EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

CentoSure-SARS-CoV-2 RT-PCR Assay

CENTOGENE US, LLC.

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

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

INTENDED USE

The CentoSure 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 swab specimens from individuals without symptoms or other reasons to suspect COVID-19. Testing is limited to the laboratories designated by CENTOGENE US. LLC located at 99 Erie Street, Cambridge MA 02139 which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in oropharyngeal 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 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 CentoSure SARS-COV-2 RT-PCR Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The CentoSure SARS-COV-2 RT-PCR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The CentoSure-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 N1 and N2 regions of the SARS-CoV-2 nucleocapsid gene (and the human RNase P in dry oropharyngeal swab specimens from patients as recommended for testing by public health authority guidelines. The CentoSure SARS-CoV-2 test is run in a multiplex format that detects all three genes with different fluorophores.

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 LightCycler 480 II is using Software Version 1.5.1.62 or higher.

INSTRUMENTS USED WITH TEST

The CentoSure-SARS-CoV-2 RT-PCR 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 or MagMAX Viral/Pathogen Kit	Macherey-Nagel ThermoFisher	744200.4 A42352
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
PCR Reagents and Equipment		
CoviDetect COVID-19 Multiplex RT-PCR reagents	Pentabase, 5000 Odense; Denmark	8014
2019-nCoV_N Positive Control	IDT, Belgium	10006625
LightCycler 480 II	Roche	N/A
LightCycler 480 Multiwell Plate 384	Roche	05102430001

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

Internal Control (IC):
In order to ensure successful extraction, for each sample, the human RNase P (RP) gene is assayed within the multiplex reaction. The EC monitors inhibitory substances present in the extracted RNA sample. It also monitors for improper sampling or sample storage and failures of the extraction process that result in insufficient amounts or quality of the samples RNA.

No template control (NTC):
For each RT-PCR plate containing maximum of 374 samples, 2 replicates of the NTC are used (empty swabs used through the extraction process) per extraction batch of 94 samples to control for reagent contamination.

Positive template control (PTC):
RT-PCR control is an artificial viral RNA to monitor RT-PCR reaction and detection performance. For each RT-PCR plate containing a maximum 372 samples, 2 replicates of the PTC are included (pipetted instead of patient RNA for RT-PCR Mix). This PTC is provided by IDT and contains the target regions of the test (N1 and N2 genes, article no. 10006625).

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.

Note: The Ct value of samples obtained with the CentoSure SARS-COV-2 RT-PCR Assay do not include the first 13 amplification cycles and therefore Ct results do not compare to other conventional Real-Time PCR tests.

a. Control result interpretation:

For the control interpretation the raw data are first analyzed for PC and NTC. If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. The test run is invalidated, and all samples and controls are re-tested.

- In case NTC are showing amplicons for N1, N2 or RP, this identifies contamination and sample results must not be interpreted. The RT-PCR plate must be repeated.
- In case the PTC is missing amplification of N1 or N2, the samples must not be interpreted, and the RT-PCR must be repeated.

Afterwards, the individual sample results are inspected for RP amplification (IC). Samples without amplification of the IC are invalidated and re-tested in another RT-PCR plate. If still no amplification detectable, a new sample has to be requested.

Table 2: Assay result interpretation for controls

Control type	Code	N1	N2	RP	Expected Ct values
No template control	NTC	-	-	-	No Ct for any of three targets
Positive template control	PTC	+	+	-	Ct < 26 for N1 and N2, no Ct for RP
Internal control in Patient Samples	IC	variable	variable	+	Ct =<41 for RP, N1 and N2 detection depends on the patient sample

b. Patient sample result interpretation:

- If RP is detected with Ct < 32 and N1 and N2 viral targets are both ≥ 26 or undetermined, a negative report is issued.

If no RNase P (RP) signal with Ct < 32 is detected in patient samples and both N1 and N2 show no amplification, 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 RP, a new sample is requested to repeat the assay from the start with a new specimen.

- in the presence of both viral targets, N1 and N2, amplification with Cts < 26, the sample is reported as *positive*, irrespective of whether RP amplification is detectable or not.

- in the presence of amplification of only one viral target (N1 and N2) with Ct < 26, the sample RNA is re-tested with a different test (the CentoFAST SARS-CoV-2 RT-PCR Assay previously EUA authorized), irrespective of whether RP amplification is detectable or not. If E and RdRP are positive as described in the EUA, the sample is reported positive. If none or only E or RdRP are tested below the threshold Ct of the EUA, the result is inconclusive, and a new sample is requested.

Table 3: Result Interpretation for Patient Samples

N1 gene	N2 gene	RP gene	Interpretation	Action report
Ct ≥ 26 or undetermined	Ct ≥ 26 or undetermined	Ct < 32	Negative	Report as negative
Ct ≥ 26 or undetermined	Ct < 26	Ct < 32 or undetermined	Inconclusive	Re-test in RT-PCR with CentoFast SARS-CoV-2 RT-PCR Test (EUA201018) RT-PCR for E and RdRP gene; (1) If E and RdRP are positive, positive report is issued. (2) If E and RdRP are detectable but above the Ct threshold of the EUA, the result is inconclusive, and a new sample is requested. (3) If E and RdRP are not detectable (Ct undetermined), a negative report is issued.
Ct < 26	Ct ≥ 26 or undetermined			
Ct undetermined	Ct undetermined	Ct undetermined	Invalid	Retest sample with the CentoSure SARS-CoV-2 RT-PCR Test and if still no RP or N1, N2 signal detected, request for new sample
Ct < 26	Ct < 26	Any Ct (Ct < 32, ≥ 32, or undetermined	Positive	Report as positive

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

To determine the LoD of the CentoSure SARS-CoV-2 RT-PCR different concentrations of the AccuPlex SARS-CoV-2 Verification panel were prepared in PBS. The solutions spiked at different concentrations were then added to the dry oropharyngeal swab/s taken from a subject who tested negative for SARS-CoV-2. Swabs were processed according to the SOP of the test with both, the NucleoMag Vet (Machery-Nagel) and the MagMax Viral/Pathogen Kit (ThermoFisher). All replicates were individual swabs that were individually processed.

a. Tentative LoD

For the tentative LoD a dilution series of 1 copy/µL to 100 copies/µL has been tested in triplicates with two extraction methods. The tentative LoD was determined to be 2 copies/µl with both, the NucleoMag Vet Kit (Machery-Nagel) and the MagMax Viral/Pathogen Kit (ThermoFisher).

b. Confirmatory LoD

The two lowest dilutions, 2 and 3.2 copies/µL have then been assessed with 20 replicates each (as described above with one swab per replicate):

Table 4: Confirmatory LoD for the CentoSure SARS-CoV-2 RT-PCR Assay

Target Level	Valid Tested Replicates	SARS-CoV-2 N1 gene Positive			SARS-CoV-2 N2 gene Positive			Human gene RP Positive		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
Nucleomag Vet Kit										
2 cp/µL	**20**	**19**	**24.4**	**95%**	**20**	**23.0**	**100%**	**20**	**20.4**	**100%**
3.2 cp/µL	20	20	23.4	100%	20	22.1	100%	20	21.6	100%
MagMax Viral/Pathogen Kit										
2 cp/µL	**20**	**19**	**22.9**	**95%**	**20**	**22.9**	**100%**	**20**	**21.2**	**100%**
3.2 cp/µL	20	20	22.3	100%	20	22.0	100%	20	22.1	100%

The final LoD for the CentoSure SARS-CoV-2 RT-PCR Assay was determined to be 2 viral copies/µL for both extraction methods.

2) Analytical Inclusivity/Specificity:

Viral primer and probe sequences have been *in silico* assessed by the manufacturer of the primer and probe set (Pentabase, Denmark) against SARS-CoV-2 strains in the GISAID database. Primers and probes were blasted against 21,085 sequences of SARS-CoV-2. The mismatch frequencies for the individual targets are: N1 = 0.5 % N2 = 0.3 %. No SARS-CoV-2 sequence shows mutation(s) in BOTH the N1 and N2 targets.

N1 Fwd Primer: 5 different SNP were found in a total of 23 sequences, all were single bp mismatches in the middle of the primer that are unlikely to impact amplification.
N1 Rev Primer: 1 type of SNP was found in 3 sequences which was a single bp mismatch in the middle of the primer that is unlikely to impact amplification.
N1 Probe: 10 different SNPS were found in a total of 79 sequences, all were single bp mismatches in the middle of the probe that are unlikely to impact the probe's binding to the target sequence.
N2 Fwd Primer: 8 different SNP were found in a total of 16 sequences, all were single bp mismatches in the middle of the primer that are unlikely to impact amplification.
N2 Rev Primer: 4 type of SNP was found in 19 sequences which were all single bp mismatches in the middle of the primer that is unlikely to impact amplification.
N2 Probe: 3 different SNPS were found in a total of 24 sequences, all were single bp mismatches in the middle of the probe that are unlikely to impact the probe's binding to the target sequence.

Due to the parallel multiplex testing of two viral targets, any potential mismatch leading to a reduced or non-detection of a positive individual in one viral target will be detected in the 2nd and according to the result interpretation rules (chapter 4) be re-tested with additional viral targets to ensure 100% detection of SARS-CoV-2 positive individuals.

3) Cross-reactivity (Analytical Specificity)

The pathogens listed in Table 5 have been analyzed *in-silico* using the published reference sequences (GenBank) compared to each individual viral target primer or probe. The highest homology (percentage nucleotide matches in percent of total primer/probe size) was detected for the following organisms:

- N1 forward primer: *Pseudomonas aeruginosa*, homology 75%
- N1 reverse primer: *Candida albicans*, homology 71%
- N1 probe: *Pseudomonas aeruginosa*, homology 63%
- N2 forward primer: *Pseudomonas aeruginosa*, homology 65%
- N2 reverse primer: *Candida albicans*, homology 65%
- N2 probe: *Mycobacterium tuberculosis*, homology 70%

None of the analyzed organisms showed homology of 80% or more with any viral primers or probes used in this assay. As such cross reactivity is not expected with these organisms.

Table 5: Analysis of Potentially Cross-Reactive Organisms (*in silico*)

GenBank taxid	Description	GenBank taxid	Description
11137	Human coronavirus 229E	12059	Rhinovirus
31631	Human coronavirus OC43	83558	*Chlamydia pneumoniae*
290028	Human coronavirus HKU1	727	*Haemophilus influenzae*
277944	Human coronavirus NL63	446	*Legionella pneumophila*
1335626	MERS-coronavirus	1773	*Mycobacterium tuberculosis*
694009	SARS-coronavirus	1313	*Streptococcus pneumoniae*
10533	Human adenovirus 1	1314	*Streptococcus pyogenes*
162145	Human metapneumovirus	520	*Bordetella pertussis*
12730	Human Parainfluenza virus 1	2104	*Mycoplasma pneumoniae*
1979160	Human Parainfluenza virus 2	42068	*Pneumocystis jirovecii*
11216	Human Parainfluenza virus 3	5476	*Candida albicans*
1979161	Human Parainfluenza virus 4	287	*Pseudomonas aeruginosa*
11320	Influenza A virus	5476	*Candida albicans*
11520	Influenza B virus	287	*Pseudomonas aeruginosa*
12059	Enterovirus	1282	*Staphylococcus epidermidis*
12814	Respiratory syncytial virus	1304	*Streptococcus salivarius*

4) Clinical Evaluation

The clinical evaluation was performed by comparing the results of the investigational CentoSure SARS-CoV-2 RT-PCR test with an EUA authorized RT-PCR comparator test with different target genes (E- and RdRP-gene). Both assays were run per their laboratory SOP. A total of 25 positive and 100 negative dry oropharyngeal samples from asymptomatic individuals were tested with both tests. Out of 25 positive samples six (24%) were low positive by the comparator test. For both the positive and negative patient specimens, 100% concordance was obtained with the EUA authorized RT-PCR comparator test.

Table 6: Confirmed positive and negative oropharyngeal samples by EUA authorized RT-PCR test.

		Comparator EUA		Total
		Positive	Negative	
CentoSure SARS-COV-2 RT-PCR Assay	**Positive**	25	0	25
	Negative	0	100	100
Total		25	100	125

Table 7: Mean Ct and Range of Clinical Samples

Target	Comparator EUA		CentoSure SARS-COV-2 RT-PCR Assay*	
	E-gene	RdRP-gene	N1 gene	N2 gene
Mean Ct	30.5	31.8	19.1	19.5
Range	23.9 - 35.3	26.7 – 35.7	13.4 -25.2	13.6 – 25.4
Positive Percent Agreement (PPA): 25/25 = 100% (95% CI: 86.28% – 100%) **Negative Percent Agreement (NPA): 100/100 = 100% (95% CI: 96.38% – 100%)**				

*Note that the first 13 cycles of the PCR amplification cycles do not record fluorescence data.

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 CentoSure SARS-COV-2 RT-PCR Assay. The KingFisher Flex device and MagMAX Viral/Pathogen Kit (Thermo Fisher) were used for RNA extraction, and the LightCycler 480 II (Roche) was used for the RT-PCR. The results are summarized in Table 8 and should only be compared to other dry swabs.

Table 8: Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

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

In addition to standard laboratory warning and precautions the following EUA and assay specific warning and limitation statements are included in the laboratory SOP:

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 CENTOGENE US located at 99 Erie Street, Cambridge MA 02139 and CENTOGENE AG (Rostock, Germany).
- This test has been authorized only for the detection of nucleic acid from SARSCoV-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 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.
- Reagents should not be used beyond their expiration date.

LIMITATIONS

- Clinical performance was established using dry swab specimens 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.
- The Ct value of samples obtained with the CentoSure SARS-COV-2 RT-PCR Assay do not include the first 13 amplification cycles and therefore Ct results do not compare to other conventional Real-Time PCR tests.