WUHAN COVID-19

Qualitative Real -Time RT-PCR for detection of SARS-CoV-2

- for “In Vitro Diagnostic” use only -

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REF. WUCO19.CE
25/50/100/150 Tests

WUHAN COVID-19

A. INTENDED USE

The **Wuhan Covid-19** Real-Time RT-PCR kit coded **WUCO19.CE** is intended for the specific qualitative detection of SARS-CoV-2 in human samples (respiratory specimens, sera, whole blood and urine) with a simultaneous control of the extraction/amplification reaction through the RNA sequence of an endogenous Internal Control (IC).

The kit has been adapted for the use on the Real-Time Thermacyclers and ABI 7500 Sequence Detection System® (Software SDS version 1.3.1, Applied Biosystems™*) and CFX96 Real-Time System (Software CFX manager version 1.7, Biorad™**)

* Applied Biosystems is a registered trademark and ABI PRISM® is a trademark of Applera Corporation or its subsisdiaries in the US and/or certain other countries.
** Biorad is a registered trademark.

B. INTRODUCTION

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Respiratory disease caused by the novel coronavirus, first detected in Wuhan City, China, has been named *coronavirus disease 2019* (COVID-19) and the virus has been named SARS-CoV-2.

The SARS-CoV-2 virus is a betacoronavirus, like MERS-CoV and SARS-CoV; they are enveloped, positive-sense, single-stranded RNA viruses of zoonotic origin.

WUCO19.CE RT-PCR assays is able to detect viral RNA reducing sample handling steps, providing fast results and a really low risk of cross-contamination for the sample under evaluation.

C. PRINCIPLE OF THE TEST

The **WUCO19.CE** Kit is based on a Real Time chemistry which uses specific Primers and Probes.

The SARS-CoV-2 RNA**,** recovered from the biological sample under investigation through an extraction step, is retrotranscribed and amplified using the Real Time amplification system. The amplified product is detected using a fluorescent reporter dye probe specific for a SARS-CoV-2 genomic sequence (RdRp region).

Endogenous Internal Control (IC) extracted and amplified meanwhile, serves as an Collection/Extraction/Amplification control for each individually processed specimen aiming the identification of good respiratory sample collection and the eventually identification of reaction inhibitors.

D. COMPONENTS

The standard format of the product code WUCO19.CE contains reagents for 50 tests.

Component	Labelling and Contents	WUCO19.CE 50 Reactions
A **CODED: WUCO19/RA** **COLOR CODE: YELLOW**	Concentrated Reaction Mix	N°2 vials/0.20 ml (to be diluted by adding B)
B **CODED: WUCO19/RB** **COLOR CODE: RED**	Reaction Mix diluent	N°2 vials/1,5 ml
NTC **CODED: ALL/NTC** **COLOR CODE: WHITE**	Negative Control	N°1 vials /1.5 ml
CTRL Positive Control **CODED:WUCO19/CTRL** **COLOR CODE: VIOLET**	Amplification DNA Positive Control	N° 2 vials/0.20 ml
Package Insert	Instruction for Use	1

Important note: Upon request, Dia.Pro can supply reagents for 25, 100, 150 tests, as reported below **:**

1. Component A 2. Component B 3. NTC 4. CTRL 5. Pack. insert	n°1 vial/0.20 ml n°1 vial/1,5 ml n°1 vial/1.5 ml n°1 vial/0,20 ml n°1 vial	n°4 vial/0,20 ml n°4 vial/1,5 ml n°2 vial/1.5 ml n°3 vial/0,2 ml n°1 vial	n°6 vial/0,20 ml n°6 vial/1,5 ml n°2 vial/1.5 ml n°4 vial/0,20 ml n°1 vial
Number of tests	**25**	**100**	**150**
Code	**WUCO19.CE.25**	**WUCO19.CE.100**	**WUCO19.CE.150**

E.STORAGE AND STABILITY

The kit WUCO19.CE must be stored at -15°C/-22°C. The not used reconstituted reaction mix (A+B) must be discard at the and of the test. If the components are to be used only intermittently, they should be frozen in aliquots, repeated thawing and freezing should be avoided. Only two defreezing are allowed.

F. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (0.5 µl < volume <1000 µl)
2. RNA extraction kit
3. MG EtOH
4. PBS
5. Thermal Block
6. Microcentrifuge
7. Tube racks
8. Sterile filtered tips with aerosol barrier
9. Nuclease-Free Microtubes
10. 0,2 ml Microtubes or PCR Microplates recommended from the Real-Time PCR instruments manufacturers
11. Disposable gloves, powder-free
12. Real-Time PCR Thermalcycler (*)
13. Absorbent paper tissues.
14. Vortex or similar mixing tools.

(*) Attention: A valid calibration of the pure dyes (Pure Spectra Component File) and of the background (Background Component File) must be done routinely.

G. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. The technical personnel must be deeply trained in the use of Real-Time thermalcyclers, in the manipulation of Molecular Biology reagents and skilled in the Real-Time PCR amplification protocols.
3. The kit has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
4. All the personnel involved in performing the assay have to wear protective laboratory clothes, powder-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the

National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
5. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
6. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and the Components and when performing the test.
7. Components A is light sensitive. Protect them from strong light exposition.
8. Avoid vibration of the bench surface where the test is undertaken.
9. Upon receipt, store the kit at -15°C/-22°C into a temperature controlled freezer.
10. Do not interchange components between different lots of the kits. It is recommended that components between kits of the same lot should not be interchanged.
11. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
12. Avoid cross-contamination between samples by using disposable tips and changing them after each sample.
13. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
14. Do not use the kit after the expiration date stated on the external container label.
15. Treat all specimens as potentially infective. All human specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
16. Store and extract specimens separately from the other reagents and use a separate room for their handling
17. Carry on all the working operations as quickly as possible maintaining the components on ice or in a cooling block.
18. The laboratory Workflow must proceed in an unidirectional way, beginning in the Extraction Area and moving to the Amplification and Data Analysis Areas. Do not return samples, equipment and reagents to the area where previous steps have been performed.
19. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring of components into automated workstations, in order to avoid cross contamination.
20. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from sample extraction procedures, has to be treated as potentially infective material and inactivated before waste. Do not put in contact the extraction waste with bleach.
21. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
22. Other waste materials generated (example: tips used for samples) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

H. SPECIMEN: PREPARATION AND RECOMMENDATIONS

For initial diagnostic testing for COVID-19 the Center for Disease Control, Atlanta, U.S. (CDC) recommends collecting and testing **upper respiratory** (nasopharyngeal and oropharyngeal swabs), and **lower respiratory** (sputum, if possible) for those patients with productive coughs. Induction of sputum is not recommended. Specimens should be collected as soon as possible once a Person Under Investigation (PUI) is identified, regardless of the time of symptom onset.

1.Collect 2-3 ml bronchoalveolar lavage and tracheal aspirate sample into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C (≤ 48h).
2. Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C(≤ 48h).
3.For Nasopharyngeal (NP) and Oropharyngeal (OP) specimen use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media. NP and OP specimens should be kept in separate vials. Refrigerate specimen at 2-8°C (≤ 5 days).
4.Collect 2-3 ml nasopharyngeal wash/aspirate or nasal aspirate into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8°C (≤ 48h).
5.Avoid any addition of preservatives to samples.
6. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
7.Samples who want to be stock for longer period must be store at -70°C. Avoid repeated freezing / thawing cycles.
8.When using frozen samples, thaw the samples just before the extraction in order to avoid cases of nucleic acid degradation.
9.Serum samples collection (acute sample and convalescent sample) are recommended for further serological test.
10.All specimens collected for laboratory investigations should be regarded as potentially infectious.
11.The Health Care Workers (HCWs) who collect or transport clinical specimens should adhere rigorously to infection prevention and control guidelines and national or international regulations for the transport of infectious substances to minimize the possibility of exposure to pathogens.

I. PREPARATION OF COMPONENTS AND WARNINGS

WUCO19/RA:
Component A. Mix well by inverting before use and centrifuge briefly to collect the whole volume.

WARNING: Component A is light sensitive. Protect it from strong light exposition.

WUCO19/RB:
Component B. Ready to use

Negative Control :
NTC. Ready to use.

WUCO19/CTRL:
Component CTRL. Mix well by vortexing before use and centrifuge briefly to collect the whole volume

L. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-5%.
2. **Extraction Device:** The WUCO19.CE Kit is intended to be used in combination only with QIAamp Viral RNA Mini kit Coded:52904 (QIAGEN) and NucleoSpin Virus Kit Coded: FC140983 (Macherey-Nagel).The end users must strictly follow the Instruction for use supplied by the manufacturers.
3. **Real-Time Thermocyclers.** The WUCO19.CE Kit is intended for the use in combination only with the Real Time Thermal cyclers ABI 7500, software SDS version 1.3.1 (Applied Biosystems), and CFX96 Real-Time System, Software CFX manager version 1.7, Biorad™

4. The end users must strictly follow the Instruments Instruction for use supplied by the manufacturers.

M. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the components are not contaminated by naked-eye visible particles or aggregates. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box.
3. Turn the Thermalcyclers on, check settings and be sure to use the right assay protocol.
4. Follow strickly the Instruments Manual supplied by the manufacturers for the correct setting of the Real-Time Thermalcyclers.
5. Check that the micropipettes are set to the required volume.
6. Check that all the other equipment is available and ready to use.
7. In case of problems, do not proceed further with the test and advise the supervisor.

N. ASSAY PROCEDURE

The assay has to be carried out according to what reported here below.

N.1 RNA extraction

The extraction step of the Wuhan COVID-19 genomic RNA has to be carried out exclusively in combination with the following kits:

Material	Description	Kit code	manufacturer
Respiratory specimens; serum; whole blood; urine	Nucleospin Virus kit	FC140983	MN™
	QIAamp Viral RNA mini kit®	52904	Qiagen™

The RNA isolation must be carried out only according to the Instruction Manual supplied by the Manufacturer (QIAGEN™; MN™).

***WARNING**: The following volumes have to be strictly used in the NucleoSpin Virus extraction procedures (MN™):*

Sample Extraction volume : 400 µl
Elution Volume: 60 µl

The RNA collected from the samples, not used in the run , has to be stored frozen (-18°C/-22°C).

Important note:
The Internal Control Ct value for the negative sample is used to evaluate if the sample collection and the RNA extraction procedures have been performed correctly (see section Q).

N.2 Setting up of the reaction

The WUCO19.CE Kit is intended for the use in combination only with the Real Time Thermal cyclers ABI 7500, software SDS version 1.3.1 (Applied Biosystems), and CFX96 Real-Time System, Software CFX manager version 1.7, Biorad™

N.2.1 Preparing the RT-PCR

***Important:** An example of dispensation scheme is reported in Section O. Please, refer to it before starting the operations described here below.*

- Prepare the components as described in Section I;
- Prepare the required number of reaction tubes or a 96-well reaction plate for the samples under evaluation and for the Positive control (prepared as described in section I).

***Important note:** Use only optical tubes or microplates suggested by the Real-Time thermalcyclers manufacturers.*

- Consider that the samples, if possible, should be tested in duplicate;
- Include at least 1 tube for the NTC (negative control)
- Prepare the **Reaction Mix** for **Samples, NTC and positive control (CTRL)** as table below:

Preparation of the Reaction Mix

Number of Reactions		x1	x10
A	Concentrated Reaction Mix	7.0 µl	70 µl
B	Reaction Mix diluent	8.0 µl	80 µl
Tot vol.		**15 µl**	**150 µl**

- Dispense 15 ul of the amplification mix in each reaction tube or microplate well
- Add 10 ul of the **Samples, NTC, CTRL** to the reaction tubes.
- Close firmly the reaction tubes
- Centrifuge briefly the reaction tubes at 2000 rpm
- Don't leave the reaction tubes at room temperature (RT) for more than 30 minute and at light exposure (cover the tubes).
- Load the reaction tubes in the Real-Time Thermacycler Thermoblock Holder.
- After the setting operations described in the Sections N3 (Instrument Programming) start the Thermacycler run.

Important note:
The not used volume of reconstituted reaction mix (A+B) must be throw away. CTRL can be freeze at -15°C/-22°C and used as described in Section E.

N.3 Instrument programming

For programming the instrument refer to the Instrumentation Instruction Manual provided by the manufacturers.

N.3.1 RT-PCR Thermal Profile

The thermal profile is reported in the table below:

Step	Cycle	Temp.	Time
1	1	45°C	20 min
1	1	95°C	10 min
2	50	95°C	15 sec
		58°C **(*)**	45 sec

IMPORTANT NOTE: (*) step for the real time data collection.
WARNING: Keep attention to set up the Real-Time Thermacycler with the correct Thermal Profile following the Instrument Manual supplied by the Instrument manufacturer.

N.3.2 Selection of the Detectors

Following the Instruction manuals of the Real-Time thermalcyclers suggested (CFX96 and ABI7500) select the Detectors reported in the table here below:

Detection	Reporter	Quencher
COVID-19	FAM	Non Fluorescent
Internal Control (IC)	JOE/VIC	Non Fluorescent
Passive Reference (only for ABI7500)	ROX	

WARNING: Keep attention to set up the Real-Time Thermacycler with the correct settings following the Instruments Manual supplied by the manufacturer.

O. ASSAY SCHEME

An example of dispensation scheme for the Analysis is reported here below:

Microplate or tubes

	1	2	3
A	**CTRL**		
B	NTC		
C	Sample 1		
D	Sample 2		
E	Sample 3		
F	Sample 4		
G	Sample 5		
H			

Legend: NTC = Negative Control CTRL = Wuhan COVID-19 DNA Positive Control, Sample 1,2,3 = Samples under evaluation.

P. INTERNAL QUALITY CONTROL

P.1 Pre - Analysis Settings

Before starting the analysis:

- Set the "Baseline" (the background fluorescence level) as reported below:

"Baseline"	
ABI™PRISM® 7500 SDS	**Auto Baseline**
BIORAD™ CFX96®	**Auto Calculated Baseline**

- Set manually the FAM/JOE/VIC fluorescence "Threshold"

FAM fluorescence "Threshold"	
ABI™PRISM® 7500 SDS	**0.1**
BIORAD™ CFX96®	**200**

JOE/VIC fluorescence "Threshold"	
ABI™PRISM® 7500 SDS	**0.1**
BIORAD™ CFX96®	**100**

P.2 Data analysis

A check is carried out on the Positive Control any time the kit is used in order to verify whether their Ct values are as expected and reported in the table below.

Check	**Requirements**
CTRL	30 ≤ Ct (Threshold Cycle) ≤ 33

Q. INTERPRETATION OF THE RESULTS AND TROUBLESHOOTING

For each samples the FAM fluorescence (positive/negative Ct values) and the Internal Control JOE fluorescence are assumed to validate SARS-CoV-2 RNA extraction and detection as described in the table below:

COVID-19 - FAM	**Internal Control - JOE**	**Assay Result**
SAMPLE POSITIVE	+	CORRECT
	-	CORRECT*
SAMPLE NEGATIVE	Ct < 40	CORRECT
	Ct > 40 or undetermined	INVALID**

*High Initial concentration of SARS-CoV-2 RNA in the sample (Positive FAM Signal) can lead to a REDUCED or an ABSENT Fluorescent Signal for the Internal Control IC due to the reagents Competition.

** Problems may be occurred during the extraction step (presence of inhibitors or initial sample containing an insufficient number of cells) or during the amplification step (inefficient or absent retrotransciption/amplification) leading to an incorrect result.

The test procedure must be repeated starting from the Extraction step using a fresh sample coming from the patient.

The results obtained with this product must be interpreted taking consideration of the clinical symptoms and the other laboratory parameters related to the patient conditions.

The following results are possible:

Troubleshooting table

	FAM	**JOE**	**Result**	**CHECK**
SAMPLE unknown	+	+/-	CORRECT RESULT *Positive*	***IMPORTANT***: High Initial concentration of SARS-CoV-2 RNA (Positive FAM Signal) can lead to REDUCED or ABSENT Fluorescent Signal of Internal Control I.C. due to the reagents Competition.
SAMPLE unknown	-	-	ATTENTION ! POSSIBILITY OF: Inhibition, error in the procedure or misfunctioning of the Instruments	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3. that the selected detection dyes are corrected FAM for the SARS-CoV-2 detection and JOE for the I.C. detection; 4. that the Analysis has been run with the correct Instrument settings; 5. that the kit has been stored correctly; 6. that no potential PCR inhibitors have been contaminated the tube 7. that the Extraction procedure have been executed correctly;
SAMPLE unknown	-	+	CORRECT RESULT *Negative*	
CTRL	+	-	CORRECT RESULT	***IMPORTANT***: NO Internal Control I.C. signal on CTRL because endogenous control.
CTRL	-	-	ATTENTION ! POSSIBILITY OF: Error in the pipetting or in the procedure	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3. That the FAM dye is selected for the SARS-CoV-2 detection 4. that the Analysis has been run with the correct Instrument settings; 5. that the kit has been stored correctly; 6. that no potential PCR inhibitors have been contaminated the tube
NTC	-	-	CORRECT RESULT	***IMPORTANT***: NO Internal Control I.C. signal on NTC because endogenous control.
NTC	+	+/-	ATTENTION ! POSSIBILITY OF: Contamination	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3.that the work space and Instruments are decontaminated at regular intervals; 4. that the kit has been stored correctly;

If one of more of the problems described in the table above happen, after checking, report any residual problem to the supervisor for further actions.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to an informatics centre, attention has to be paid to avoid erroneous data transfer.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Internal Technical Specifications or ITS.
The performance evaluation was carried out in DiaPro's laboratories on materials supplied by the reference clinical labs.

R.1 ANALYTICAL SENSITIVITY

Analytical sensitivity may be expressed as **Limit of Detection**.
Limit of detection (LOD): it is the lowest amount of target that can be detected by the system with a stated probability.
For the NAT tests it is expressed as the smallest concentration of the **analyte** that tested in multiple repetitions gives a positive result.
The **limit of detection (LOD)** is determined by testing serial dilutions containing known concentrations of a Synthetic specific RNA sequence.
The **LOD** is the lowest concentration of analyte that can be consistently detected (e.g. in $\geq$ 95% of samples under routine laboratory conditions).
The results were analized by a **Probit** analysis, to determine the detection limit at 95%.

The results are the following:

Detection Limit (p=0.05)	
ABI™PRISM® 7500 SDS	
BIORAD™ CFX96®	

R.2 ANALITYCAL SPECIFICITY

The Analytical specificity is the ability of the method to detect only the target RNA sequence.
The analytical specificity of Wuhan SARS-CoV-2 RNA assay has been studied as follow:

1. The primer/probe Set has been choose analysing the genome target sequences with appropriate software (BioEdit Sequence Alignment Editor, Oligo Analyzer and Primer Express v.3.0" supplied by Applied Biosystem Inc.).
2. The primer/probe Set and the target genome sequence has been controlled by the "BLAST" software, in order to check if any of the nucleotide sequences deposited in the worldwide genomic banks has any homology with Wuhan Covid-19, and by the "ClustalW" software, in order to compare the genome target sequences of the different genotypes of Toxoplasma.
3. The specificity was improved through the selection of stringent reaction conditions.

R.4 PRECISION

Precision shows the degree of the system's reliability. Every measurement procedure has an inherent random variation called "random error". Random error does not have a number value but it is determined by dispersion of measurement as standard deviation (DevST) and coefficient variation (CV%). Usually precision of an assay refers to the agreement between replicate measurements of the same material.
In the kit WUCO19.CE, **precision** was expressed as intra-assay variability and inter-assay variability.
8 replicates of the CTRL-H and CTRL-L were tested in the same run (intra-assay) and in three different runs (inter-assay).
On the basis of the results obtained Intra and inter-assay variability were then calculated.

In absence of established International parameters we have identified the following value of acceptability for the WUCO19.CE Kit:
Intra-Assay Coefficient Variation (CV%) ≤ 10%.
Inter-Assay Coefficient Variation (CV%) ≤ 10%.

S. LIMITATIONS

The user of this kit is advised to carefully read and understand this package insert. Strict adherence to the protocol is necessary in order to obtain reliable test results. In particular, accurate sample and reagent pipetting, application of a correct workflow along with careful programming of thermalcycling steps are essential for an accurate and a reproducible SARS-CoV-2 RNA detection.
The SARS-CoV-2 RNA determination in a patient sample has extensive medical, social, Psychological and economical implications.

Detection of a possible human case of emerging pathogen causing severe acute respiratory disease should be immediately notified to local and national public health authorities. In line with the International Health Regulation (IHR) 2005 the national health authority must notify WHO within 24 hours of all events that may constitute a public health emergency.

It is recommended that confidentiality, appropriate counselling and medical evaluation be considered as an essential aspect of the testing sequence.

T. BIBLIOGRAPHY

1. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. V.M. Corman et al. www.eurosurvellance.org
2. Real-Time RT-PCR panel for dection 2019-novel coronavirus. Center for disease control and prevention (CDC).
3. Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR. HKU ; LKS faculty of medice school of public health.
4. Interim guidelines for collecting, handling and testing clinical specimens from person under investigation (PUIs) for coronavirus disease 2019 (COVID-19). Center for disease control and prevention (CDC).
5. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. World Health Organization (WHO) 17th of January 2020.
6. Polminite da nuovo coronavirus (2019-nCoV) in Cina. 0001997-22/01/2020-DGPRE-DGPRE-P. Ministero della Salute, direzione generale della prevenzione sanitaria.
7. Development of a quantitative assay for SARS coronavirus and correlation of GAPDH mRNA with SARS coronavirus in clinical specimens. S.C.C. Wong et al.; J Clin Pathol 2005;58:276-280.

5. Symbols

LEGENDA			
REF	Product code	t	Storage temperature
IVD	In Vitro Diagnostic Device		See use instructions
LOT	Lot number		Manufacturer
	Expiry date	s	Number of tests
0	CE conformity mark	m	Date of manufacturing

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