REF IDNCOV2q-100
IDNCOV2q-500

IDNCOV-2

Instructions for use

Σ 100 tests
500 tests

Triplex Real-time RT-PCR assay for the **qualitative** detection of extracted **SARS-CoV-2 RNA**

Sample: Nucleic acids extracted from human biological samples

CE

IVD

Intended for *in vitro* use

0767

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SUMMARY

1 INTENDED USE

The **IDNCOV-2** kit is an *in vitro* diagnostic test based on real-time RT-PCR (or RT-qPCR) technology for the qualitative detection of viral RNA for severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) after sample extraction from patients in the context of suspected SARS-CoV-2 infection.

The **IDNCOV-2** kit is intended for clinical laboratories receiving nasopharyngeal samples, oropharyngeal swab samples, saliva samples and sputum samples (performed by a licensed healthcare professional) from patients in whom detection of SARS-CoV-2 is indicated. This test cannot be used directly on samples from patients. Thus, an extraction of nucleic acids must be carried out before proceeding to the test. The kit should be used with a real-time PCR thermocycler.

The results of the **IDNCOV-2** kit make it possible to detect the presence of RNA from SARS-CoV-2 and therefore helps in the diagnosis and management of Covid-19 patients. However, it should not be used alone, but in combination with other diagnostic techniques deemed necessary by the clinician.

The **IDNCOV-2** kit should be used only by laboratory health professionals, who have received instructions and training in real-time PCR techniques related to the thermocycler used and *in vitro* diagnostic procedures.

The **IDNCOV-2** kit is intended for *in vitro* diagnostic use.

2 TEST PRINCIPLE

1. Type of samples

The samples used with the **IDNCOV-2** are nucleic acid extracted from nasopharyngeal, oropharyngeal swab samples, saliva samples and sputum samples.

2. Extraction of nucleic acids

The SARS-CoV-2 viral RNA and the DNA corresponding to the endogenous internal control must be extracted from the samples before being amplified by RT-qPCR.

For extractions from nasopharyngeal, oropharyngeal swab specimens, and sputum samples, it is important to ensure that the nucleic acid extraction system used is compatible with real-time PCR technology. The quality of the extracted RNAs is essential to ensure the performance of the entire test. The suitability of the nucleic acid extraction procedure for use with the **IDNCOV-2** kit must be validated by the user.

Regarding extractions from saliva samples, the performance of the **IDNCOV-2** test has only been validated with the following extraction systems:

ID SOLUTIONS	Magnetic bead extraction system	ID Gene™ Mag Fast Extraction Kit	MAGFAST-768 MAGFAST-384
		ID Gene™ Mag Virus Extraction kit	MAGVIRUS-250
		ID Gene™ Mag Patho Extraction kit	MAGPATHO-12000

When using extraction systems recommended by ID SOLUTIONS, the performance of the **IDNCOV-2** test will be identical to that obtained in studies carried out by ID SOLUTIONS.

If another extraction system is used, performance cannot be guaranteed by ID SOLUTIONS. Thus, it is important to ensure that the nucleic acid extraction system used is compatible with real-time PCR technology. The quality of the extracted RNAs is essential to ensure the performance of the entire test. The suitability of the nucleic acid extraction procedure for use with the **IDNCOV-2** kit must be validated by the user.

3. Real-time amplification and detection

- **Principle**

The technology used for the test is real-time RT-PCR, which first allows reverse transcription of the extracted RNA into complementary DNA (cDNA), then, in a second step, the amplification of specific sequences from the targeted genome and the endogenous internal control. The presence of nucleic acids is then detected by an increase in fluorescence due to the hydrolysis of specific probes during the amplification step (technique of 5 ' nuclease hydrolysis probes).

The endogenous control corresponds to a human gene which is constitutively expressed in the cells present in the samples taken from the patients. Its detection makes it possible to evaluate the presence of nucleic acids to be tested in the sample, the implementation of the entire procedure and to evaluate whether amplification inhibitors are present.

- **Amplified sequences of SARS-CoV-2**

The primers used in the **IDNCOV-2** test make it possible to amplify two target sequences specific for the strain of the coronavirus SARS-CoV-2, at the level of the nucleocapsid protein (N) coding sequence, hereinafter named N1 and N2.

IDNCOV-2 is a qualitative triplex system which allows, for each sample, the simultaneous amplification of target RNAs (fluorescence read in the FAM ™ channel for the N1 target and in the VIC® / HEX ™ channel for the N2 target) and an endogenous internal control (fluorescence read in the Cyanine 5 - Cy5 channel), which corresponds to a human gene constitutively expressed in the cells.

- **Real-time PCR instruments**

The **IDNCOV-2** kit was developed and validated to be used with the real-time PCR thermocyclers listed in Table 1.

Table 1. LIST OF REAL-TIME PCR INSTRUMENTS VALID WITH THE KIT **IDNCOV-2**

Manufacturer	Model
Applied Biosystems	QuantStudio 5
	QuantStudio 7
	ABI 7500
	ABI prism®7500 Fast SDS
Roche	LightCycler 96
	LightCycler 480 (System II)
Bio-Rad	CFX96 Touch real-Time PCR detection System
Qiagen	Rotor-Gene Q
Agilent	Aria Mx Real-time PCR

⚠ Ensure that instruments and equipments have been checked and calibrated in accordance with the manufacturer's recommendations.

4. Controls

- **Extraction controls**

Negative Extraction Control (NEC)
This control is not present in the kit. It must be included during the extraction step.

This control corresponds to the replacement of the sample from a patient with either a **negative matrix (NEC-matrix); a new swab, for example,** or **nuclease-free water (NEC-H_2O).**

The control must be extracted and amplified at the same time and according to the same protocol as the patient samples.

*Note: When using a **NEC-matrix**, refer to the extraction kit protocol for the matrix in question.*

It detects the possible presence of cross contamination during extraction and amplification steps.

Positive Extraction Control
This control is not present in the kit. It must be included during the extraction step.

This control corresponds either to commercial positive controls like VLP "virus like particle" or to nasopharyngeal samples, oropharyngeal swab samples and sputum samples from patients found to be positive.

It validates the extraction step, mainly the extraction of viral RNA, the reverse transcription step and determines the presence of inhibitors during PCR amplification.

- **Amplification control**

The following controls should be used for each run of analysis:

Positive Amplification Control (PAC)

This control is present in the kit (PAC-NCOV-2). It contains a mixture of genomic and synthetic DNA from each target (respectively endogenous and pathogenic target N1 and N2), calibrated to the values mentioned on the quality control sheet for the corresponding batch.
It validates the amplification stage of each target.

Negative Amplification Control (NAC)

This control is not present in the kit. It corresponds to the deposition of 8 µl of amplification reaction mixture (ARM-NCOV-2) and 5 µl of nuclease-free water.
The control must be amplified at the same time and according to the same protocol as the samples extracted from patients. It allows to check the absence of contamination during the amplification step.

3 REAGENTS AND MATERIAL

1. Kit composition

The **IDNCOV-2** kit is available under two references: IDNCOV2q-100 (100 reactions) and IDNCOV2q-500 (500 reactions). The kit composition is presented in the table below:

TABLE 2. IDNCOV-2 KIT COMPONENTS

COMPONENT		KIT REFERENCE 100 reactions **IDNCOV2q-100**	KIT REFERENCE 500 reactions **IDNCOV2q-500**	COLOR CAP	DESCRIPTION
Designation	Name				
PAC-NCOV-2	Positive Amplification Control	90 µL 1 tube	90 µL 2 tubes	Red	Ready-to-use mixture of DNA and plasmid specific of SARS-CoV-2 and of the human endogenous control
ARM-NCOV-2	Amplification Reaction Mix	800 µL 1 tube	800 µL 5 tubes	Green	Ready-to-use reaction mix containing the Reverse Transcriptase, the Taq polymerase, the primers and the hydrolysis probes for the detection of SARS-CoV-2 and of the human endogenous control

2. Preparation and storage conditions of the reagents

The **IDNCOV-2** kit must arrive frozen upon receipt. If one of the kit components is not frozen on arrival or if one of the tubes was damaged during transport, please contact **ID-Solutions**.

Before and after opening the kit	Reagents should be stored between -16 °C and -26 °C and protected from light
After thawing	The reagents must be kept on a cooling block (+2 °C/+8 °C) or on ice during all their use.
Before use	The reagents must be completely thawed, then they must be homogenized.
After use	The reagents must be replaced as quickly as possible between -16°C and -26°C protected from light.

TABLE 3. CONDITIONS OF PREPARATION, STORAGE AND USE OF REAGENTS OF THE KIT **IDNCOV-2**

DESIGNATION	NAME	PREPARATION	CONDITIONS OF STORAGE AND USE
PAC-NCOV-2	Positive Amplification Control	Ready-to-use	Do not exceed 3 freeze/thaw cycles
ARM-NCOV-2	Amplification Reaction Mix	Ready-to-use	Do not exceed 3 freeze/thaw cycles. Must be protected from light (during storage and when thawing). It is sensitive to temperature variations. Once thawed, do not leave the tube at room temperature. Use a cooling block (+2 °C/+8 °C) or ice for its handling and place the tube as soon as possible at a temperature between -16 °C and -26 °C after use.

When stored under the specified storage conditions, the kit is stable until the stated expiration date. It is recommended to store the PCR reagents in a pre-amplification area (green tube) and the control (red tube) in a post-amplification area (manipulation of DNA).

The kit is stable for 3 months after opening in the absence of contamination.

⚠ **The ARM-NCOV-2 is very sensitive to temperature variations, scrupulously follow the instructions for storage and use conditions in the table above.**

3. Material needed but not provided in the kit

- Systems or kits suitable for nucleic acids extraction, follow the manufacturer's instructions (refer to principle of the test)
- Real-time PCR thermocyclers validated with the kit (refer to principle of the test).
- Centrifuge for reaction tubes or plate
- 96 well PCR plates or PCR reaction tubes with adhesive films or caps for closure adapted to each validated thermocycler.
- Cooling block or ice
- Precision pipettes and Nuclease-free tips with filters adapted to the volume to be pipetted.
- 1.5 ml tubes
- Distilled or Nuclease-free water (DNase and RNase free)

4 INSTRUCTIONS AND PRECAUTIONS OF USE

The use of this product is limited to qualified personnel trained in real-time PCR techniques and *in vitro* diagnostic procedures. Good laboratory practice should be followed.

Please read the instructions before starting the test.

1. Hygiene and safety guidelines

- Wear protective clothing, including a lab coat, eye and face protection, and disposable gloves (synthetic latex-free gloves are recommended). Handle the kit reagents and the patient's samples in accordance with the required Good Laboratory Practices. Wash your hands thoroughly after completing the test.

- Do not smoke, drink, or eat in areas where samples or reagents of the kit are handled.

- Surfaces contaminated with a contaminating liquid must be thoroughly cleaned with an effective disinfectant. The material used for cleaning must be disposed of in a special container for contaminated waste.

- ⚠ Do not put solutions containing bleach in an autoclave.

- Dispose of all samples and material used to perform the test as if they were potentially infectious. The disposal of hazardous chemical and biological waste must be carried out in accordance with local regulations.

For more information, see the appropriate safety data sheet (SDS. It is available upon request at the address: **info@id-solutions.fr**.

2. Precautions of use

- The infectious and/or dangerous risks must be considered during handling, in accordance with the laboratory's current safety procedures.

⚠ **Patient samples and RNA samples stored in poor conditions may yield to erroneous results.**

- Do not use the kit or any of the kit reagents beyond the indicated expiration date.

- Do not mix reagents from other kits with different batch numbers.

- Do not use reagents that have been poorly preserved.

- All reagents should be thawed at +18 °C/+25 °C and homogenized before use. After thawing, handle the reagents on a cooling block at +2 °C/+8 °C or on ice and replace them as quickly as possible at -16 °C/-26 °C. The ARM-NCOV-2 reagent contains fluorophore-marked probes and must be protected from light especially during thawing.

Pay attention to all the points listed below:

- Follow the usage recommendations carefully to ensure that the test is performed correctly. Any discrepancies may affect test performance.

- Before use, check that the product and its components are not damaged, are compliant (number of tubes, volume), are properly labeled, and have been frozen upon receipt of the product.

- Use work areas separate from each other for the different testing stages: preparation of the samples, and preparation of amplification/detection reaction. It is recommended to adopt "a step forward" when completing the various stages. Wear gloves in each work area and change them before entering a different area.

- Use dedicated equipment and consumables for each work area and do not move them from one area to another.

- Avoid DNase and RNase contamination of the sample and kit components that could result in RNA/MATRIX DNA degradation, or PCR contamination by transfer, which could result in a false positive signal. Use nuclease-free laboratory equipment (pipettes, pipette tips, reaction vials) and wear gloves when performing the test. Use aerosol-resistant pipette tips for all pipetting steps.

- Separate and do not put positive and/or potentially positive material in contact with kit components.

- Do not open the tubes/reaction plates after amplification to avoid contamination by the amplicons.

- Do not reuse reaction tubes after a PCR (e.g. autoclaving tubes). Amplified nucleic acids will not be degraded.

5 PROTOCOL

1. Protocol for the extraction of nucleic acids and storage conditions for RNA samples

For each extraction, include a negative extraction control (NEC-H_2O) and a positive extraction control (see Test Principle).

Because of the risk of contamination by ribonucleases (RNases), RNA extraction is more difficult than DNA extraction. Strict precautions should be taken to prevent post-extraction RNA degradation. Once extracted, RNA samples should remain at a minimum on the ice if used immediately after or be stored at –80 °C for longer storage.

Regarding saliva samples, please follow the pre-analytical conditions recommended by local authorities and regulations.

2. Real-time detection protocol

- **Preparation of amplification reaction by RT-qPCR**

The sample to be amplified corresponds to the eluate product obtained after nucleic acid extraction.

Note: The amplification reaction has to be performed in the area dedicated to amplification.

1. Prepare an analysis plan for the experimental samples and controls, taking care to keep the positive amplification control (PAC-NCOV-2) away from samples. It is also advisable to frame the samples with two NAC controls, at the beginning and at the end of the sample series.
2. Thaw the **IDNCOV-2** kit at a temperature of +18 °C/+25 °C and away from the light. Once thawed, keep them on a cooling block (+2 °C/+8 °C) or on ice.

⚠ **After use, place the reagents, ARM-NCOV-2 and the PAC-NCOV-2, as soon as possible at a temperature of -16 °C/-26 °C.**

3. Homogenize (vortex) the **ARM-NCOV-2** and **PAC-NCOV-2** tubes and centrifuge them briefly.
4. Add **8 µL of ARM-NCOV-2** per well.

⚠ **Please use equipment (96 wells plates or reaction tubes with adhesive films or caps for closure) suitable for the thermocycler to be used. Follow the manufacturer's instructions.**

5. Add to the reaction mix either:
   - 5 µl of extracted RNA, for each sample to be analyzed
   - 5 µl of PAC-NCOV-2
   - 5 µl of NEC extract
   - 5 µl nuclease-free water (NAC)
6. Cover the plate or tubes with suitable adhesive films or caps.
7. Centrifuge for 1 minute at 1000 rpm to collect all the reagents.

- **Programming the PCR amplification phase in the thermocycler**

Note: Please consult the manuals for the use of the different real-time PCR instruments for general information on their programming.

1. Program the reading of the following detectors on the thermocycler for each of the wells to be analyzed

Table 4. THERMOCYCLER READING PARAMETERS

TARGET	READING CHANNEL	WAVELENGTH	QUENCHER
SARS-CoV-2-N1	FAM™	530 nm	non fluorescent
SARS-CoV-2-N2	VIC®/HEX™	560 nm	non fluorescent
Endogenous control	Cy5	670 nm	non fluorescent

Warning: For instruments requiring an internal reference, the reaction amplification mixture already contains ROX.

2. Enter the following amplification program on the thermocycler:

Table 5. RT-qPCR PROGRAM

STEPS		PROGRAM	CYCLES	ACQUISITION OF FLUORESCENCE				
				Applied Biosystems validated	Roche validated	CFX96	Aria Mx Real-Time PCR	RotorGene Q
(1) Reverse Transcription		10 min 50 °C	1	---	---	---		
(2) Activation of Taq Polymerase		2 min 95 °C	1	---	---	---		
(3) Amplification	DNA denaturation	10 sec 95 °C	40	---	---	---		
	Hybridization and elongation	30 sec 60 °C		FAM CY5 VIC	FAM (465-510) Cy5 / Cy5.5 (618-660) VIC/HEX/Yellow555 (533-580)	FAM Cy5 HEX	FAM Cy5 HEX	FAM Cy5 HEX

*Note: Fluorescence reading is performed at the **end** of the elongation phase at 60 °C.*

3. Select a final volume of **13 µl per PCR.**
4. Place the plate or tubes in the thermocycler and start the program.

6 ANALYSIS AND INTERPRETATION OF THE RESULTS

Thanks to real-time PCR, the entire amplification kinetics is measurable (above the baseline) and may be quantified.

The fluorescence may then be expressed on a logarithm scale. The exponential phase is linearized.

Note: Ct = Threshold cycle, Cq = quantification cycle or CP = Crossing point

1. Analysis of the results

- **Data analysis on validated Applied Biosystems instruments**

- Check that **ROX** has been selected in the **PASSIVE REFERENCE** field (the amplification mix contains a passive reference).

- The targets N1 and N2 are analyzed once the **FAM** and **VIC** detectors / reporters have been selected in the **DETECTOR/REPORTER** field.

- The endogenous target analysis is performed once the **CY5** detector/reporter has been selected in the **DETECTOR/REPORTER** field.

- Let the software set the thresholds automatically.

- For each positive sample, a Ct is calculated in **FAM** or **VIC**. Negative samples or controls marked as **UNDETERMINED** are displayed in the **Ct** column.

- For FAM and VIC negative samples, check the presence of a CY5 Ct.

- **Data analysis on validated Roche Instruments**

- Target analysis is carried out in **ABSOLUTE QUANTIFICATION** mode in **FAM and HEX**.

- Analysis of the endogenous target is carried out in **ABSOLUTE QUANTIFICATION** mode **in Cy5**.

- For each positive sample, a **CROSSING POINT** (CP) is calculated in **FAM** and **HEX**.

- For each negative sample a **CROSSING POINT** (CP) is calculated in **Cy5**.

- Use the **FIT POINTS** method to determine the state (positive / negative) of the samples and controls.

- Analysis using the Fit Points method:

Analysis using the **FIT POINTS** method takes place in three stages: **CYCLE RANGE; NOISE BAND**; **ANALYSIS**.

In **STEP 1**, define the background noise range; for this, define the background between 2 and 16 cycles.

In **STEP 2**, select the automatic definition of the background noise (noise band) and make sure that the horizontal line eliminates the background noise and crosses all the curves at the start of their exponential phase.

Then, in **STEP 3**, select the automatic definition of the threshold.

- **Data analysis on the CFX96 instrument**

- The targets are analyzed under the **QUANTITATION** tab, leaving only the **FAM** and **VIC** buttons checked.

- The endogenous target is analyzed under the **QUANTITATION** tab, leaving only the **Cy5** button checked.

- In **SINGLE THRESHOLD** mode, manually move the threshold line so that it is:

- above the background noise
- in the exponential phase of each amplification curve, which generally corresponds to 5 to 10% of the final fluorescence of the sample.

- A Cq in FAM and VIC is calculated for each positive sample. Negative samples or controls are designated by the indication **N/A** in the column **Cq**. For negative samples in FAM and VIC, check the presence of a Cq in CY5.

- **Data analysis on the Aria Mx Real-Time PCR instrument**

- Check that **ROX** has been selected in the **REFERENCE DYE** field of the **Plate Setup** menu (the amplification mix contains a passive reference).

- Select the **FAM, VIC** and **Cy5** (Dye Name) channels in the **Plate Setup** menu and add the name of the targets for each fluorophore (Target Name).

- The targets are analyzed in the **Analysis** menu via the **Analysis Criteria** tab (visualization of Cq) and **Graphical Display** (display of curves and modification of the threshold).

- In the **Graphical Display** tab, manually move the threshold line so that it is:

- above background noise
- in the exponential phase of each amplification curve, which generally corresponds to 5 to 10% of the final fluorescence of the sample.

- A Cq in FAM and VIC is calculated for each positive sample. Negative samples or controls are designated as **No Cq** in the **Cq** column. For FAM and VIC negative samples, check for the presence of a Cq in CY5.

- **Data analysis on the Rotor Gene Q instrument**

- Select the **FAM** (Green Channel), **HEX** (Yellow Channel) and **Cy5** (Red Channel) channels in the **Analysis** menu.

- In the **Threshold** tab, manually move the threshold line so that it is:

- above the background noise
- in the exponential phase of each amplification curve, which generally corresponds to 5 to 10% of the final fluorescence of the sample.

- A Ct in FAM and HEX is calculated for each positive sample. Negative samples are designated by an empty box in the **Ct** column. For negative samples in FAM and HEX, check for the presence of a Ct in CY5

2. Validation and interpretation of results

- **Validation of the RT-PCR test**

The test is only validated if all the validation criteria described below are met.

Table 6. VALIDATION CRITERIA FOR **IDNCOV-2** KIT RT-PCR ASSAY

CONTROL	EXPECTED RESULT	VALIDATION CRITERIA
PAC-NCOV-2	SARS-CoV-2 N1 specific signal detected in **FAM™**	Presence of three characteristic curves. Refer to the Cq value shown on the quality control sheet (FCQ) of the corresponding batch.
	SARS-CoV-2 N2 specific signal detected in **VIC™/HEX™**	
	Endogenous control specific signal detected in **Cy5**	
NEC	**No detection in FAM™ and VIC®/HEX™**	If NEC is a negative sample, possible detection of the Cy5 signal. if NEC is H_2O, no signal is expected
NAC	**No detection**	Total absence of a characteristic curve

If all the criteria are met, **the test is validated, and the results can be interpreted.**

If all the criteria are not met, **the test is invalidated**. The user can decide to repeat the test after having checked the elements described in section 9.

- **Interpretation of results**

The presence or absence of SARS-CoV-2 RNA in the test sample is determined **qualitatively** using the Cq values obtained for each sample for each of the targets.

For each sample analyzed, the results must be interpreted according to the following criteria:

Table 7. INTERPRETATION OF RESULTS

SARS-CoV-2 N1	SARS-CoV-2 N2	Endogenous Control	INTERPRETATION	CONCLUSION
+	+	+ / -	SARS-CoV-2 specific RNA detected	Sample contains detectable amounts of SARS-CoV-2 specific RNA
+	-	+ / -	Doubtful result	Repeat the test or re-extract and then repeat the test.
-	+	+ / -		
-	-	+	SARS-CoV-2 specific RNA not detected	Sample does not contain detectable amount of SARS-CoV-2 specific RNA
-	-	-	Not interpretable	Repeat the test or re-extract and then repeat the test.

- The **presence of Cq in FAM and VIC/HEX** and a **characteristic curve** coupled with the presence or absence of Cq for endogenous control (Cy5) corresponds to a **positive sample**.
- The **absence of Cq in FAM and VIC / HEX** coupled with the **presence of Cq for endogenous control (Cy5)** corresponds to a **negative sample**.
- The **presence of Cq only in FAM or VIC/HEX** coupled with the presence or absence of Cq for endogenous control (Cy5) makes the **result doubtful.**
- The **absence of any signal** makes the **result uninterpretable**.

7 EVALUATION OF ANALYTICAL PERFORMANCE

The analytical performance of the **IDNCOV-2** kit was determined using *in silico* analyses and positive samples made from synthetic DNA fragments representing the different targets sought by the test. The following performance was achieved in accordance with the conditions described in the procedure and with the use of the QuantStudio 5 thermocycler (Applied Biosystems).

1. Analytical Specificity

Inclusivity

The sequences targeted by the **IDNCOV-2** test are recommended and validated by the World Health Organization **(WHO).** These are specific sequences of the SARS-CoV-2 strain determined by the Centers for Disease Control and Prevention (CDC) in the U.S.A.

The specificity of the primers and probes allowing the detection of SARS-CoV-2 of the IDNCOV-2 test was determined by an *in silico* analysis of the sequences on the databases of the NCBI. This analysis shows that the test specifically detects the strain SARS-CoV-2.

Exclusivity and biological interference

The specificity of the primers and probes allowing the detection of SARS-CoV-2 of the IDNCOV-2 assay has been demonstrated through an *in silico* analysis and an experimental analysis carried out on a commercial panel containing pathogens susceptible to '' be found in samples from patients (see list below).

The pathogens were tested:

- Without co-infection with SARS-CoV-2 (exclusivity study).
- Co-infected with SARS-CoV-2 at 3 x LoD (study of biological interference).

No cross-reaction or interference was observed for the following pathogens:

- **Viruses:** human coronavirus: 229E, NL63, OC43, HKU1, SARS and MERS-CoV, adenovirus (type 1; 3; 31), human metapneumovirus (MPVh), parainfluenza virus type 1 to 4, influenza A, influenza B, respiratory syncytial virus (RSV), and rhinovirus.

- **Bacteria:** *Chlamydia pneumoniae, Bordetella pertussis, Bordetella parapertussis, Mycoplasma pneumoniae*

2. Analytical Sensitivity - Detection Limit

The analytical sensitivity (or limit of detection at 95%, LoD) of the **IDNCOV-2** kit was determined on a range of dilutions of synthetic DNA titrated by digital PCR. These artificial samples were then amplified with the **IDNCOV-2** kit using QuantStudio5 (Applied Biosystems). The LoD has been determined to be the lowest matrix concentration that can be reliably detected with 95% confidence.
Each sample was tested 24 times*. The table below expresses the results on the average of the 24 replicates for each of the targets.

Table 8. RESULTS OF THE ANALYTICAL SENSITIVITY STUDY

Target	Concentration (copies/PCR)	Average Cq	Standard deviation	% detected
N1	100*	31.954	0.289	100%
	50*	33.179	0.437	100%
	25	33.770	0.985	100%
	12,5	**34.327**	**1.465**	**100%**
	6,25	34.390	0.958	92%
N2	100*	30.497	0.259	100%
	50*	31.519	0.644	100%
	25	32.587	1.361	100%
	12,5	**33.319**	**1.630**	**96%**
	6,25	33.564	1.248	75%

**Tested on 8 replicates only. N1 and N2 target the N gene of SARS-CoV-2.*

The limit of detection at 95% of the **IDNCOV-2** kit is **12.5 copies/PCR.**
Detection limit values have also been confirmed with the following thermocyclers: QuantStudio 7, ABI 7500, ABI prism 7500 Fast SDS, LC96, LC480, CFX96, Rotor-Gene Q and AriaMx Real-time PCR.

3. Characterization of the complete method on saliva and sputum

In the absence of titrated reference material, the approach to the detection limit of the complete method was carried out using assayed biological samples.

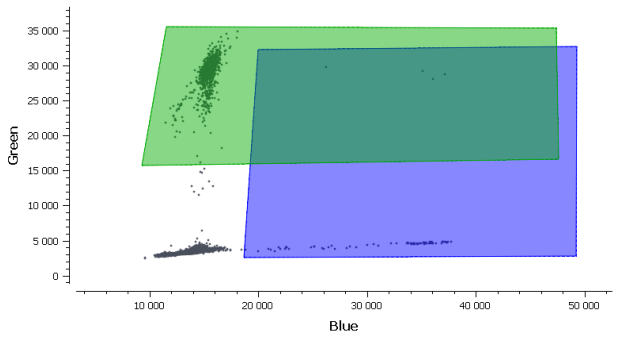
Method description:

Native saliva and sputum samples have been collected from patients with suspected respiratory infection.

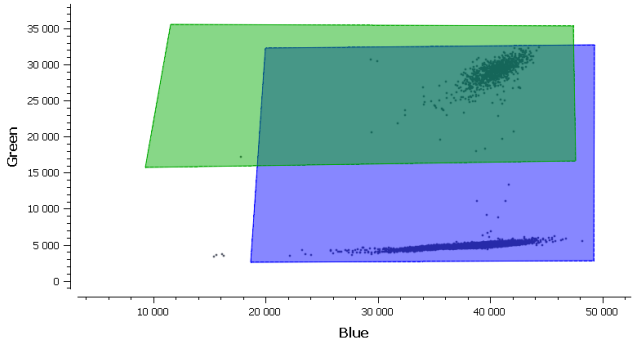
They were quantified in the number of copies of target sequence per ml and are used as reference samples for the spiking in a matrix of the same nature that do not contained the desired target.

The saliva and sputum samples were identified as being strongly detected by PCR. They were assayed by digital PCR (internal method ID SOLUTIONS). These native samples were then amplified with the IDNCOV-2 kit using the Roche LightCycler 96 thermal cycler.

Saliva :



Sputum :



Results:

- Quantification by digital PCR gives: **6500 copies / ml** of sample for **saliva** and **19,150,000 copies / ml** of sample for **sputum**

Experimental method for approaching the detection limit of the method:

The LDMETHODE is estimated to be the last level of dilution where the 8 repeats are detected. The limit thus estimated is not the absolute limit of detection of the method which is at a lower concentration of target sequences.

Experimental design for the estimation of LDMETHODE:

Minimum number of sessions	Minimum number of operators	Minimum number of replicates per dilution
2	1	4

The validated methods are:

MAGFAST-384/768: ID GENE ™ MAG FAST Extraction Kit. Rapid extraction of nucleic acids from pathogens by magnetic beads.

MAGVIRUS-250: ID GENE ™ MAG Virus Extraction Kit. Kit for rapid extraction by magnetic beads of pathogen nucleic acids.

MAGPATHO12000: ID GENE ™ MAG Pathogen Extraction Kit. Rapid extraction of nucleic acids from pathogens by magnetic beads.

Each method was validated using the IDNCOV-2 Amplification Kit.

The analytical performances of the 3 extraction kits mentioned above give identical performances, we present below the results of MAGFAST-384/768 for the saliva matrix.

Results target N1, MAGFAST-384/768 for the saliva matrix:

Table 9. RESULTS OF THE LD METHOD ON SALIVA FOR THE TARGET N1

	Nb of copies / ml samples	250	125	62.5	31.25
Session 1	Replicate 1	34.2	34.3	-	-
	Replicate 2	35.1	37.1	35.6	36.1
	Replicate 3	34.5	35.0	38.4	-
	Replicate 4	35.2	37.9	37.4	-
	Mean	34.8	36.1	37.1	NA
	CV %	1.4	4.7	3.8	NA
	% of detection	**100%**	100%	75%	25%
Session 2	Replicate 1	34.7	36.0	36.8	37.4
	Replicate 2	34.1	39.1	36.5	35.8
	Replicate 3	35.1	-	37.1	39.1
	Replicate 4	35.2	34.9	36.4	36.6
	Mean	34.8	36.7	36.7	36.7
	CV %	1.4	6.0	0.8	3.9
	% of detection	**100%**	75%	100%	100%

Results:

The detection limit of the MAGFAST-384/768 method on saliva is **250 copies/ml of sample** for the **N1 target**

Results target N2, MAGFAST-384/768 for the saliva matrix:

Table 10. RESULTS OF THE LD METHOD ON SALIVA FOR THE TARGET N2

	Nb de copies / ml d'échantillon	250	125	62.5	31.25
Séance 1	Réplique 1	35.9	34.1	-	-
	Réplique 2	35.4	-	-	36.9
	Réplique 3	36.6	36.2	-	-
	Réplique 4	35.5	37.6	36.9	-
	Moyenne	35.9	36.0	NA	Na
	CV %	1.5	4.8	NA	NA
	% de détection	**100%**	75%	25%	25%
Séance 2	Réplique 1	37.0	-	-	37.2
	Réplique 2	35.6	-	-	36.2
	Réplique 3	36.4	37.2	36.9	37.2
	Réplique 4	35.6	37.6	37.6	37.1
	Moyenne	36.2	37.4	37.2	36.9
	CV %	1.9	0.7	1.3	1.4
	% de détection	**100%**	50%	50%	100%

Results:

The detection limit of the MAGFAST-384/768 method on saliva is **250 copies/ml of sample** for the **N2 target**

4. Accuracy

Accuracy studies include an assessment of repeatability (intra-assays) and intermediate fidelity (inter-assays). The accuracy of the assay was assessed on the Positive Amplification Control (PAC-NCOV-2) and on a range of dilutions of synthetic DNA titrated in digital PCR. These artificial samples were then amplified with the **IDNCOV-2** kit using QuantStudio5 (Applied Biosystems).

- The intra-test accuracy (Table 9) was evaluated on 8 replicates of 3 panel samples at concentrations > 3x the LoD.
- The inter-assay studies (Table 10) were conducted on the same batch of reagents, tested on 2 different QuantStudio5 instruments, by the same user on 3 three independent runs; with 8 repetitions per sample, on 3 samples, including 2 samples presenting concentrations close to the LoD.

The average Cq, the standard deviation, and the intra-assay and inter-assay accuracy (% CV) for each of the samples from the panel are presented in the following tables.

Table 9. TEST REPEATABILITY

Target	Concentration (copies/PCR)	Repeatability		
		Average Cq	Standard deviation	%CV
N1	PAC (500)	28.71	0.14	0.5%
	100	31.95	0.29	0.9%
	50	33.18	0.44	1.3%
N2	PAC (500)	27.07	0.13	0.5%
	100	30.50	0.26	0.9%
	50	31.52	0.64	2.0%
Endo	PAC (400)	29.53	0.15	0.5%
	200	30.53	0.35	1.1%
	100	31.72	0.31	1.0%

N1 and N2 target the N gene for SARS-CoV-2; The Endo targets a human gene

Table 10. INTERMEDIATE FIDELITY OF THE ASSAY

Target	Detection Level (copies/PCR)	ASSAY 1 (robot #1)		ASSAY 2 (robot #2)		ASSAY 3 (robot #1)		ASSAY 1 + 2 + 3		
		Average Cq	SD	Average Cq	SD	Average Cq	SD	Average Cq	SD	%CV
N1	25	33.87	0.95	34.37	0.97	33.35	0.80	33.77	0.98	2.9%
	12,5	34.52	0.79	35.21	1.91	33.25	0.71	34.33	1.46	4.3%
	PAC (500)	28.71	0.14	28.78	0.16	28.32	0.07	28.63	0.22	0.8%
N2	25	32.16	0.90	34.04	0.89	30.49	6.23	32.59	1.36	4.2%
	12,5	32.70	1.18	34.96	1.16	32.14	0.91	33.32	1.63	4.9%
	PAC (500)	27.07	0.13	28.64	0.17	26.31	0.86	27.27	0.97	3.6%
Endo	25	31.72	0.31	32.24	0.12	30.96	1.06	31.31	1.02	3.3%
	12,5	32.66	0.42	33.17	0.20	31.04	0.34	32.29	0.98	3.0%
	PAC (400)	29.80	0.14	30.21	0.02	28.53	0.07	29.59	0.68	2.3%

N1 and N2 target the N gene for SARS-CoV-2; The Endo targets a human gene

Accuracy data show that the test is accurate in values > 3 times the LoD (%CV < 2%), and varies on values close to the LoD (%CV < 5%), or between thermocyclers. However, the LoD is not impacted by these variations.

8 EVALUATION OF CLINICAL PERFORMANCE

1. Nasopharyngeal swab samples

The clinical evaluation of the **IDNCOV-2** test designed to detect the SARS-CoV-2 coronavirus by real-time RT-PCR, was carried out on a retrospective study including 94 RNA samples extracted from patient samples taken as part of a suspected SARS-CoV-2 infection.

The performance of the **IDNCOV-2** test was evaluated in comparison with a reference RT-PCR method, CE-IVD marked, routinely used for the characterization of samples.

The **IDNCOV-2** test was performed on the same extract as the reference method, after undergoing a single freezing/thawing cycle.

Table 11. CLINICAL EVALUATION RESULTS

		RT-PCR reference (CE-IVD marked)		
N=94	**Samples Status**	**Positive**	**Negative**	**Total**
IDNCOV-2	**Positive**	21	0	21
	Negative	0	73	73
	Total	21	73	94

The results obtained with the **IDNCOV-2 test** kit completely agree with the results of the reference RT-PCR method, CE-IVD marked, used routinely for the characterization of samples extracted from patients in the context of a suspicion with SARS-CoV-2 infection.
Relative sensitivity of the IDNCOV-2 test: 100% (21/21).
Relative specificity of the IDNCOV-2 test: 100% (73/73).
Overall match of the IDNCOV-2 test: 100% (94/94).

2. Comparison of clinical performance between nasopharyngeal and saliva samples

The comparison of the clinical sensitivity of the **IDNCOV-2** assay was performed on 165 nasopharyngeal and saliva samples collected concomitantly.
These samples were extracted following the recommendations of the MAGFAST-768, MAGVIRUS-250 or MAGPATHO-12000 Extraction Kit Instructions for Use. The extracted nucleic acids were then amplified with the **IDNCOV-2** kit on different thermal cyclers (CFX96, LC480, Aria Mx, QS5 and QS7).

Extraction kits	Number of samples
MAGFAST-768	112
MAGVIRUS-250	32
MAGPATHO-12000	21

Thermocyclers	Number of samples
CFX96	47
LC480	21
Aria Mx	25
QS5	11
QS7	61

A nasopharyngeal sample was considered positive if the N1 and N2 targets are amplified with a Cq value less than 34 corresponding to the LoD with a 95% confidence interval of the **IDNCOV-2** kit (see page 12).

Tableau 12 RESULTS OF THE COMPARISON OF CLINICAL PERFORMANCE BETWEEN NASOPHARYNGE AND SALIVARY SAMPLES

		Nasopharyngeal samples		
N=165	**Sample status**	**Positive**	**Négative**	**Total**
Saliva samples	**Positive**	82	1	83
	Négative	9	73	82
	Total	91	74	165

Results :

- **Positive agreement of IDNCOV-2** kit between nasopharyngeal and saliva samples: 90% (82/91) with a confident interval of 95% of [84.0%-96.2%].
- **Negative agreement of IDNCOV-2** kit between nasopharyngeal and saliva samples : 99% (73/74) with a confident interval of 95% of [96.0%-100%].
- **Overall agreement** of **IDNCOV-2** kit between nasopharyngeal and saliva samples : 94% (155/165) with a confident interval of 95% of [90.3%-97.6%].

9 TROUBLESHOOTING

INVALID ASSAYS

Potential cause	Solution
The characteristic amplification curves do not appear due to the degradation of the reagents in the kit, including the amplification mix.	Check that the storage conditions and handling of the reagents are scrupulously followed (see section 3). Check reagent expiration dates.
Error in the implementation of the test protocol.	Check the entire test protocol followed against the protocol described in these instructions for use
Contamination of reagents.	Check the conditions of storage and conditions of use of reagents. Validate the absence of contamination of the reagents. Decontaminate equipment and premises.

PATHOGEN PRESENT BUT NOT DETECTED IN SAMPLES: FALSE NEGATIVES

Potential cause	Solution
Degradation of the reagents in the kit, including the amplification mix.	Check that the conditions of storage and handling of the reagents are scrupulously followed (see section 3). Check reagent expiration dates Check that the validation criteria for controls are met.
Problems collecting, transporting, or storing of the sample	Check that the recommendations of the laboratory and / or the manufacturer of the sampling equipment have been observed. Check the time between the sampling and the analysis.
Potential cause	**Solution**
Problem encountered during the extraction.	Check that the extraction conditions and maintenance operation of the extraction devices have been respected in accordance with the manufacturer's protocol. Validate the results of the extraction control (if it was carried out as recommended). Check that the samples have been homogenized before starting the extraction step.
Error in the implementation	Check the entire test protocol followed against the protocol described in these instructions for use.
Programming error	Check all the programming parameters of the thermocycler as recommended in these instructions for use. Contact info@id-solutions.fr for more details.
Problem during the amplification step	Validate thermocycler compliance.

	Validate all the elements implemented in the reaction (consumables used, properly sealed plate, etc.). Refer to the equipment manufacturer's manual for more details.
Problem of results analysis	Validate the positioning of the positivity threshold Contact info@id-solutions.fr for more details.
Problem interpreting the results	Validate that all validation criteria are met.

PATHOGEN NOT PRESENT BUT DETECTED IN SAMPLES: FALSE POSITIVE

Potential cause	Solution
Contamination	Check the conditions of storage and use of reagents. Check that the validation criteria for controls are met. Validate the absence of contamination of the reagents. Decontaminate equipment and premises.
Error in the implementation	Check the entire test protocol against the protocol described in these instructions for use.
Programming error	Check all of the thermocycler's programming parameters as recommended in these instructions for use. Contact info@id-solutions.fr for more details.
Problem of analysis of results	Validate the positioning of the positivity threshold Contact info@id-solutions.fr for more details.
Problem interpreting the results	Validate that all validation criteria are met.

INHIBITION OF SAMPLES

Potential cause	Solution
Problem encountered during the extraction.	Check that the extraction conditions have been respected in accordance with the manufacturer's protocol. Validate the results of the extraction control (if it was carried out as recommended). Check the validity of the batches of reagent used. Check that the samples have been homogenized before starting the extraction step.

10 LIMITATIONS

- The product is intended to be used only by healthcare professionals who have received instructions and training in real-time PCR techniques related to the thermocycler used and *in vitro* diagnostic procedures.
- Respect good laboratory practices to guarantee the proper functioning of this test. Preserve the purity of the kit components when preparing the samples, which is a source of contamination. Laboratory and reagents must be monitored to prevent them from being contaminated or containing impurities. If a reagent is suspected, the laboratory should proceed with its disposal.
- Do not use this test directly on the patient sample, an adequate nucleic acid extraction method must be used before performing the test. It is important to assess the quality of the extraction step before testing.
- The reagents provided with the kit are ready to use, so no dilution should be made as performance would be affected.
- If the sample or reagents are not used according to the instructions for use, the test result may be incorrect. The repetition of the test in a new sample from the same patient should be considered if Covid-19 is suspected or if an error has occurred during the procedure.
- The procedure and interpretation of the results described for the test must be followed when performing RNA detection in the samples. It is recommended that the user of the kit read the instructions for use carefully before carrying out the test. The test procedure must be strictly followed.
- Obtaining a negative result on samples does not exclude the diagnosis of Covid-19. Samples should be taken regularly from patients with suspected Covid-19 in order to perform tests again.
- Positive results do not exclude bacterial infection or co-infection with other viruses.
- The result of the molecular analysis should not lead to the final diagnosis of SARS-CoV-2 infection but should always be considered in the context of the patient's medical history and symptoms.
- The procedures for collecting, transporting, storing and processing patient samples must be scrupulously observed according to the recommendations given by the manufacturer of systems or kits for the extraction of nucleic acids, in order to ensure optimal performance of the test.
- Potential mutations in the targeted areas of the virus genome recognized by the primers and / or the probes of the test can prevent its detection.

11 TECHNICAL SUPPORT AND DOCUMENTATION

For any questions or technical support, please contact us at the following address: **info@id-solutions.fr.**

12 SYMBOLS USED

The following symbols may appear on the packaging and labelling:

	Contains enough reagents for n reactions
	Batch number
	Reference number
	Positive control
	Expiration date
	This product meets the requirements of European Directive 98/79 EC for *in vitro* diagnostic medical devices.
	*In vitro* diagnostic medical device
	Refer to the instructions for use before any use
	Manufacturer
	Keep away from the sun
	Temperature limit
	Warning

13 LAST REVISION

VERSION	EDITING DATE	REFERENCES	DESCRIPTION OF THE MODIFICATION
0221-2	26/02/2021	DOC767	Addition of use of saliva samples and associated clinical performances

Note:

Note