-CoV-2 RT-PCR Assay is a real-time RT-PCR-based test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in dry oropharyngeal swabs from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to CENTOGENE US, LLC or other laboratories designated by CENTOGENE US, LLC that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high-complexity test.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in oropharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The CentoFast-SARS-CoV-2 RT-PCR Assay is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The CentoFast-SARS-CoV-2 RT-PCR Assay is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The CentoFast-SARS-CoV-2 RT-PCR Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the E and the RdRP genes of SARS-CoV-2 in dry oropharyngeal swab specimens from patients as recommended for testing by public health authority guidelines. The device tests in a tiered approach in which samples positive for the E-gene template are followed up with a confirmatory testing for E and RdRP targets. The E-target is the same in both tiers. The FAM channel is used for viral gene signal detection for both targets (run in separate reactions).

In addition, the test also detects an internal control derived from sequences of the Equine Arteritis Virus (EAV) genome. The internal control is added to each sample in the PCR step and is amplified by its own specific primers and Atto 647 labeled probe set. In addition, the test utilizes external controls as described on page 3 below (low titer positive control and a negative control).

RNA from dry oropharyngeal swabs is eluted from the swab at room temperature in PBS and is subsequently combined with a lysis buffer that includes proteinase K. After lysis and protein digestion the RNA is extracted and purified using the commercial NucleoMag VET kit. RNA is eluted in 50 µl elution buffer of which 2.5 µl are used in the subsequent RT-PCR reaction in a total reaction volume of 10 µl. Selective amplification of target nucleic acid from the sample is achieved by reverse transcription of the SARS-CoV-2 RNA genome and subsequent PCR run on the QuantStudio 5 Real-Time PCR System in a 384-well plate format.

INSTRUMENTS USED WITH TEST

The CentoFast-SARS-CoV-2 Assay is to be used with KingFisher Flex (Thermo Fisher) for RNA extraction and the LightCycler 480 II (Roche) for the RT-PCR. The LightCycler 480 II is using Software Version 1.5.1.62 or higher.

EQUIPMENT, REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test:

Table 1: Reagents and Materials

Reagents	Manufacturer	Catalog #
Nucleic Acid Extraction Reagents		
NucleoMag VET Kit	Macherey-Nagel	744200.4
KingFisher deep-well 96 plates	Thermo Fisher Scientific	95040450
KingFisher 96 plate 200µl	Thermo Fisher Scientific	97002540
KingFisher 96 tip comb for DW magnets	Thermo Fisher Scientific	97002534
Dulbecco's Phosphate Buffered Saline	Sigma Aldrich	D8537
Ethanol, minimum 99.8% p.a.	Carl Roth GmbH	9065.4

Reagents	Manufacturer	Catalog #
PCR Reagents and Equipment		
TaqMan Fast-Virus 1-Step Master Mix	Thermo Fisher Scientific	4444434
LightMix SarbecoV E-gene plus EAV control*	TibMolBiol	40-0776-96
LightMix Modular SARS-CoV-2 (COVID19) RdRP*	TibMolBiol	53-0777-96
LightCycler 480 II	Roche	N/A
LightCycler 480 Multiwell Plate 384	Roche	05102430001

* published by Corman et. al, 2020: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045

CONTROLS TO BE USED WITH THE CENTOFAST-SARS-CoV-2 ASSAY

- **An Empty Swab Control (EC)** is used as a negative full process negative control for the entire procedure. For each batch of 94 samples, 2 sterile swabs, not used for sampling, are processed per SOP through the entire testing procedure including swab elution, extraction, and RT-PCR. These samples are used together with the NTC to monitor for any reagent contamination during sample processing.

- **A Positive Template Control (PC)** is used as a full process positive control to verify that the assay run is performing as intended. The PC provided by the manufacturer of the PCR kit (TibMolBiol) is based on artificial RNA covering the tests target sequences of the SARS-CoV-2 genome. The positive control is used once for each run.

- **A Negative Template Control (NTC)** serves as a negative PCR control to monitor for PCR reagent contamination and sample carryover with SARS-CoV-2 sequences that could occur during the PCR set up and amplification process. The NTC consists of water and is run once for every PCR plate. The NTC also contains the internal EAV control (see below).

- **An Internal Amplification Contro**l will be performed on every clinical sample as an endogenous control. The internal control is added to each sample during the RT-PCR step of the test. It consists artificial RNA sequences of the Equine Arteritis Virus (EAV). It is be used to monitor reverse transcription and PCR amplification for each sample.

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the positive or negative controls are not valid, the patient results cannot be interpreted and all patient specimens need to be retested after a root cause has been identified and eliminated.

a. Control result interpretation:

For the control interpretation the EAV target Cp-values are first analyzed by a scientist for all controls:

- If no EAV signal is detected in the no template control, the no template control is invalid and extraction and PCR have to be repeated for all controls and patient samples.
- In case NTC are showing amplicons for E or RdRP this identifies contamination and patient sample results must not be interpreted. The RT-PCR plate must be repeated.
- If any signal (SARS-CoV-2 targets or EAV) is detected in the empty swab control (EC), this indicates contamination of extraction or PCR reagents. In this case the control is invalid and patient results cannot be interpreted.
- In case the PC is missing amplification of E or RdRP targets or EAV, the patient sample must not be interpreted, and the RT-PCR must be repeated.

Table 2: Assay result interpretation for controls

Control type	Code	E-gene	RdRP-gene	EAV	Expected Ct values
No template control	NTC	-	-	+	No Ct for E and RdRP, Cp < 41 for EAV
Empty swab control	EC	-	-	-	No Cp for E, RdRP, and EAV
Positive control	PC	+	+	+	Cp < 41 for all targets

b. Patient sample result interpretation:

Samples are interpreted as follows; for all samples the EAV control is analyzed first and then the amplification of the SARS-specific target sequences:

EAV-Interpretation 1st Tier:

- If no EAV signal is detected in patient samples during Tier 1 testing:
  - in the absence of an E-gene amplification with Cp < 38, the sample is considered invalid and extraction and PCR have to be repeated (result is not passed on for medical evaluation and reporting) using the extracted RNA. If repeat is negative again for EAV, a new sample is requested to repeat the assay from the start with a new specimen.
  - in the presence of E-gene amplification with a Cp < 38, then this sample is will be tested in Tier 2 with E, RdRP, and EAV.

- After the EAV interpretation for samples, review results for E and report as follows:

Table 3: Tier 1 Result Interpretation for Patient Samples

Tier 1		Interpretation	Action Report
E-Gene	**EAV**		
Cp ≥ 38 or undetermined	Cp < 41	Negative	Report as negative
undetermined	Cp ≥ 41	Invalid	Do NOT report, Retest Tier 1
Cp ≥ 36 to < 38	Cp < 41 or Cp≥ 41	Inconclusive	Do NOT report Test Tier 2, if repeat inconclusive request new sample
Cp < 36	Cp < 41	Positive	Do NOT report Test Tier 2
	Cp ≥ 41	Positive	Do NOT report Test Tier 2

EAV-Interpretation 2nd Tier:

- If no EAV signal is detected in patient samples undergoing Tier 2 testing:
  - in the absence of an E-gene amplification with Cp <36, the sample is considered *invalid* and PCR has to be repeated (result is not passed on for medical evaluation and reporting) using the extracted RNA. If repeat is negative again for EAV, a new sample is requested to repeat the assay from the start with a new specimen because RNA may have degraded.
  - in the presence of both, E and RdRP gene amplification with Cp values < 36, the sample is reported as *positive*.
  - in the presence of amplification of one target, E or RdRP gene, the sample is reported as *inconclusive* if the Cp of the one target is < 36.
- After the EAV interpretation for samples, review results for E and RdRp and 1st tier test results (if applicable) and report as follows:

Table 4: Tier 2 Result Interpretation for Patient Samples

Tier 2			Interpretation	Action Report
E-Gene	**RdRP**	**EAV**		
Cp ≥ 36 or undetermined	Cp ≥ 36 or undetermined	Ct < 41	Negative	Report as negative
Cp < 36	Cp < 36	Any	Positive	Report as positive
Cp ≥ 36 or undetermined	Cp < 36	Ct < 41 or undetermined	Inconclusive	Retest Tier 2, if repeat inconclusive request new sample
Cp < 36	Cp ≥ 36 or undetermined	Ct < 41 or undetermined		

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

To determine the LOD of this assay, the commercially available standard "AccuPlex SARS-CoV-2 Verification panel; SeraCare, Gaithersburg MD 20878" has been used based on the concentration of viral copies per µl as given by the manufacturer. Various concentrations (as shown inn Table 5 below) of the AccuPlex SARS-CoV-2 Verification panel were prepared in PBS. For each replicate the AccuPlex SARS-CoV-2 spiked PBS solution was spiked onto a dry oropharyngeal swab/s that was taken from a subject who tested negative for SARS-CoV-2. The swab replicates were then individually processed according to the SOP of the test.

a. Tentative LoD

For the tentative LoD a dilution series of 1 copy/µl to 100 copies/µl was tested in triplicate. Results are shown in Table 5 below.

Table 5: Tentative LoD Testing Results

Target Level	No. Valid tested replicates	SARS-CoV-2 E-Gene Positive			SARS-CoV-2 RdRP Gene Positive			Internal Control (EAV) Positive		
		n*	Mean Cp	Detection Rate	n*	Mean Cp	Detection Rate	n*	Mean Cp	Detection Rate
1 cp/µL	3	0	39.4	0%	0	38.9	0%	3	28.5	100%
2 cp/µL	3	1	37.2	33%	**3**	**35.5**	**100%**	3	28.7	100%
3.2 cp/µL	3	2	36.0	66%	3	35.0	100%	3	28.7	100%
5 cp/µL	**3**	**3**	**34.6**	**100%**	3	34.2	100%	3	28.4	100%
10 cp/µL	3	3	34.1	100%	3	33.4	100%	3	28.6	100%
31.6 cp/µL	3	3	32.4	100%	3	32.0	100%	3	28.5	100%
100 cp/µL	3	3	29.9	100%	3	29.9	100%	3	28.5	100%

* This column contains only valid *positive* replicates based on the cutoff

b. Confirmatory LoD

The two lowest dilutions, 3.2 and 5 copies/µl were then assessed with 20 replicates each (as described above with one swab per replicate); confirmatory results are shown in Table 6 below. The final LOD was determined to be 5 viral copies/µl.

Table 6: Confirmatory LoD Testing Results

Target Level	No. Valid tested replicates	SARS-CoV-2 E-Gene Positive			SARS-CoV-2 RdRP Gene Positive			Internal Control (EAV) Positive		
		n*	Mean Cp	Detection Rate	n*	Mean Cp	Detection Rate	n*	Mean Cp	Detection Rate
3.2 cp/µL	20	16	35.8	80%	20	34.9	100%	20	28.8	100%
5 cp/µL	20	20	34.4	100%	20	34.2	100%	20	29.1	100%

* This column contains only valid *positive* replicates based on the cutoff

2) ***Analytical Inclusivity/Specificity:***

a. Inclusivity

An inclusivity study was conducted and for this test and published by "Corman *et. al*, 2020: Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR" https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045. According to the publication and manufacturer's instructions of the RT-PCR kit:

- The E-gene specific primers & probes detect SARS and SARS-CoV-2 virus (bat-associated SARS-related Sarbecovirus).
- The RdRP gene primers & probes are specific for the detection of the Wuhan origin 2019-nCoV pneumonia virus (SARS-CoV-2).

b. Cross-Reactivity

i. Wet Testing by Reference:

The sponsor refers to the publication of Corman et. al, 2020 (Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR) that tested the following organisms (wet testing). This testing is applicable to this test. No cross-reactivity was observed in the wet testing of the organisms listed in Table 7, including cross reactivity with other coronaviruses NL63, 229E, HKU, OC43, or MERS.

Table 7: Testing of Potentially Cross-Reactive Organisms (Wet Testing)

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	Adenovirus
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A(H1N1) pdm09
MERS-coronavirus	Influenza A(H3N2)
	Influenza A (untyped)

Other high priority pathogens from the same genetic family	High priority organisms likely in circulating areas
	Influenza A(H5N1)
	Influenza A(H7N9)
	Influenza B (Victoria or Yamagata)
	Rhinovirus/enterovirus
	Respiratory syncytial virus (A/B)
	Parainfluenza 1 virus
	Parainfluenza 2 virus
	Parainfluenza 3 virus
	Legionella spp.
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius
	Human Bocavirus

ii. *In Silico* Analysis:

Additionally, the pathogens in the following table were analyzed by *in-silico* analysis using the published reference sequences (GenBank) compared to each individual viral target primers or probe. The highest homology (percentage nucleotide matches in percent of total primer/probe size) was detected for *Mycobacterium tuberculosis* with the E-Gene reverse primer; the detected homology was 64%. None of the analyzed organisms showed a homology of 80% or more with any viral primers & probes used in this assay.

Table 8: Analysis of Potentially Cross-Reactive Organisms (*in silico*)

Accession	Description	Target 1 (E)			Target 2 (RdRP)		
		FWD P	REV P	PROBE	FWD P	REV P	PROBE
AY278741	SARS-coronavirus	none	none	none	none	none	none
CP054014.1	*Mycobacterium tuberculosis*	none	64%	46%	59%	none	none
JF896312.1	Enterovirus (e.g. EV68)	none	none	none	none	none	none
AE009440.1	*Chlamydia pneumoniae*	none	none	none	none	none	none
LR590465.1	*Haemophilus influenzae*	none	none	none	none	54%	none
LN831051.1	*Streptococcus pneumoniae*	none	none	none	none	none	none
LN831034.1	*Streptococcus pyogenes*	none	none	none	none	none	none

Accession	Description	Target 1 (E)			Target 2 (RdRP)		
		FWD P	REV P	PROBE	FWD P	REV P	PROBE
HE965805.1	*Bordetella pertussis*	none	none	none	none	none	none
CP010546.1	*Mycoplasma pneumoniae*	none	none	none	none	none	none

3) Clinical Evaluation:

The clinical evaluation was performed by in-house comparative testing with another EUA RT-PCR test. Thirty positive and thirty negative dry oropharyngeal samples were tested in a blinded and randomized manner with the CentoFast-SARS-COV-2 RT-PCR Assay and the comparator.

For both the positive and negative patient specimens, 100% concordance was obtained with the EUA authorized test (i.e., PPA=100%, NPA=100%).

Table 9: Confirmed positive and negative oropharyngeal samples by EUA authorized test.

		Comparator	
		Positive	Negative
Centogene	Positive	30	0
	Negative	0	30

Table 10: Mean Cp and Range of Clinical Samples

	Comparator	CENTOGENE SARS-COV-2 RT-PCR	
Target	**N1/N2**	**E-Gene**	**RdRP-Gene**
Mean Cp	27.9	30.9	31.4
Range	24.9 – 31.9	27.3 – 35.9	27.6 – 35.9
Positive Percent Agreement (PPA):	**30/30 = 100% (95% CI: 88.65% – 100%)**		
Negative Percent Agreement (NPA):	**30/30 = 100% (95% CI: 88.65% – 100%)**		

There were no samples above a Ct of 35.9 with the investigational device and the highest Ct for the comparator was 31.9.

WARNINGS:

- For *in vitro* diagnostic use
- Rx only
- For use under Emergency Use Authorization (EUA) only
- This test has not been FDA cleared or approved;

- This test has been authorized by FDA under an EUA for use by the 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; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests 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.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Reagents should not be used beyond their expiration date.

LMITATIONS:

- The performance of this test was established using dry oropharyngeal swab specimens. Dry nasal swabs also considered acceptable specimen types for use with the test.
- Clinical performance was established using dry swabs processed directly without elution in viral transport media (VTM). Use of conventional swabs eluted/transported in VTM will result in decreased detection of low positive samples that are near the limit of detection of the test because of the higher dilution volume of the VTM in the conventional collection systems.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. For each study, mock swabs were prepared by pipetting 50 µL of each diluted virus stock onto a swab. Dry swabs were allowed to dry for 20 minutes, and the swabs were tested following the Instructions for Use for CentoFast-SARS-CoV-2 RT-PCR Assay. The extraction method used was NucleoMag VET Kit (Machery Nagel) on the KingFisher Flex instrument and the assay was run on the LightCycler 480 II (Roche). The results are summarized in the following Table 11 and should only be compared to other dry swabs.

Table 11. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Pane l

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Oropharyngeal Swab	$5.4x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL:RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected