genestore™

Détection Expert 1S™ SARS-CoV-2

ONE STEP REAL TIME REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION KIT FOR QUALITATIVE DETECTION OF SARS-CoV-2

INSTRUCTIONS FOR USE V.3.1

REF GS.D1F.31519.2.1000 Σ 1000

REF GS.D1F.31519.2.100 Σ 100

QUALITATIVE IN VITRO DIAGNOSTICS

www.genestore.org

Version: February 2021
GS.D1F.31519.2.IFU.EN.V.3.1

CE IVD

COMMITTED TO HIGH QUALITY IN NUCLEIC ACID TECHNOLOGIES

GeneStore is a genomics diagnostics focused company head-quartered in Provence, France.

GeneStore currently operates R&D and manufacturing facilities across Europe, Middle-east, South America, and South Asia.

We strongly believe in providing advanced, and high quality products across the globe to ensure a method that produce simple and robust results.

For more information on GeneStore visit: www.genestore.org.

PRINCIPLE

The kit "Détection Expert 1S™ SARS-CoV-2" produced by Genestore is a qualitative test for the detection of SARS-CoV-2 using RT-PCR[1].

The detection is made from viral RNA present in the upper respiratory tract of human beings. The extraction of viral RNA from individuals suspected of being carriers of the virus is performed by means of swabs introduced into the nasopharyngeal respiratory tract, by aspiration of samples from the same tract.

The expected results are the identification of SARS-CoV-2 RNA (targets genes N1 and N2). This RNA is usually detectable in swab samples from the nasopharyngeal airways during the acute phase of infection.

Positive results indicate the presence of SARS-CoV-2 RNA in the specimens. The clinical correlation between the patient's medical history and the diagnostic information is necessary to determine if the patient is infected or not.

Even positive results do not exclude the presence of bacterial infection or co-infections with other viruses. The detected agent alone should not be considered the sole cause of the disease.

Laboratories are required to inform the competent public health authorities of all positive results.

Negative results do not preclude SARS-Cov-2 infection and should not be used as the sole approach to patient care decisions. Negative results should be combined with clinical findings, patient history, and epidemiologic information.

The tests using the Détection Expert 1S™ SARS-CoV-2 kit are intended for use by medical

INTENDED USE

The kit "Détection Expert 1S™ SARS-CoV-2" is a real-time RT-PCR test for the qualitative detection of SARS-CoV-2 nucleic acid in a nasopharyngeal swab taken from persons suspected by their physician of being infected with COVID-19.

- The results identify SARS-CoV-2 RNA. Positive results indicate the presence of SARS-CoV-2 RNA.
- The assay consists of a one-step RT-qPCR (both reactions in the same tube) by reverse transcription of the viral RNA target to cDNA, followed by amplification of the assay target and detection by the qPCR hydrolysis probe method.

The kit "Détection Expert 1S™ SARS-CoV-2" contains the following reagents:

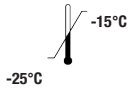
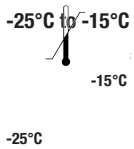
A. A box named "Pack réactifs d'amplification" (Amplification reagent pack) containing:
- Mélange enzymatique (Enzymatic Mix)
- Sondes/Amorces (Primers / Probes mix)

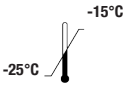
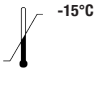
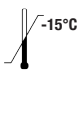
B. A box named "Controle positif (N1 + N2)" containing:
- Contrôle positif SARS-CoV-2 - Régions Cibles N1 et N2 (positive control SARS-CoV-2 - Target Regions N1 and N2)

SYMBOLS [2]

Σ <n>	Contains enough reagents for (n) tests
	Expiry date
IVD	In vitro diagnostic medical device
REF	Catalogue number
LOT	Lot number
	Refer to instructions for use
	Caution / Warning
COMP	Component
CONT	Contents
NUM	Number
	Temperature Limits (storage)
	Manufacturer of the medical device
CE	CE mark - European Conformity
CONTROL +	Positive Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack

KIT COMPOSITION

"Détection Expert 1S™ SARS-CoV-2" 100 Tests REF GS.D1F.31519.2.100			
Componant	**Description**	**Quantity (Volume)**	**Storage conditions**
A. Pack or box **Pack Réactifs d'amplification** **AMPLIFICATION REAGENT PACK**	**Sondes/Amorces (primers/probes mix)** • N1 [FAM] • N2 [HEX] • RNaseP [CY5]	**454.50 µL** **Number of tubes: 01**	**-25°C to -15°C** 
	Mélange enzymatique (Enzymatic mix)	**1212.00 µL** **Number of tubes: 01**	**-25°C to -15°C** 
B. Pack or box **CONTROLE POSITIF (N1 + N2)** **Positive control (N1 + N2)**	**POSITIVE CONTROL**	**100.00 µL** **Concentration:** 10,000 copies/µL **Number of tubes:** 01	**-25°C to -15°C** 

"Détection Expert 1S™ SARS-CoV-2" 1000 Tests REF GS.D1F.31519.2.1000			
Componant	**Description**	**Quantity**	**Storage conditions**
A. Pack or box **Pack Réactifs d'amplification** **AMPLIFICATION REAGENT PACK**	**Sondes/Amorces (primers/probes mix)** • N1 [FAM] • N2 [HEX] • RNaseP [CY5]	**454.50 µL** **Number of tubes:** 10	**-25°C to -15°C** 
	Mélange enzymatique (Enzymatic mix)	**1212.00 µL** **Number of tubes:** 10	**-25°C to -15°C** 
B. Pack or box **CONTROLE POSITIF (N1 + N2)** **Positive control (N1 + N2)**	**CONTROLE POSITIF (Positive control)**	**100.00 µL** **Number of tubes:** 01 **Concentration:** 10,000 copies/µL	**-25°C to -15°C** 

STORAGE OF REAGENTS, HANDLING AND STABILITY

1. Store reagents at -25°C to -15°C.
2. Always check expiry dates before use. Do not use expired reagents.
3. Protect fluorescent probes from light.
4. Primers and probes (including aliquots), and the enzyme mix should be thawed on a refrigerated block during kit preparation and use.
5. Do not refreeze master mix (mix obtained by mix of primers/probes and enzymatic mix).
6. Controls and their aliquots should be thawed on ice during the entire preparation and use of the kit.
7. For in vitro diagnostic use only by qualified laboratories and for professional use.

Stability of closed reagents: See expiration date on package label.

REQUIRED MATERIAL (NOT SUPPLIED)

1. UTM : steril tubes with transport medium
2. Vortex, Mixer
3. Micro Centrifuge
4. Micropipettes (2 or 10µl, 200µl and 1000µl)
5. Multichannel pipettes (5 to 50 µl)
6. Racks for 1.5 ml microtubes
7. 2 x 96-well cold plates (-20°C)
8. Thermal cycler (multi-channel) with analysis software
9. water for molecular biology (without RNAse)
10. 10% concentrated bleach (10g/100ml)
11. DNAZap™ (Ambion,cat.#AM9890) or equivalent
12. RNAse Away™ (Fisher Scientific; cat.#21-236-21) or equivalent
13. Gloves (powder-free) and surgical gowns
14. Micropipettes Tips with filters
15. 1.5 ml microtube (without RNAse and without DNAse)
16. 0.2 ml PCR tubes (packed in rows or plates)
17. Optical 8-cap Strips (in case of strip tubes)
18. Dry bath or oven

INSTRUMENTS VALIDATED FOR USE WITH THIS KIT:

This kit has been validated for use with the following instruments:

- CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
- BIOER linegene 9660
- ABI® 7500 (Applied Biosystems)

genestore™

⚠ WARNINGS AND PRECAUTIONS

- For in vitro use only.

- Follow standard precautions, each patient sample and positive control should be considered potentially infectious and treated accordingly.

- Do not eat, drink, smoke, wear make-up, or handle contact lenses in areas where reagents and/or human specimens are handled. Handle human specimens with precaution (infectious risks), using good laboratory practices.

- Refer to the laboratory biosafety instructions for handling and processing of samples in contact with COVID-19.

- Processing of samples should be performed in accordance with national biosafety regulations.

- In case of suspected infection, established on a clinical or epidemiological screening criterion advised by public health authorities, samples should be collected with appropriate infection control precautions.

-Performance characteristics have been determined with human upper respiratory tract specimens or upper respiratory tract in humans or the lower respiratory tract, which shows signs and symptoms of respiratory infections. Perform all manipulations with specimens containing live virus with a minimum of a Class II microbiological safety cabinet. Use personal protective equipment, such as (but not limited to) gloves, eye protection, gowns.

- Application technologies such as PCR (Polymerase Chain Reaction) are sensitive to accidental addition of PCR products from previous amplification reactions.

- Incorrect results may occur if clinical samples or reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). The workflow in the laboratory should be unidirectional.

- Maintain separate work areas for testing and handling of nucleic acids.

- Always check the expiration date before using the products. Do not use an expired expired reagent. Do not substitute or mix reagents from different kit lots, or from another manufacturer.

- Change pipette tips with filters before each pipetting of liquids.

- During sample preparation, compliance with GLP (Good Laboratory Practices) is essential to minimize the risk of cross-contamination between samples and samples, and inadvertent introduction of nucleases into samples during after the extraction procedure. Appropriate cleaning and sanitization techniques should always be used when using nucleic acids.

⚠ WARNINGS AND PRECAUTIONS

- Keep separate, and designate each piece of equipment to a task (pipettes, microcentrifuges) as well as consumables (tubes, tips, ...) for test preparation and nucleic acid extraction work.

- Wear a clean gown and powder-free gloves (never used) during test preparation.

- Change gloves during sample preparation and whenever contamination is suspected.

- Keep reagents and tubes, used for the reaction, capped or covered as much as possible.

- Primers (genomic primers), probes (stained probes, including aliquots), and enzymes in the Master Mix should be thawed and kept in cold blocks during preparation and use.

- Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with a cleaning agent such as 10% bleach to minimize the risk of nucleic acid contamination. Bleach residue can be removed with 70% ethanol.

- RNA should be kept in a cold block or on ice during preparation and use to ensure stability.

- Dispose of used reagents and human samples according to applicable regulations.

LIMITATIONS

- The kit “Détection Expert 1S™ SARS-CoV-2” can only be used with specimens obtained from swabs introduced into the nasopharyngeal airway by aspiration of specimens from the same airway.

- Any other type of sample has not been evaluated and should not be tested in this manner.

- Samples should be collected, transported and stored using proper conditions and procedures. Improper collection, transport, or storage of samples may impair the ability of the assay to detect target sequences.

- Extraction and amplification of nucleic acids from clinical specimens should be performed in accordance with the recommendations described in this manual. Other extraction approaches or extraction systems have not been evaluated.

A false-Negative result may result from:

- Improper sample collection

- Degradation of SARS-CoV-2 RNA during transport or storage

- Use of unauthorized extraction or reagents

- Presence of RT-PCR inhibitors

- Mutation of the SARS-CoV-2 virus on genes N1 and N2 silmutaneously.

- Failure to follow the instructions in this manual.

- Negative results alone should not exclude infection with SARS-CoV-2 and should not be the sole basis for the patient’s future decision.

LIMITATIONS

A false positive result may result from:

- Cross-contamination during sample handling and preparation. Cross-contamination between samples from different patients. Mixing of samples.

- Contamination of the sample during preparation.

- The impact of vaccines, antivirals, antibiotics, chemotherapeutic or immunosuppressive drugs has not been evaluated. Genestore’s “Détection Expert 1S™ SARS-CoV-2” RT-PCR kit cannot exclude disease caused by other pathogenic bacteria or viruses.

- Laboratories are required to notify health authorities in the event of positive results.

genestore™

CONTROLS TO BE USED WITH THE KIT

"Détection Expert 1S™ SARS-CoV-2"

FOR RT-PCR

Patient samples should be collected in accordance with Good Laboratory Practice (GLP) recommendations. Positive and negative test controls should be included to accurately interpret patient results.

Include the following controls:

Control	Used for the control	Test
N1 ET N2 (Mix of primers probes included in the test of RT PCR) POSITIVE CONTROL (N1 ET N2): substrate provided	RT-PCR reaction setup and integrity of the reagent.	"Détection Expert 1S™ SARS-CoV-2"
RNase P *(Mix of primers porbes included in the test of RT PCR) Controle of human origin substrat not provided	The performance: extraction of acids nucleic acids	
NEGATIVE CONTROL (not supplied with the kit, use nuclease-free water or physiological serum)		

* The RNase P marker included in the SARS-CoV-2 RT PCR multiplex assay is used to measure the performance of the nucleic acid extraction method and also as an internal control.

The RNase P test is based on the presence of samples of human genomic DNA or RNA from an extraction, amplification will be observed if the performance of the extraction is respected.

PREPARATION OF THE POSITIVE CONTROL ⚠

Precautions: This reagent should be handled with care, in an area dedicated to the handling of nucleic acids, to prevent possible contamination. Freeze/thaw cycles should be avoided as much as possible. Keep on ice when thawed.

Include the following controls:

- Prepare several aliquots (of approximately 30µl) and store at approximately -20°C.
- Thaw a single aliquot of positive control for each experiment, and store on ice until added to the plate. Discard any unused aliquots.

HUMAN SOURCE CONTROL (HSC) (not provided)

Human Source Controls (HSC), or those listed as "acceptable" alternative extraction controls, should be prepared and used with each sample extraction reading.

CONTROL WITHOUT MATRIX (NTC) (not supplied)

1. Use sterile nuclease-free water

2. Make small volume aliquots (30ul)

3. Use NTC to check for contamination during sample extractions and/or plate preparation.

ASSAY PROTOCOL

SAMPLE COLLECTION

Samples tested with this kit must have been collected and transported according to WHO recommendation. These samples should be upper respiratory specimens collected from individuals suspected of having COVID-19 by their physician. Ensure that the sample is stored properly and kept away from sources of contamination/pollution.

PREPARATION OF EQUIPMENT

Clean and decontaminate all surfaces, pipettes, centrifuges, and other equipment before use. Decontamination products should be used including 10% bleach, 70% ethanol, to minimize the risk of contamination with nucleic acids.

SAMPLE PREPARATION WITHOUT EXTRACTION

The GeneSotre "Détection Expert 1S™ SARS-CoV-2" kit allows a direct amplification and qualitative detection of SARS-CoV- 2 RNA by multiplexed real-time PCR.

For the use of the detection kit without extraction, it is very important that the sample has not been deactivated by any chemical product, as these inhibit the PCR reaction. The sample can be prepared by dipping swab directly in physiological serum[3] (NaCl 0.9%) or with water Rnase free 500ul to 1ml. If the laboratory needs to inactivate the virus, the inactivation method should be: HEAT ONLY (incubate the sample at 56°C for 30 minutes [3] or 95°C for 5 minutes on a dry bath).

SAMPLE PREPARATION WITH EXTRACTION

The performance of the RT-PCR assay is dependent on the quantity and quality of RNA purified from the human sample. Use commercially available RNA extraction kits and proceed as described and validated by the manufacturer to recover and purify viral RNA from nasopharyngeal specimens collected by aspiration using a swab. The manufacturers' recommended procedures for use should be followed for RNA extraction from these specimens.

METHODS TO BE FOLLOWED FOR PREPARATION OF RT-PCR

Important Note :

- Prepare the read plate on ice and keep on ice until reading by RT-PCR.
- Start reading as soon as the plate preparation is finished. Failure to do so may result in RNA degradation.
- To avoid contamination, prepare PCR reagents in a dedicated work area or equivalent amplicon-free work area. Do not use the same pipettes for controls and samples, and always use pipette tips with filters.
- Maintain an RNase-free working environment.
- Protect assays from light.
- Keep samples and reagents on ice during use.
- Include a positive and a negative control in each plate, and set up and read the plate in the RT-PCR.

RT-PCR ASSAY PREPARATION

Note: The plate plan may vary with the number of samples and organization of the day. Negative and positive controls should be included in each read.

1. If frozen, thaw nucleic acid samples and reagents on ice.

For non-extraction sample preparation: if the sample is a swab that has dried, rehydrate with 0.5 to 1ml of physiological serum[3] (NaCl 0.9%) or with water Rnase free and vortex gently for 15 seconds. If the sample reaches the lab in UTM, vortex gently for 15 seconds.

2. Gently mix the samples and reagents, then centrifuge briefly to recover the mixture from the bottom of the plate wells/ or the bottom of the tubes.

genestore™

PREPARATION OF THE MASTER MIX

- For each reading, combine the following reagents in sufficient quantity for the number of tests desired, add one positive and one negative control.

Component	Volume to one sample or to one controle (µL)	Volume to N samples to N control
RT PCR ENZYME MIX	12.00 µL	12.00 *(N+1) µL
PROBE MIX	4.50 µL	4.50 *(N+1) µL
Total reactionnal volume	16.50 µL	

1. Dispense the reagents into each annotated microtube respectively. After adding the reagents, mix gently by pipetting up and down. Do not vortex.

2. Centrifuge for 5 seconds to collect all the mixture at the bottom of the tubes, and place the tubes in a cold block.

3. Place the tubes or plates in a cold 96-well block.

4. Dispense 16.5µl of each Mix into the appropriate wells (including samples and controls).

5. Before moving the nucleic acids to the work area, prepare the reaction with the negative control per column#1 in the assay preparation area.

6. Pipet 3.5µl of nuclease-free water into the wells reserved for the negative controls. As a safety precaution close the wells before proceeding.

7. Completely cover the plate and tubes, move the assembly to the nucleic acid handling work area.

ADDITION OF NUCLEIC ACID

8. Gently mix the nucleic acids in the tubes for 5 seconds.

9. Centrifuge the tubes for 5 seconds to collect the mixture at the bottom of the tube.

10. After centrifugation, place the tubes containing the nucleic acid extracts in a cold block.

11. Carefully pipet 3.5 µl of the first sample and deposit the pipetted volume into the set of wells reserved for this sample (Tube 1: N1 + N2 + RNaseP multiplexed). Keep the remaining wells covered while adding samples. Change the pipette tip after pipetting.

12. Close the caps of the column where the samples were deposited to prevent cross-contamination, and keep track of the samples.

13. Change gloves frequently and as soon as necessary to eliminate the risk of contamination.

14. Repeat steps 11 and 12 for the remaining samples.

ONE STEP CYCLE CONDITIONS FOR RT-PCR

- Set up and run the following rRT-PCR cycle program on your rRT-PCR instrument (follow the manufacturer's instructions on your instrument's software setup).

First incubation : 42 °C for 5 mins
Second incubation : 95 °C or 5 mins

Cycle time : 40 cycles with the following conditions:

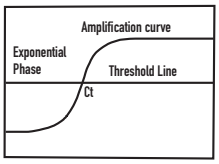
- Denaturation 95°C for 15 seconds
- Hybridization 58°C for 30 seconds

Color of detection channels:

- N1 is FAM (green color)
- N2 is HEX (yellow color)
- RNase P is CY5 (red color)

DATA ANALYSIS [4]

Note: Refer to the instructions in the manual of the device used to generate the amplification curves and Ct values for each sample.



Note: To determine the Ct values adjust the threshold line until it is within the exponential phase of the curves and above the background signal.

INTERPRETATION OF RESULTS & REPORT

Negative control (NTC)

The NTC consists in the use of RNase-free water in the RT-PCR reaction instead of RNA. NTC reactions for all primers and probes should not have amplification curves that exceed the threshold line. If an NTC reaction shows an amplification curve that crosses the threshold line, sample contamination must have occurred. The assay is not validated and should be repeated with strict adherence to the recommendations for use in the manual.

Positive control

The positive control consists of plasmids containing the gene sequence of the SARS CoV 2 nucleocapsid regions 1 (N1) and 2 (N2). The positive control should yield a positive result with the following primers/probes: N1 and N2 only.

Human Extraction Control (HEC) (RNase P)

The RNase P like HEC (see previous section in assay preparation), is used as a control of the RNA extraction procedure, to demonstrate that the RNA has been extracted correctly as well as the integrity of the extraction reagents. A successful nucleic acid purification should show a positive result with the primer probe of RNAse P.

INTERPRETATION OF RESULTS AND REPORT

Summary table of expected results for the diagnosis of SARS-CoV-2 by RT-PCR:

Nature of the Control	Region 1 of SARS-CoV-2 (N1)	Region 2 of SARS-CoV-2 (N2)	RNaseP	Ct Values expected
Positive	[+]	[+]	[-]	< 35.00
Negative	[-]	[-]	[-]	no detec-tion
HEC	[-]	[-]	[+]	< 35.00

Important Note:
If any of the above controls do not perform as described in the table, the test may not have been prepared and/or performed properly, and/or reagents and/or equipment malfunction may have occurred. The assay should not be validated and should be repeated in integrality.

RNAse P Extraction Control

All clinical samples should show increasing fluorescence curves with the RNAse P reaction, these should exceed the threshold line before reaching 35 cycles (<35 Ct). If this is the case, then this indicates the presence of the RNAse P gene. Failure to detect the presence of the RNAse P gene in clinical samples indicates that:

- Nucleic acid extraction from clinical samples failed, either by loss of RNA or by degradation of the RNA.
- Human specimens were not collected in sufficient quantity, or are missing, either due to low collection or loss of specimens.
- The test was poorly performed or poorly prepared.
- Reagents and equipment are malfunctioning.

2. If the RNAse-P test does not produce positive results with human clinical specimens, the results can be interpreted as follows:

- If the N1 and N2 markers are positive even in the absence of the RNAse P positive control, then the results are still validated. It is possible that some samples did not show increasing curves due to the small amount of cells present in the clinical sample. A negative RNAse-P result does not preclude the presence of SARS-CoV-2 RNA in clinical samples.
- If all virus and RNase P markers are negative with clinical specimens, the results should be considered non-validated for the specimens tested.

If a sample is non-validated, then the extraction procedure should be repeated and the test repeated as well. If all markers come back negative after retesting, the results should be reported as invalidated and a new collection of samples should be done if still possible.

genestore™

INTERPRETATION OF RESULTS & REPORT

SARS-CoV-2 Markers (N1 and N2)

1. When the controls show the expected results, a sample is considered negative if both N1 and N2 markers show increasing curves that DO NOT cross the threshold line before 35 cycles (below 35 Ct); and the increasing curves of RNAse-P do cross the threshold line before 35 cycles (below 35 Ct).

2. When the controls show the expected results, a sample is considered positive if both markers N1 and N2 show increasing curves that CROSS the threshold line before 35 cycles (below 35 Ct). The RNAse-P curve may or may not be positive as described above, in the 2 cases the result is validated.

3. When all controls show the expected results, and the increasing curves of the N1 and N2 markers and the RNAse-P marker DO NOT cross the threshold level before 35 cycles (below 35 Ct), the result is not validated. RNA extracted from human samples should be retested. If no more RNA extract is available, a new extraction must be repeated and tested. If the test is again negative for all markers (including RNAse-P), the result is not validated and a new collection of human samples should be considered.

4. If HSC is positive for N1 or N2, there may be contamination during sample extraction or processing. Invalidate all results from samples collected with HSC. Re-extract samples and HSC and repeat.

GUIDE TO INTERPRETATION OF RESULTS

The following table lists the results expected from the GENESTORE's Détection Expert 1S™ SARS-CoV-2 test. If a laboratory does not obtain the expected results for the controls, or if invalid or inconclusive results are obtained that cannot be resolved after retesting, please contact your GENESTORE representative for consultation and re-testing if necessary.

Region 1 of SARS-CoV-2 (N1)	Region 2 of SARS-CoV-2 (N2)	RNaseP	Interpretation of results	Report	Actions
[+]	[+]	[+/-]	SARS-CoV-2 detected	Positive for SARS-CoV-2	Report to local health authorities and to the the sender
If one or both targets are positives		[+/-]	SARS-CoV-2 detected	Positive for SARS-CoV-2	Report to local health authorities and to the the sender.
[-]	[-]	[+]	SARS-CoV-2 not detected	Not detected	Send the results back to the to the sender. Patient can be tested for another respiratory virus.
[-]	[-]	[-]	Invalid results	Invalid	Repeat extraction and RT-PCR. If the results are again invalid, start a new sample collection on the patient.

Laboratories should report their diagnosis as appropriate and in accordance with their specific reporting system. The optimal viral peak level of SARS-CoV-2 and its duration has not been determined in different types of samples. Collection of different samples from the same individual is therefore necessary to detect the virus. The possibility of a false-negative result should be particularly considered if the patient has been exposed to the virus or has clinical signs suggesting possible SARS-CoV-2 infection, and tests for other respiratory diseases are negative. If SARS-CoV-2 infection is still suspected, retesting should be considered in consultation with public health authorities.

QUALITY CONTROL

- Quality control requirements must be followed in accordance with applicable regulations, accreditation requirements, and adherence to quality control procedures by laboratory personnel. For more information on quality control procedures, refer to the government accreditation body.
- Quality control procedures are designed to ensure product and test compliance.
- Test the positive controls first, before testing samples for each new batch of kit, to ensure that the reagents and kit compounds work properly.
- Good laboratory practice (GLP) recommends including a positive extraction control in each batch of nucleic acid isolation. Although HSC is not included in the SARS-CoV-2 rRT-PCR diagnostic panel, the HSC extraction control should go through nucleic acid isolation per batch of samples to be tested.
- Always include a negative control, and the appropriate positive control in each PCR. All clinical samples should be tested for the presence of the RNAse P gene, to control the quality of the sample and its extraction.

Performance Characteristics INCLUSIVENESS: IN SILICO ANALYSIS [4]

- The analytical sensitivity of the assay should be further evaluated by FDA recommended reference material, using FDA developed protocols if applicable or possible. Simulation of the analyses for the Primers and Probes sequences.

- An alignment was performed with the primers and oligonucleotide probe sequences by real-time RT-PCR of the SARS-CoV-2 Diagnostic Panel with all publicly available nucleic acid sequences for SARS-CoV-2 available to GenBank as of February 1, 2020 to demonstrate the expected inclusivity of the SARS-CoV-2 Diagnostic Panel real-time RT-PCR. All alignments show 100% identity of the CDC panel to the available SARS-CoV-2 sequences, with the exception of one nucleotide mismatch with the direct N1 primer in a given sequence. The risk of a single mismatch resulting in a significant loss of reactivity and a false-negative result is low, due to the design of primers and probes with melting temperatures > 60°C, and the test conditions with a second incubation at 58°C, so that one to two mismatches can be tolerated.

SPECIFICITY / EXCLUSIVITY TEST: IN SILICO ANALYSIS

- BLASTn analysis queries of the SARS-CoV-2 rRT-PCR assays for primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows:

1) The nucleotide collection consists of GenBank + EMBL + DDBJ + PDB+ RefSeq, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 , HTGS sequences, and sequences larger than 100 Mb;

2) The database is not redundant. Identical sequences were merged into a single entry, while preserving the membership, GI, title and taxonomy information for each entry;

3) The database was updated on 10/03/2019;

4) Search parameters were automatically adjusted for short entry sequences and the expected threshold is 1000;

5) The match and mismatch scores are 1 and -3, respectively;

6) The penalty for creating and extending a space in an alignment is 5 and 2 respectively.

SPECIFICITY / EXCLUSIVITY TEST: IN SILICO ANALYSIS (SARS-CoV-2 N1 TEST)

The probe sequence of the SARS-CoV-2 rRT-PCR assay showed sequence homology with the SARS coronavirus and the bat coronavirus genome. However, the forward and reverse primers did not show sequence homology with the SARS coronavirus and the bat coronavirus genome. When combining primers and probes, there are no significant homologies to the human genome, other coronaviruses or human microflora may give possible false positive results by rRT-PCR.

SPECIFICITY / EXCLUSIVITY TEST: IN SILICO ANALYSIS (SARS-CoV-2 N2 TEST)

The direct primer sequence of the rRT-PCR SARS-CoV-2 N2 showed a high sequence homology sequence with bat coronavirus.
The reverse primer and probe sequences did not show significant homology to the human genome, other coronaviruses or human microflora. By combining primers and probes, there is no possibility of false positive rRT-PCR results. In summary, the SARS-CoV-2 N1 and N2 rRT-PCR assay, designed for the specific detection of SARS-CoV-2, showed no significant combined homology to the human genome, to other coronaviruses, or to microflora that would predict potential false positive rRT-PCR results.

LIMIT OF DETECTION (LOD): INSTITUT PASTEUR STUDY [5]

The objective of the evaluation is to test the analytical sensitivity of the Détection Expert 1S™ SARS-CoV-2" - GeneStore kit for the detection of SARS-CoV-2 by comparison with the reference technique used at the CNR of the Institut Pasteur, using

- RNA extracted from SARS-CoV-2 positive respiratory specimen pools covering a wide range of Ct up to the limit of detection (pools 2, 3, 4, 5, 6, 7, 8 and 9).

- RNA extracted from SARS-CoV-2 negative respiratory specimen pools (Negative pool).

Panel of sample tested

- Nine pools of nasopharyngeal respiratory specimens from patients with similar Ct values, one of which consisted of negative sera. The most concentrated pools (pools 2, 3 and 4) were tested once. The lowest concentration pools (pools 5, 6, 7, 8 and 9) and the negative pool are tested in triplicates.

- RNA extracted from a 1000-fold diluted viral culture supernatant as a positive control.

Reference technique CNR*

Extraction with the NucleoSpin Dx Virus Extraction kit (Ref. Macherey Nagel 740895.50). SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Ref. Invitrogen 1732-020). Two targets: IP2 and IP4. Assay size: 5 µL

Technique evaluated according to the GeneStore manual

Test sample size of 3.5 µL. ABI 7500 System

Study Conclusion :

The CNR considers and validates that the GeneStore "Détection Expert 1S™ SARS-CoV-2" Kit has acceptable sensitivity for the detection of SARS-CoV-2.

CLINICAL VALIDATION : SENSITIVITY AND SPECIFICITY [3]

A clinical evaluation study was conducted to assess the performance of the GeneStore (Détection Expert 1S™ SARS-CoV-2) test using patient nasopharyngeal swab (NP) specimens:

A total of twenty one (21) specimens were tested:

- 11 positive nasopharyngeal swab specimens

- 10 negative nasopharyngeal swab specimens

The protocol used in this study aims to validate the performance of the test in the detection of the presence of the virus directly on samples taken without the RNA extraction step of RNA extraction.

The results of the study:

- Sensitivity: 100%.

- Specificity: 100%.

- Accuracy of the analysis: excellent (dispersion not exceeding 1.5% on the whole population tested)

- Efficiency of the measurement goes beyond a dilution to 1/ 10,000 of the sample to be tested.

- Time saving (generated by the simplified analysis process with the GeneStore test)

genestore™

PRECISION [6] : REPEATABILITY

Repeatability is defined as the variation in readings or the closeness of measured values when the same person measures the same sample several times using the same equipment and method under the same conditions.

Repeatability was measured by analyzing all 5 replicates of each sample dilution in a single run. Repeatability was calculated as the percentage coefficient of variance (% CV) of Cqs of a sample within a single run.

	SARS-CoV-2 N1			SARS-CoV-2 N2		
Template Dilution	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**
2857 copies per µl	27.59	100%	0.21	27.17	100%	0.25
285.7 copies per µl	30.49	100%	0.67	29.95	100%	0.57
28.57 copies per µl	33.80	100%	0.60	33.13	100%	0.95

PRECISION [6]: INTER-BATCH REPRODUCIBILITY

Reproducibility is an inter-batch study that determines whether the test can be successfully performed on multiple lots of the medical device under test and produce the same results. A relative standard deviation of less than 35% is acceptable. These values reflect the inherent variability of biological systems.

Reproducibility was measured by analyzing the 5 replicates of each sample dilution over three assays on one instrument using the three separate manufacturing lots/batches of the assay. Repeatability was calculated as the percentage coefficient of variance (% CV) of Cqs of a sample between the three runs configured on an instrument using the same RT-PCR machine.

	SARS-CoV-2 N1			SARS-CoV-2 N2		
Template Dilution	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**
2857 copies per µl	28.32	100%	2.22	27.89	100%	2.23
285.7 copies per µl	31.26	100%	2.13	30.73	100%	2.23
28.57 copies per µl	34.57	100%	1.91	34.00	100%	2.30

PRECISION [6]: DAILY TIME REPRODUCIBILITY

Daily time reproducibility is a study that determines whether the test can be successfully performed to produce the same results over several days. A relative standard deviation of less than 35% is acceptable. These values reflect the inherent variability of biological systems.

Reproducibility was measured by analyzing 3 replicates of different sample dilutions on 4 different days. Repeatability was calculated as the percentage coefficient of variance (%CV) of the Cqs.

	SARS-CoV-2 N1			SARS-CoV-2 N2		
Template Dilution	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**
2857 copies per µl	27.78	100%	1.65	27.17	100%	1.56
285.7 copies per µl	31.39	100%	1.39	30.81	100%	1.21

PRECISION [6] : INTER-OPERATOR REPRODUCIBILITY

Reproducibility is an inter-technician study that determines whether the test can be performed successfully by all technicians and produce the same results. A relative standard deviation of less than 35% is acceptable. These values reflect the inherent variability of biological systems.

Reproducibility was measured by analyzing the 5 replicates of each sample dilution in a single run. Repeatability was calculated as the percent coefficient of variance (%CV) of Cqs of a sample between the two runs setup by two different technicians on the same instrument RT-PCR machine.

	SARS-CoV-2 N1			SARS-CoV-2 N2		
Template Dilution	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**
2857 copies per µl	28.30	100%	2.18	27.86	100%	2.13
285.7 copies per µl	30.72	100%	2.59	30.16	100%	2.47
28.57 copies per µl	34.53	100%	1.85	33.86	100%	1.88

ANALYTICAL SPECIFICITY [6] : LIMIT OF DETECTION (LOD)

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected.

This concentration therefore serves as the limit of detection of the test (LOD).

Reproducibility was measured by analyzing all 30 replicates of the same sample dilution in a single run. Repeatability was calculated as the percentage coefficient of variance (%CV) of the Cqs of the 30 samples.

	SARS-CoV-2 N1			SARS-CoV-2 N2		
Template Dilution	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**	**Mean Cq**	**% Replicate Detection**	**Coefficient of Variance (%)**
28.57 copies per µl	32.50	100%	1.6	32.10	100%	1.2

genestore™

BIBLIOGRAPHY

1. European Centre for Disease Prevention and Control (ECDC). Laboratory support by specialised laboratories in the EU/EEA. At: ecdc.europa.eu/en/novel-coronavirus/laboratory; updated 8 February 2020. Accessed 19 March 2020.

2. ISO15223-1: 2016 - Medical devices Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements

3. Rapport de validation Clinique – Laboratoire investigateur * - Octobre 2020, échantillons testés par le Kit GENESTORE sur le thermocycleur Bioer Line-Gene 9660.

4. Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2, https:// www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2, https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC7392423/

5.https://www.sfm-microbiologie.org/wp-cont-ent/uploads/2020/09/Rapport_GENESTORE_200907.pdf

6. GeneStore France - Analytical performance Study of the GeneStore test (Precision: Repeatability and reproducibility, analytical specificity/ LOD) - 2021/2022 - France

*** For more details on the clinical validation study, please send your request to the following email address:** qa@genestore.eu

NOTICE TO USERS

Any serious incident related to the device should be reported to GENESTORE France (e-mail: qa@genestore.eu, contact: +33 4 88 70 01 65) and to the competent authority of the Member State in which the user and/or patient is established.

TECHNICAL SUPPORT / COMPLAINTS

To obtain information on this kit, or for any complaint, contact us by email qa@genestore.eu, or by phone at +33 4 88 70 01 65

WARRANTY LIMIT

Genestrore France SAS and affiliated Genestore sites around the world warrant their products according to the terms and conditions of sale described on www.genestore.org/terms-and conditions.html. If you have any questions, please contact us at: qa@genestore.eu.

GeneStore France SAS
800 Avenue du Château de Jouques,
Gémenos, Provence-Alpes-Côte
d'Azur, 13420, France

genestore™