Viga Sars-CoV-2 Molecular Diagnostic Kit,

One-step Multiplex RT-PCR Assay

Molecular SARS-CoV-2 (Covid-19) Diagnostic kit based on Real-Time-PCR

For In Vitro Diagnostic Use

BY ROJE

Edition, 01/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality, affordable Molecular and diagnostic Kits and reagents comparable to the world leaders to research centers, laboratories, clinics, hospitals, and diagnostic centers all over the world.

Contents

Kit Content

Components	100 Preps
Q-ROMAX, 4X	500µl
ProI Mix	400µl
RTase, Recombinant Reverse Transcriptase, RNase H-(200 U/µl)	100µl
Positive Control	150µl
Negative control	150µl

Introduction

It was the last days of 2019 when a novel strain of Coronavirus caused a pneumonia epidemic worldwide. Since the number of people who died from Covid-19 exceeded 1000, the WHO named the disease caused by SARS-CoV-2, COVID-19. The virus was initially termed 2019-nCoV (novel Coronavirus) and renamed SARS-CoV-2 by the "International Committee on Taxonomy of Viruses." It was the first time this kind of virus-infected human beings massively. Based on the first estimations, the death rate for SARS-CoV-2 was 2 to 3%. The most common symptoms of infectors include fever, dry cough, and respiratory system problems such as shortness of breath or severe breathing difficulty, sore throat, and runny nose. Although the first cases of covid-19 were diagnosed in a seafood market in the Wuhan region, China, and the first assumption suspected publics to the animal to human transmission, the World Health Organization considered it human-to-human transmission.

Storage

ROJETechnologies checked shipment conditions. After arrival, all reagents should be kept in darkness, at -20 ±5ºC. Do not freeze-thaw the Kits frequently. When storage condition is as directed, all reagents are stable until the expiration date, as indicated on the kit box.

Intended Use

Viga Sars-CoV-2 Molecular Diagnostic kit is based on a reverse transcription-PCR assay specific to detect the virus and its detection by Real Time-PCR. This Kit is intended for the qualitative detection of nucleic acid from SARS-COV-2 in samples of a nasopharyngeal swab, oropharyngeal (throat) swab, anterior nasal swab, nasal washes, and nasal aspiration from people of suspected COVID-19 by their health care provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendment. Based on the Ministry of Health rules and guidelines, the selling of COVID-19 diagnostic kits is just being allowed to the licensed laboratories. The presented kit is intended for emergency use and for In Vitro

Diagnostic use (IVD). This test also is used for identifying the genome of SARS-COVID-2. The genome of SARS-COVID-2 is recognizable in samples of patients with an acute viral infection. The positive result shows the presence of the genome of SARS-COVID-2; the clinical history of patients and other medical conditions is needed for accurate diagnosis. It should be noted that Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of the disease. Laboratories must report all positive cases to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used solely as for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Guarantee & Warranty

ROJETechnologies guarantee the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any of the products do not meet your satisfaction, please contact our technical support team for reasons other than misuse. If the problem is due to the manufacturing process, the ROJE team will replace the kit for you.

Notice to Purchaser

This product is only for experimental and not for commercial use of any kind. There is no right to resell the kit or any of its components. For information about our licensing or distributors, contact the ROJE business team.

Warning and Precautions

Material Safety Data Sheet (MSDS) for all products and reagents are accessible online on www.rojetechnologies.com.

Dear users, please follow laboratory safety rules.

Please study the guideline precisely before use.

Notice that all samples of patients and positive controls are potentially infectious.

- Avoid eating, drinking, smoking, chewing gum, applying cosmetics, and taking medicine during working with hazardous materials and human samples in laboratories. Consider all samples of patients and positive control potentially infectious.
- Before starting, study all safety guidelines in relation to the sample of COVID-19 on this website https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html .

- The Viga Sars-CoV-2 Molecular Diagnostic kit is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in real-time PCR and in vitro diagnostic procedures. Each step of procedures such as sampling, storage, shipping, and laboratory tests must follow biosafety and laboratory information management systems (LIMS).
- Due to this test, a separate and private workstation in a laboratory is needed:
    - Place 1: preparation area- the components of test preparation.
    - Place 2: sample processing- isolation and controlling are done.
    - Place 3: amplification area where Real Time-PCR test is done.
- The clinical laboratories must be equipped with instruments and operators following the guidelines of the Ministry of Health.
- Provided kit contents are specific to test COVID-19; changing or replacing any kit content may compromise the product performance.
- Before starting the tests, each component must be melted, vortexed, and centrifuged briefly. Avoid repeated freeze-thaw cycles.
- All pipette tips and microtubes must be sterile and DNase-RNase-free. To avoid contamination, filter pipette tips are needed. Change pipette tips after adding any substance or samples.
- Dispose of hazardous and biological waste only in compliance with local and national regulations to avoid environmental contamination. Use decontaminants like Sodium hypochlorite %10, ethanol %70, and RNZO in nucleic acid contaminations. Avoid putting the combination of PCR-COVID 19 in exposure to sunlight.
- Positive results should be reported to the health authorities.

Quality control

According to clinical and laboratory standards institute and WHO, Viga Sars-CoV-2 Molecular Diagnostic kit is tested against predetermined experiments on a lot-to-lot basis to ensure consistent product quality. For more information about the results, enter the labeled REF and LOT number in the "certificate of analysis" on www.rojetechnoloes.com.

Procedure

This kit is designed for qualitative diagnosis of RdRp genes and N of SARS COVID-19 in respiratory specimens. After isolation by RNJia Virus Kit (Cat No: RN983072) or other commercial kits approved by the Ministry of Health and verified sample collection procedure, viral nucleic acid should be added to the master mix primer/probe RTase enzyme to perform the reaction. Internal control targets the human RNase gene by which quality of sample

collection, nucleic acid isolation, and the whole process of RT-PCR reaction can be checked and controlled to avoid false-negative results. The lowest sensitivity of the present kit is 100 copies per ml.

Types of controls

Negative control: A "no template" (negative) control is used to monitor contamination for the RT-PCR process and is used in each detection run.

Positive control: A positive template control monitors whether the RT-PCR process works properly and is used in each detection run.

Internal control (RNase P): An internal control for the RNase P gene is used to monitor the sample collection, handling, and RT-PCR process and is used in each sample amplification.

Equipment & Reagents to Be Supplied by User

- You need a Nylon or Dacron swab with an aluminum or plastic shaft.
- DNase-RNase-free microtubes for sampling (1.5ml)
- PCR microtube 0.1ml or 0.2ml strip
- Various models of pipette and pipette tips (10µl, 100µl, and 1000µl of filter pipette tips)
- Surface sanitizing solution like RNZO (Cat No: RN983048)
- Disposable Powder- Free gloves and surgical gown
- Different types of Real-Time PCR Instruments (with green, yellow, and orange channels)
- Centrifuge (can reach 13000 rpm)
- Microcentrifuge
- Vortex
- Coolbox

Real-Time PCR instruments

This kit can be run on the following instruments:

- Rotor-Gene Q, 5plex
- Corbett Rotor-Gene 3000&6000
- Mic qPCR Cycler
- ABI StepOne & StepOnePlus
- Biorad CFX96 Real-Time PCR
- Roche LightCycler® 96 Real-Time PCR System

- Anatolia Montania 484 Real-Time PCR Instrument

Applications

The Viga SARS COV-2 Molecular Diagnostic Kit is a Real-Time reverse transcription-polymerase chain reaction (rRT-PCR) test. The 2019-nCoV primer and probe sets are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients suspected of COVID-19 by their healthcare provider. This kit is used to detect the RdRp and N genes of SARS-CoV-2 RNA qualitatively. By one simple step of centrifugation and lysis, the sample mixture can be directly added to the 2019-nCoV-PCR master mix (Q-ROMAX+ RTase+ ProI) to carry out rRT-PCR amplification. RNJia Virus Kit (Cat No: RN983072) can be used as an alternative extraction method. Internal control targets the RNase P gene monitor the sample collection, sample handling, and rRT-PCR process to avoid false-negative results. The LoD of the kit is 100 copies/ml.

Sample Storage and Preparation

specimen collection

Viga Sars-CoV-2 Molecular Diagnostic kit is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens. The collection should avoid possible contamination in collection, storage, and transportation. The specimen should be presumed contagious and be handled according to relevant regulations. Consider potentially infectious samples and transfer the samples under the biosafety guidelines. The collection swab should have a synthetic tip, such as nylon or Dacron, and an aluminum or plastic shaft. A cotton swab with wooden shafts is not recommended at all. After sampling, swabs should be stored in a proper virus transport medium immediately.

storage and delivery of specimens

The specimen should be tested within 24 hours if stored at 4ºC. Samples that cannot be tested within 24 hours should be stored at -70ºC or below (in the absence of -70ºC storage condition, specimens can be stored at -20ºC for ten days, the nucleic acid can be stored at -20±5ºC for 15 days). Multiple freeze-thaw cycles must be avoided.

specimen isolation

For viral nucleic acid isolation use the RNJia Virus Kit (Cat No: RN983072) or other kits with confirmation from the ministry of health.

Pathogenicity

Considering the type of Coronavirus, symptoms can cause illnesses ranging from the common cold to more severe diseases like fever, cough, shortness of breath, and breathing difficulties.

It can also cause a cough that lasts for a few days, evidently for no reason. MERS-COVID, contrary to SARS COVID, damages the respiratory system and vital organs like kidneys and liver. Digestive disorders like diarrhea, severe acute respiratory syndrome, coagulopathy, and renal failure have been reported in acute cases. Patients with these symptoms may need Hemodialysis.

A new version of coronavirus that leads to COVID-19 typically appears a few days after exposure to the virus. But in some cases, symptoms appear a little later. According to statistics and research that have been conducted, symptoms can include fever (in 43.8% of cases upon hospital admission and in %88.7 of cases when hospitalized), dry cough (67.8% of cases), respiratory disorder, fatigue, and, myodynia (in 11 to %14 of patients) diarrhea (in %3.8 of cases). The average incubation period for COVID-19 is four or five days. About %56.4 of ground-glass opacity cases (in their incubation period) are diagnosed in chest CT scans. %17.9 of patients experiencing mild symptoms, and %2.9 patients with severe symptoms haven't shown up any problems in their radiology or chest CT scan. In %83.2 of patients, Lymphopenia or the decreased number of Lymphocytes in blood circulation during hospital admission has been reported. Some people experience mild or even no symptoms, while in some other cases, COVID-19 can cause serious problems such as Pneumonia or hypoventilation that might lead to death. Patients with an underlying medical condition are at greater risk.

How is coronavirus transmitted?

The disease is caused by the SARS-CoV-2 virus, which spreads differently. In some cases, the ways of human-to-human transmission of Coronavirus are similar to Flu. Both viruses are spread mainly by airborne particles made when people cough or sneeze. However, being outdoors is a lower risk of Coronavirus transmission than indoors. Human-to-human transmission can occur when people stay indoors for a long time with close contact with infected cases like hospitalized COVID patients. There is still no evidence whether the first transmission of COVID-19 occurred from animal-to-human or contaminated surfaces.

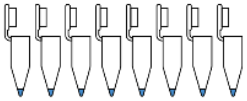
Workstation preparation

All work surfaces, pipettes, centrifuges, and other supplies must be cleaned and sanitized before use. Use sanitizers like %70 Ethanol or %10 Sodium Hypochlorite to minimize the risk of nucleic acid contamination.

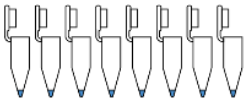
protocol

Step 1:
Isolated nucleic acid

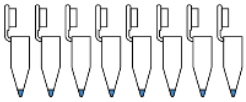
Step 2:
Add molecular biology grade water



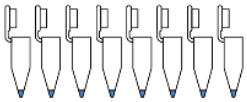
Step 3:
Add Q-ROMAX, 4



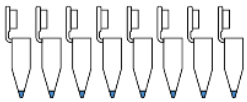
Step 4
Add pro I mix



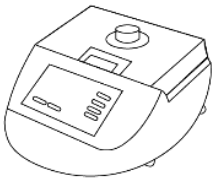
Step 5:
Add RTase enzyme



Step 6:
Add isolated nucleic acid



Step 7:
Run the PCR program



Step 8:
Interpret the PCR results

Results	Cq HEX (IC)	Cq Texas Red (N)	Cq FAM	
A	+/-	Ct<40	Ct<40	Covid 19 positive
A	+/-	Ct<40	-	Covid19 positive
A	+/-	-	Ct<40	Covid19 positive
R	-	-	-	False Negative
A	-	-	-	Negative Control
A	Ct<40	-	-	Negative clinical sample

Figure 1: preparation of reagents, the addition of isolated DNA, PCR run, and interpretation of results.

process

Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate to room temperature, then briefly vortex each for later uses. The whole volume of the isolated sample in this test should be 10µl. Based on table 1, prepare the reaction components (Master Mix) and perform Real Time-PCR according to table 2.

Table1: Regent's preparation per one single reaction.

Components	Volume
Q-ROMAX	5µl
RTase, Recombinant Reverse Transcriptase, RNase H- (200 U/µl)	1µl
Pro I Mix	4µl
Isolated RNA	10µl

Table 2: PCR program for one-step Multiple Real Time-RT-PCR.

Step	time	Temperature	Number of cycles
cDNA synthesis	20min	50ºC	1
Polymerase enzyme activation	3min	95ºC	1
Denaturation	10s	95ºC	45 cycles
Annealing and extension of nucleic acid and measurement of fluorescence in green, yellow, and orange channels	40s	60ºC	

Interpretation of clinical results

- Data analysis for each gene should be performed separately by using a manual threshold.
- The threshold for each sample should be in the exponential phase of the fluorescence curves and above any background signal.
- FAM Fluorophore (green) for the RdRp gene, Texas Red Fluorophore (orange) for the N gene, and HEX Fluorophore (Yellow) for the RNase P gene (internal control).
- A negative control is used as contamination control. The magnitude increase of the Fluorescence curve in the negative control does not cross the threshold. If Ct is less than 35 (Ct<35), it is considered possible contamination. Strong signals above 35 in

the NTC can be PCR artifacts, and in these cases, the shape of the curve can be considered (the S-shaped curve is typical for a positive result).

- Internal control or RNase P gene should be positive for all clinical specimens at Ct 35 or less than 35, indicating sufficient nucleic acid from the human RNase gene and the sample has acceptable quality.
- Internal control curve or RNase P gene Ct>40 or without Ct indicates low sample concentration or inhibitors in the reaction (the isolated sample is recommended to dilute at least ½). If the test result is not acceptable again during the retest, another new sample should be taken from the patient, and the test must be repeated.
- A positive clinical specimen should have Ct≤40 for genes or have two positive genes.
- If the expected positive reaction is not achieved (typical S-shaped curve), the performed test is not acceptable, and the test must be repeated by following the kit instructions accurately.
- Determine the reason for the failure of positive control, take the corrective action, and document corrective action results.

Table 3: Control conditions for a valid PCR Run

RdRp (FAM)	N (Texas Red)	IC (HEX)	Results
+	+	Not considered	**Positive control***
+	-		
-	+		
-	-	+	**Negative control**
-	-	-	**Invalid and not accepted**

Result of (-): Ct value >40 or Undetermined, Result of (+): Ct value ≤ 40*

Test limitations

- A false-negative result is obtained in case of low titration of virus in the patient sample, improper transportation, and lack of quality of sample isolation.
- Coronavirus genome is a type of RNA that shows significant genetic diversity. Although attempts have been made to design primer or probe for viral genome-protected areas, this genetic diversity can cause a poor primer/probe binding to the target sequence, leading to false-negative results.
- All controls must be checked before the interpretation of the results. If the controls are not valid, the patient's results cannot be interpreted. The diagnostic limit of this

kit equals Ct≤40, and also, the user must review the fluorescence curve before final interpretation. All positive curves must have an amplification peak.

- Non-observance of proper storage conditions of the kit can lead to false-negative results.
- Handling this kit needs experienced and trained personnel. Any personnel error may lead to invalid results.
- The results of this diagnostic kit are only acceptable if they are along with clinical evidence for diagnosing SARS-COV-2. Definitive diagnosis and treatment of patients must be based on a combination of this test with other test results, medical records, and how to respond to treatment. The final concentration of positive samples for N and RdRp is in the table below.

Performance evaluation

A standard sample preparation

RNA was isolated from a sample contaminated with SARS-COV-2 (200000 copies per ml). From the same sample, serial dilutions of 20, 200, 2000, and 20000 copies per ml were prepared. Using Novel Coronavirus (2019-nCOV) Nucleic Acid Diagnostic Kit (Sansure Biotech Inc.), the average of three replicates was set for each dilution using the Real time-PCR (Rotor-Gene Q-Qiagen). The final concentration of positive samples for N and RdRp is in the table below.

Table 4: Average Ct results of Sansure Biotech Novel Coronavirus (nCOV-2019) Nucleic Acid Diagnostic Kit detecting N gene and RdRp gene.

Target genes	concentration (copies/mL)	Average of Cts three repeats	Average of Cts three repeats for a limit of detection in Kit (200copies/mL)
N	200000	22.9587	**36.17**
	20000	26.61745	
	2000	30.65462	
	200	36.17117	
	20	38.99604	
RdRp	200000	24.41095	**37.21**
	20000	27.98925	
	2000	31.68987	
	200	37.21188	
	20	38.9026	

The same dilutions were evaluated by Viga Sars-CoV-2 Molecular Diagnostic kit, and the results are summarized in Table 5:

Table 5: Average Ct results of Viga Sars-CoV-2 Molecular Diagnostic kit.

Target genes	concentration (copies/mL)	Average of Cts three repeats	Average of Cts three repeats for a limit of detection in Kit (200copies/mL)
N	200000	24.00423	35.32749
	20000	27.65744	
	2000	31.82054	
	200	35.32749	
	20	Undetermined	
RdRp	200000	22.97669	34.46268
	20000	26.91568	
	2000	30.91477	
	200	34.46268	
	20	Undetermined	

Limit of Detection (LoD) - Analytical Sensitivity:

LoD studies were used to determine the lowest detectable concentration of SARS-CoV-2 RNA at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples. Preparation of the manufacturer's standards: RNA was extracted from a National Standard for SARS-CoV-2 RNA (Pasteur Institute of Iran) for Nucleic Acid Amplification Techniques and from a 1:100000 dilution of the same specimen using the RNJia Virus Kit (Cat No: RN983072). The RNA concentration of the neat and diluted specimen was determined by the median value of three replicates using Viga SARS COV-2 Molecular Diagnostic Kit. The final concentration of the positive sample was set as 1×10^6 copies/mL using the median value of RdRp gene and N gene.

LoD with Clinical Specimen

The LoD of the Viga SARS COV-2 Molecular Diagnostic Kit was estimated by testing standardized dilutions of the positive specimen. Serially diluted to 200 copies/mL, 100 copies/mL, and 50 copies/mL copies/mL (n= 3 each) using negative specimen matrix (a negative oropharyngeal swab specimen). The lowest concentration at which all three replicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing 20 replicates with concentrations at the tentative detection limit. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of 19 out of 20 replicates.

The LoD of the Viga SARS COV-2 Molecular Diagnostic Kit was established using RNJia Virus Kit (Cat No: RN983072). The results demonstrated that the LoD of the assay is 100 copies/mL.

LoD Detection Results of 2019-nCoV Using RNJia Virus Kit (Cat No: RN983072)

Table 6: Analytical sensitivity and Limit of Detection of Viga Sars-CoV-2 Molecular Diagnostic kit

No	Concentration (Copies/mL)					
	RdRp gene			N gene		
	200	100	50	200	100	50
1	37.33546	36.56248	Undetermined	36.7778	36.01872	Undetermined
2	35.75644	37.2996	Undetermined	34.97221	35.72504	Undetermined
3	36.15275	36.45737	40.01379	35.50759	35.77135	39.49453
4	36.12224	37.92434	39.78859	35.45836	37.33982	39.17679
5	35.43289	36.67382	Undetermined	34.66985	35.91962	Undetermined
6	36.18688	36.99414	Undetermined	35.50755	36.35858	Undetermined
7	35.79122	37.54021	Undetermined	35.2	37.05488	Undetermined
8	35.92614	36.86021	Undetermined	35.28196	36.22875	Undetermined
9	35.81124	37.07031	Undetermined	35.20401	36.47231	Undetermined
10	35.73511	37.16536	Undetermined	34.94667	36.53217	Undetermined
11	35.88995	36.75371	40.21012	35.23834	36.22711	39.60618
12	36.50063	37.4638	Undetermined	35.73087	36.7232	Undetermined
13	36.46613	37.22571	Undetermined	35.92348	36.61359	Undetermined
14	35.73911	36.62986	Undetermined	35.17391	36.03484	Undetermined
15	36.45601	37.04602	Undetermined	36.02764	36.44575	Undetermined
16	36.0982	37.04296	Undetermined	35.44752	36.35696	Undetermined
17	36.52337	37.11725	40.11924	35.36263	36.82512	39.93521
18	35.43264	37.159251	Undetermined	35.62663	37.062543	40.16625
19	35.735532	36.271552	40.03261	35.72521	36.24283	39.96241
20	36.639926	37.623919	39.76625	35.76523	36.762191	40.02371
Call rate	100%	100%	30%	100%	100%	35%

Inclusivity (analytical sensitivity)

Inclusivity of the primer/probe set used in the Viga SARS COV-2 Molecular Diagnostic Kit was analyzed in silico based on SARS-CoV-2 sequences from NCBI (3612 sequences) database accessed on August 2, 2020. The primer/probe sets for RdRp gene and N gene sequencing

alignment analysis demonstrate %100 inclusivity for SARS-CoV-2 sequences identified from patient samples.

Table 7: Representative results of In Silico Analysis for Coronavirus (2019-nCOV) primers/probe against the reported 2019-nCOV sequences in NCBI Site.

Strain	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
SARS-CoV-2/Felis catus/USA/TAMU-078/2020	N gene	MW263337.1	100	100	100
SARS-CoV-2/Canis familiaris/USA/TAMU-077/2020	N gene	MW263336.1	100	100	100
SARS coronavirus isolate Xiao Tang Shang Hospital polyprotein 1ab-like gene	N gene	AY465926.1	100	100	100
SARS coronavirus Tor2 isolate Tor2/FP1-10912	N gene	JX163923.1	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-36/2020	N gene	MW356672.1	100	100	100
SARS-CoV-2/human/ECU/Z&Z_SARS_4/2020	N gene	MW294011.1	100	100	100
BetaCoV/Wuhan/WH-01/2019	N gene	CNA0007332	100	100	100
SARS coronavirus isolate Guangdong/20SF012/2020	N gene	EPI_ISL_403932	100	100	100
SARS coronavirus isolate /South Korea/KCDC03/2020	N gene	EPI_ISL_407193	100	100	100
SARS coronavirus isolate /France/IDF0372/2020	N gene	EPI_ISL_406596	100	100	100
SARS coronavirus isolate /Finland/1/2020	N gene	EPI_ISL_407079	100	100	100

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/TX-DSHS-1535/2020	N gene	MW349166.1	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-31/2020	N gene	MW356784.1	100	100	100
SARS coronavirus isolate /Japan/AI/I-004/2020	N gene	EPI_ISL_407084	100	100	100
SARS coronavirus isolate /England/01/2020	N gene	EPI_ISL_407071	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-36/2020	N gene	MW356672.1	100	100	100
SARS coronavirus isolate /Singapore/1/2020	N gene	EPI_ISL_406973	100	100	100
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/MN-MDH-2049/2020	N gene	MW349104.1	100	100	100
SARS coronavirus isolate /Australia/VIC01/2020	N gene	EPI_ISL_406844	100	100	100

Clinical sensitivity

The wet testing of inclusivity using the RNJia Virus Kit (Cat No: RN983072) was evaluated as supplemental data by testing three SARS-CoV-2 positive specimens from Day hospital. These specimens were confirmed positive by the rRT-PCR kit. Each specimen was diluted to (≤3log10 LOD, ≤2log10 LOD, ≤1log10 in negative specimen matrix (Nasal/oropharyngeal swab specimen) and tested in triplicate.

Table 8: Clinical sensitivity results of Viga Sars-CoV-2 Molecular Diagnostic kit

Concentration	**Specimen**	**Ct N gene**	Ct RdRp gene
≤3log10 LOD	Nasal/oropharyngeal swab	25.423	22.864
		25.32	22.766
		25.472	23.01
≤2log10 LOD	Nasal/oropharyngeal swab	27.767	26.156
		27.659	26.229
		27.821	26.162
≤1log10 LOD	Nasal/oropharyngeal swab	32.20	31.91477

		32.431	31.892
		32.268	31.9371

Cross-reactivity (analytical specificity)

Cross-reactivity of the Viga SARS COV-2 Molecular Diagnostic Kit was evaluated by boh in silico analysis and by wet testing potentially cross-reactive whole pathogens or purified nucleic acid from clinical specimens. No cross-reactivity was detected. The in-silico mapping analysis of each primer/probe against several pathogens is based on the NCBI nr/nt database accessed August 12, 2020, using the online BLASTN 2.10.0+, and the representative results are shown in below table. The RdRp and N gene primer/probes may detect bat coronaviruses based on this in silico analysis. No cross-reactivity was observed for other listed respiratory pathogens in silico and wet-testing.

Table 9: The in-Silico Specificity Analysis of primer and Probe sets Coronavirus (nCOV-2019) for other Respiratory pathogens.

Pathogen (Taxonomy ID)	**Strain**	**Target**	**GenBank Acc#**	**% Homology Test FP**	**% Homology Test RP**	% Homology Test Probe
Human coronavirus 229E	camel/Abu Dhabi/B38	N gene	MG000870.1	43	36	40
Human coronavirus OC43	HCoV_OC43/Seattle/USA/SC9428/2018	N gene	MN630549.1	63	58	33
Human coronavirus HKU1	HKU1 SC2628	N gene	DQ437612.1	71	36	30
Human coronavirus NL63	HCoV_NL63/Seattle/USA/SC0179/2018	N gene	DQ462758.1	56	36	43
MERS-CoV	BtVs-BetaCoV/SC2013	N gene	MK858156.1	43	54	63
Human adenovirus D8 isolate BA_280-2008	Human	N gene	MK913814.1	78	60	40

hexon gene						
Human metapneumovirus	Human	N gene	MH482736.1	47	40	40
Paramyxoviridae	MVs/Padova. ITA/24.17/1[B3]	N gene	MK513622.1	52	48	40
Orthomyxoviridae	A/ruddy turnstone/South Carolina/UGAI18-1224/2018 (H3N1)	N gene	MN938062.1	52	48	43
Parainfluenza	NCTC10665	N gene	LR134481.1	58	65	53
Influenza A virus	A/Ross's Goose/Arkansas/AH0085761 S.4.A/2016 (H11N9)	N gene	MN253675.1	56	48	40
Influenza B virus	B/Hong Kong/CUHK21967/2000	N gene	MF955545.1	52	40	36
Respiratory syncytial virus	isolate RSVA/USA/ACRI-051/2016	N gene	MN630104.1	47	40	66
Legionella pneumophila	C9_S	N gene	CP015942.1	72	65	60
Haemophilus influenzae	IH197	N gene	MG694561.1	56	47	36
Mycobacterium tuberculosis	FDAARGOS_757	N gene	CP054013.1	84	52	43
Bordetella pertussis	J802	N gene	CP033303.1	80	No Sig.	83
Pseudomonas aeruginosa	PcyII-40	N gene	LR739069.1	60	72	46
Pneumocystis jirovecii	T551_01783	N gene	XM_018374046.1	40	48	56
Staphylococcus epidermidis	SR1	N gene	AF269311.1	52	76	46
Enterovirus	CMC718	N gene	MN629889.1	65	52	50
SARS-coronavirus	human/EGY/CUNCI-HGC9I015/2020	N gene	MW547443.1	75	64	76

Chlamydia pneumoniae	H12	N gene	LN847142.1	52	48	66
Streptococcus pyogenes	NCTC8231	N gene	LS483345.1	54	68	63
Streptococcus pneumonia	4041STDY6836170	N gene	LS483449.1	43	80	68
Cryptococcus neoformans	B-3501A	N gene	XM_767816.1	60	60	46
Candida albicans	SC5314 Mnn13p	N gene	XM_715485.2	63	44	46
Enterovirus EV68	NIE0611579	N gene	KX162706.1	50	57	56
Rhinovirus	16-J2	N gene	KY629935.1	30	45	48
Mycoplasma pneumonia	NCTC10119	N gene	LR214945.1	54	57	45
Streptococcus salivarius	ICDC3	N gene	CP018189.1	57	65	53
human genome	CHM13	N gene	CP068259.1	53	58	64

Cross-reactivity (clinical specificity)

Determination of clinical cross-reactivity was carried out by Viga Sars-CoV-2 Molecular Diagnostic kit based on a panel consisting of different concentrations of negative plasma samples. No potential cross-reactivities were observed with pathogens.

Table 10: Cross-reactivity of the novel Coronavirus (Ncov-2019) resulted from Viga Sars-CoV-2 Molecular Diagnostic kit setup.

Virus/Bacteria/Parasite	Source/ Sample type	Concentration	Ct Value (ORF1ab gene/N gene)
Adenovirus	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Influenza A	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**

Influenza B	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Legionella pneumophila	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Cryptococcus neoformans	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Chlamydia pneumonia	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Streptococcus pneumoniae	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Respiratory Syncytial Virus	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Mycoplasma pneumoniae	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Streptococcus pyogenes	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
Mycobacterium tuberculosis	AmpliRun®DNA/RNA Vircell	104 copies/ml	**-/-**
10 Pooled human genomes	Clinical sample	10 ng/µl	**-/-**

Accuracy

Accuracy assessment includes In Vitro Intra-assay and Inter-assay.

Intra-assay

Intra-assay refers to the accuracy and ability of the designed method in determining the concentration of similar repeats in one Real Time-PCR cycle, which as Mean±SD for different Cts is shown. For this purpose, three repetitions of each concentration of the control sample were examined in one reaction, and coefficient of variation values was calculated for the threshold cycle values. The result of the Run for N gene in the maximum coefficient of variation is 1.2, and the minimum coefficient of variation is %51; for the RdRp gene, the maximum coefficient of variation is 1.19, and the minimum coefficient of variation is 29%. All acceptable results must have a cv of less than %5.

Inter-assay

Inter-assay refers to the results of different Runs in Real Time-PCR or results from other laboratories expressed as Mean±SD for different Cts related to the number of copies or different concentrations of a sample. For this purpose, five repeats of each concentration of the control sample were tested on three additional days. Run result for the N gene in maximum

coefficient of variation is 2.27, and the minimum coefficient of variation is 0.8; for the RdRp gene, the maximum coefficient of variation is 1.99, and the minimum coefficient of variation is 44%. All acceptable results must have a cv of less than %10.

Clinical evaluation

The clinical function of Viga Sars-CoV-2 Molecular Diagnostic kit using 185 samples of the throat and nasal swabs (in the Virus transmission environment) collected from patients suspected of COVID-19 were evaluated. To compare and verify the diagnostic kit of Novel Coronavirus (2019-ncov) Nucleic Acid Diagnostic Kit (PCR Fluorescence Probing) (Sansure Biotech Inc), which was licensed for emergency use in the laboratory by the US food and Drug Administration, was used. Both methods were performed on Real Time-PCR (Rotor-Gene Q-Qiagen). The results of (negative percent agreement) NPA %100 and (positive percent agreement) PPA %97/64 are shown in the table below.

Table 11: Clinical evaluation between Viga SARS-COV-2 Molecular Diagnostic Kit (ROJE Technologies) and Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) as Comparator Method

Test type		**Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR Fluorescence Probing) (Sansure Biotech Inc)**		**total**
		Positive	Negative	
Viga Sars-CoV-2 Molecular Diagnostic kit	Positive	83	0	83
	Negative	2	100	102
Total		85	100	185

Positive Agreement Rate: 83/85×%100= %97/64

Negative Agreement Rate: 100/100×%100=%100

Overall Rates of Agreement: (%98/91=%100) × (2+ 100+ 0+83) / (100 + 83)

symbols

Tables 12: symbols on the Kit Label

Symbols	meaning	symbols	meaning
	Date of manufacture		manufacturer
	Expiration Date		Temperature limitation
REF	Catalog number	LOT	Lot number
		IVD	In Vitro diagnostic medical device

Troubleshooting

Here we try to cover as many problems as you may see in using this product; however, scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during the preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:2, 1:10 dilution of the extracted RNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

Ordering Information

category	Product name	Cat No.	Preps no.
Molecular diagnostic Kit	Viga Sars-CoV-2 Molecular Diagnostic kit	MD983053	100 Preps
Related product	RNJia Virus kit	RN983072	100 Preps

Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website (www.ROJETechnologies. com)
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Factory address:

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130- phone: +982191070705

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