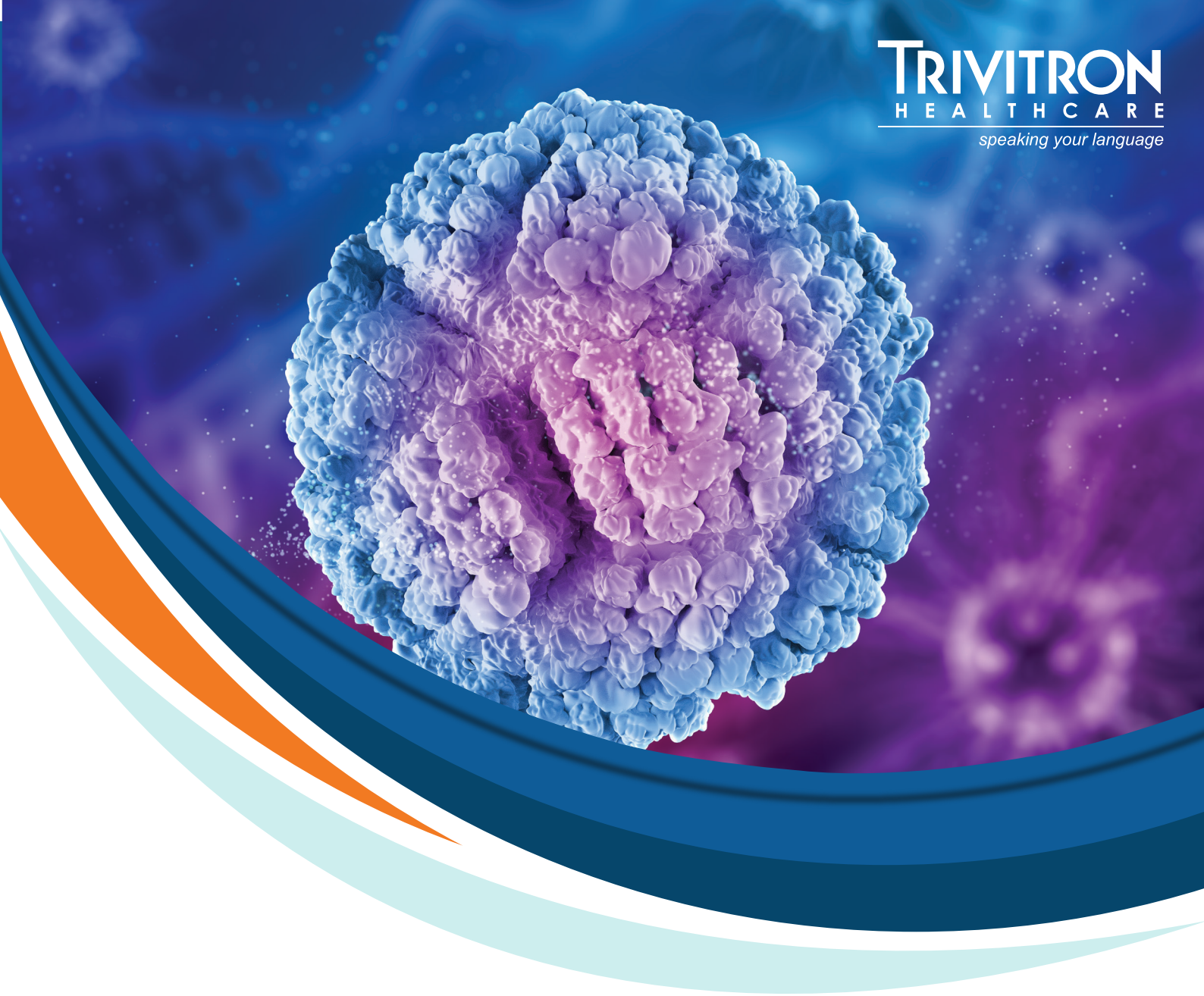
TRIVITRON HEALTHCARE
speaking your language



COVIDsure DIRECT

Multiplex RT-PCR kit with Extraction Free Technology

- Extraction Free technology based on Magic buffer (Provisional Patent Filed).
- Tedious and time-consuming RNA extraction process is replaced with one-step easy technology using RNA processing buffer (RPB).
- Cost of RNA extraction kit and instruments is saved.
- Simultaneous detection of three SARS-CoV-2 specific genes ORF1ab, N gene and S gene besides human internal control gene.
- *Kit enables the in-direct detection of SARS-CoV-2 variants, Omicron, Alpha B 1.1.7,
- Eta B.1.525 by S gene target failure mechanism (SGTF).
  Low virus load detection: < 5 copies/ µl
- Works equally well on VTM as well as extracted RNA.

Approved by

icmr
INDIAN COUNCIL OF MEDICAL RESEARCH
Serving the nation since 1911

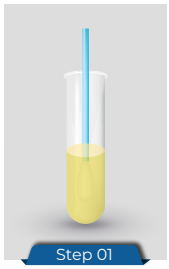
COVIDsure DIRECT

Multiplex RT-PCR Kit with Extraction Free Technology

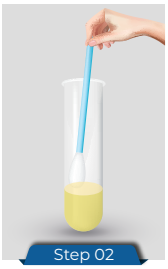
The COVIDsure DIRECT is a multiplex RT-PCR Kit for rapid qualitative in vitro detection of SARS-CoV-2 (COVID-19) directly from the VTM samples without RNA extraction from respiratory specimens (nasopharyngeal swab, oropharyngeal swab) of suspected cases in COVID-19 disease. The kit is supplied with RNA process buffer (RPB) which can be used for RNA sample preparation without extensive RNA extraction process. This kit can also detect SARS-CoV-2 virus from the RNA extracted from the VTM samples from suspected COVID-19 patients.

Highly Specific: 100% Specificity. No detectable cross reactivity with most human Coronavirus strains, respiratory pathogens, etc.
Utmost Sensitivity: 100% Sensitivity. Wider range of Covid-19 strains covered to detect most mutations (mutation variants)
Timesaving Approach: Processing time is approximately 45 mins - 60 mins
*Indirect detection of SARS-CoV-2 variants by S Gene must be verified by sequencing.

Test Procedure



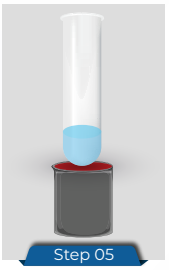
Swab sample in VTM



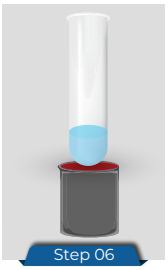
Take 6µL VTM sample

Add 2µL RPB to trhe sample

Mix sample and RPB



Heat the sample at 56°C for 10 min



Heat the sample at 95°C for 5 min

Use the sample mix as template for RT-PCR

Ordering Information

Product Code	Product Description	Pack Size	Storage	Shelf Life
DVZ-30055	COVIDsure DIRECT : Multiple RT-PCR Kit with Extraction Free Technology	100 Reactions	-20°C	1 year
DVZ-30054	COVIDsure DIRECT : Multiple RT-PCR Kit with Extraction Free Technology	500 Reactions	-20°C	1 year

*Please note: Product specifications are subject to change without prior notice owing to product modifications, improvements / up-gradation.

corporate@trivitron.com | www.trivitron.com
Customer Care Helpline: +91 98400 80008

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COVIDsure DIRECT
Multiplex RT-PCR kit with extraction free technology
INSTRUCTIONS FOR USE

REF DVZ-30055/DVZ-30054
Σ 100/500 RXN
-20 °C

INTENDED USE

The COVIDsure DIRECT is a multiplex RT-PCR Kit for rapid qualitative *in vitro* detection of SARS-CoV-2 (COVID-19) directly from the VTM samples without RNA extraction from respiratory specimens (nasopharyngeal swab, oropharyngeal swab) of suspected cases in COVID-19 disease. The kit is supplied with RNA process buffer (RPB) which can be used for RNA sample preparation without extensive RNA extraction process. This kit can also detect SARS-CoV-2 virus from the RNA extracted from the VTM samples from suspected COVID-19 patients.

KITCONTENTS

S.No	Components	100 Rxn Kit		500 Rxn Kit	
		Volume	No. of Vials	Volume	No. of Vials
1	Enzyme mix (EM)	1300 µl	1	1300 µl	5
2	Primer Probe Mix (PPM)	400 µl	1	400 µl	5
3	Positive Control	100 µl	1	200 µl	1
4	Nuclease Free Water (NFW)	200 µl	1	500 µl	1
5	RNA Process Buffer (RPB)	250 µl	1	250 µl	5

STORAGE CONDITION:

- Store all the kit components at -20 °C, after first thawing keep RPB either at 2-8 °C or aliquot and keep at – 20 °C to avoid freeze-thaw cycles.
- PPM is light sensitive and hence should not be exposed to light.
- Freeze-Thaws of the kit are not recommended. If more than 4 freeze-thaws are required, then aliquots the components.

PRINCIPLE OF THE TEST

The kit is supplied with RT-PCR enzyme mix for real-time PCR technology based upon Synthetic DNA Primers and fluorescence labelled hydrolysis probes. If the SARS-CoV-2 RNA is present in the suspected patients samples then it is converted to cDNA using reverse transcriptase followed by amplification of Orf1ab (HEX), S gene (FAM) and N gene (Rox) regions of SARS-CoV genome using dual labelled Primers & Probes set specific to respective gene sequence by Taq polymerase. Fluorescence labelled probes is cleaved during each PCR cycle and if the fluorescence crosses the threshold limit it is detected during the amplification cycles. Besides, a Primer-probe set specific to beta-globulin human gene (Cy5) serves as an internal control. The kit is based upon Single Step RT-PCR, where both Reverse Transcription by Reverse Transcriptase and PCR by Taq Polymerase take place in single tube. A signal in HEX/FAM/Rox channel suggests presence of SARS-CoV-2 (COVID-19) virus in the sample. Signal in Cy5 Channel suggests that proper processing of the RNA from the specimen and Reverse transcription reaction has worked. The kit also contains the RNA process buffer (RPB) for processing of RNA samples directly from VTM.

EQUIPMENT AND APPARATUS (Required but not supplied with the kit)

1. Biosafety Cabinet Class II A
2. Heating block/ PCR block, PCR work station
3. Appropriate Real-Time PCR Instrument
4. PCR tubes/Strip or plates compatible with the real time PCR instrument
5. Bench-top Centrifuge, Plate Centrifuge (if working with 96 or 384 well plates)
6. Vortex Mixer, Micro-Pipette (10 µL, 20 µL, 200 µL, 1ml)

PRECAUTIONS

1. All the processes should be carried out as per the WHO and CDC guidelines for sample preparation and RT-PCR assay.
2. The specimens should be handled in accordance with regulations pertaining to environment and safe laboratory practices recommended for infectious and/or bio-hazardous materials.
3. Use protective gears including, laboratory coat, disposable powder-free gloves, eye goggles, and other protective equipment while handling the specimen.
4. Keep the kit and its components away from DNase/RNase contaminants
5. Use of DNase/RNase free disposable pipette tips with aerosol barriers is recommended.
6. The working area has to be segregated and separated for each of the testing steps i.e. a) Preparation of sample b) Setting up of reaction c) Amplification and detection. The workflow should be streamlined and unidirectional. At each of these steps use of new disposable gloves and personnel protective equipment is recommended.
7. Dedicated Equipment & plastic ware (e.g. pipettes, tip boxes etc.) is recommended. Please avoid moving equipment & consumables from one area to other.
8. Separate storage of positive and/or potential positive materials are to be done. Positive and/or potential positive materials should not be brought in contact with kit components.
9. Avoid opening the tubes/plate after reaction is complete to prevent contamination from amplicons.
10. Use of additional controls may be implemented as per the guidelines of local, national or other regulatory institutions.
11. Autoclaving doesn't degrade amplicons produced from the reaction and increases the risk of laboratory contamination. Discarding un-used samples and other assay waste in bleach or as per the safety regulations and local guidelines is to be followed.
12. The COVIDsure DIRECT is a multiplex RT-PCR Kit is stable at -20 °C up to the expiry date mentioned on the label however the kits once open should be used within 30 days and the repeated/multiple free-thaw of the kit contents should be strictly avoided.

SPECIMEN COLLECTION

- Collect Respiratory specimens such as nasopharyngeal swab, oropharyngeal swab in VTM as per the applicable guidelines for COVID-19. We recommend use of Trivitron-Labsystems' **ViSure Sample Collection Kit.**
- Only use flocked or spun polyester swabs; avoid the use of calcium alginate swabs or swabs with wooden shafts, as they may contain PCR inhibitors and virus inactivating substances. If the rapid protocol is to be used, avoid collecting samples in lytic VTMs that contain high concentrations of chaotropes like GITC or VTMs that contain high concentrations of phenolic or other dyes that impart deep colors to the medium.
- The collected specimens should be tested as soon as possible.
- The specimen shall be transported using sealed foam box with ice pack with 2-8°C.
- Specimens can be stored at 2-8°C for no more than 48 hours storage of specimen is critical for the test performance.

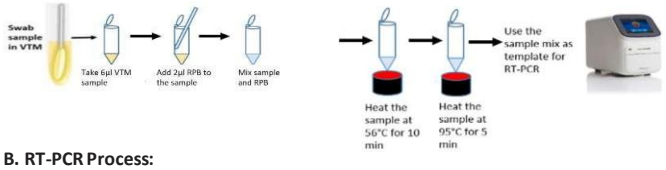
TEST PROCEDURE

Following is the protocol for RNA sample preparation for RT-PCR using RPB supplied with the kit.

A. Sample Preparation using RPB supplied with the kit:

1. Initial processing (till addition of RPB) of all specimens should be done in a validated biological safety cabinet (BSC) or primary containment device.
2. Add 6 µl of VTM sample and 2ul of RPB in the PCR tube or 96 well plate and mix well by pipetting up and down 10 times or vortexing for 10 seconds 3 times followed by spin for 15 sec.
3. Preheat the samples at 56°C for 10 mins followed by heat shock at 95°C for 5 mins either on heating block or PCR block.
4. Allow the sample to cool down, short spin.
5. This extracted viral RNA samples can also be used for detection of viral gene with this kit.

PROCESS FOR RNA SAMPLE PREPARATION USING RPB



B. RT-PCR Process:

1. Thaw all the kit components on the ice.
2. Prepare the master mix of enzyme mix and the PPM as given in the table below:

Reaction	Volume/Reaction*
Enzyme mix (EM)	13 µl
Primer Probe Mix (PPM)	4.0 µl
Processed sample/Positive Control/NTC/Extracted RNA	8.0 µl
Total	**25 µl**

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1st Floor, BIOME Building (RCC Structure), AMTZ Campus, Pragati Maidan, Nadupuru Village, Pedagantyada, Visakhapatnam A.P – 530031,

Note: ***Positive Control and Nuclease Free Water (NFW) should be used in each plate/batch***

3. Transfer 17 µl (13 µl of enzyme mix and 4 µl of PP mix) of master mix in each well of 96 well plate or PCR tubes. Avoid formation of bubbles while dispensing the same.
4. Add 8 µl of processed sample from section 'A' or extracted RNA to each well/tube.
5. Add 8 µl of Positive Control and 8 µl of Nuclease Free Water in each tubes/wells designated as positive and negative controls respectively.
6. Properly seal the plate/tubes, vortex to mix the contents and spin to collect the liquid at the bottom.
7. Transfer the plate/tube to real time PCR machine and start the run as per the PCR Program given below.
8. COVIDsure-Direct is a sensitive kit and can detect the RNA up to 10 copies/ µl.

PROGRAMING REAL TIME PCR SYSTEM

Program your Real Time PCR System for following cycling conditions as per the instructions of instrument manufacturer.

Step	Temp	Time	Cycles
Reverse Transcription	45 °C	10 min	1
Initial activation	95 °C	3 min	1
Denaturation	95 °C	15 sec	40
Annealing, Extension and signal acquisition*	59 °C	30 sec	

*Acquire Fluorescence Signals in FAM, HEX, Cy5 & ROX Channels at this step

NOTE: COVIDsure-Direct RT-PCR kit can also be used for the detection of RNA extracted from VTM sample using any approved method.

a. Manual RNA extraction kit or
b. Automated RNA extraction kit

INSTRUMENTS RECOMMENDED FOR RT-PCR

The kit has been validated on QuantStudio-5/3 and Bio-Rad CFX96, 384 and can also be used on Applied Biosystems 7300/7500, Roche Diagnostics LightCycler 96/480, Qiagen Rotor-Gene Q, Agilent MX3000, Ariamax etc.,

(Instrument should be calibrated, maintained and used as per manufacturer recommendation).

DATA ANALYSIS AND INTERPRETATION

The Ct Values should be interpreted as follows;

HEX (Orf1abGene)	Rox (N-Gene)	FAM* (SGene*)	Cy5 (Internal Control)	Interpretation
CtValue≤36	CtValue≤36	CtValue≤36	CtValue≤36	2019-nCoVRNA Detected
CtValue≤36	CtValue≤36	No amplification	CtValue≤36	2019-nCoVRNA Detected
CtValue≤36	No amplification	CtValue≤36	CtValue≤36	2019-nCoVRNA Detected
CtValue≤36	No amplification	No amplification	CtValue≤36	Presumptivepositive. Sample should be retested
No amplification	CtValue≤36	No amplification	CtValue≤36	Presumptivepositive. Sample should be retested
No amplification	No amplification	CtValue≤36	CtValue≤36	Presumptivepositive. Sample should be retested
No amplification	No amplification	No amplification	CtValue≤36	2019-nCoVRNANot Detected
No amplification	No amplification	No amplification	No amplification	Invalidresult. Re-perform Sample processing

***if ORF1b and N gene is amplified but S gene is not amplified then the sample may have mutations in S gene pertaining to delta or Omicron variant. Such samples may be confirmed by other method/s or sequencing to further investigate the mutant-variant.**

REF	Catalogue No.
LOT	Kit Lot No
	Expiry
IVD	In-vitro Diagnostics use
	Temperature limits
	Manufactured by
	Date of Manufacture
	Instruction For Use
	Do not use if package is damaged
	Keep away from sunlight
	Potential Bio hazardous material

TROUBLESHOOTING:

a. In case of peak in NTC, please clean the area and PC tips with hypochlorite, handle PC very carefully as it contaminate the plate and cause for NTC and false positive.
b. RFU below 12 Cycles should be considered baseline. In case, your Real time PCR machine does not do automatic baseline correction, please set the baseline manually.
c. Samples showing amplification below 12 Ct value should be diluted 1:1 and repeated for the confirmatory diagnosis.
d. A Ct >36 and/or ΔCt ($Ct_{Test\ Sample}$ - $Ct_{+ve\ Control}$) >15 for any target is beyond sensitivity limit of the test and should not be reported. A repeat sample should be requested in such cases.

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स्वास्थ्य एवं परिवार कल्याण मंत्रालय
भारत सरकार
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संस्थागत क्षेत्र,
नोएडा – 201 309, उत्तर प्रदेश, भारत

NATIONAL INSTITUTE OF BIOLOGICALS
Ministry of Health & Family Welfare
Government of India
A-32, Sector - 62,
Institutional Area,
NOIDA - 201 309 (U.P.), INDIA

Phones : 0120 - 2400022, 2400074, 2593600; Fax : 0120 - 2403014; E-mail : info@nib.gov.in; Website : www.nib.gov.in

By Speed Post
No. N. 13-101/2021-22-SRRD/CKTL
Dated 07/02/2022

To,
M/s Trivitron Healthcare Pvt. Ltd.,
Ground Floor, Wing A, Old No. 25,
New No. 15, Abhiramapuram, IVth Street,
Chennai – 600018, Tamil Nadu (India).

Subject: Testing of **COVIDsure Direct Multiplex RT PCR Kit with extraction free technology,** Manufactured by M/s Trivitron Healthcare Pvt. Ltd., Visakhapatnam, Andhra Pradesh, India.

Sir,

Pursuant to your Mfg. Licence No. MFG/TL/IVD/2021/000721, Dated 07/12/2021 and your letter dated 11/01/2022 on the above subject and to say that the sample of **COVIDsure Direct Multiplex RT PCR Kit with extraction free technology,** Manufactured by M/s Trivitron Healthcare Pvt. Ltd., Visakhapatnam, Andhra Pradesh, India, has been tested and found to be **Standard Quality**. The Certificate of Analysis is attached.

S.No.	Name of the Product	Batch No.	Mfg. Date	Exp. Date	Analytical Report No.
1.	COVIDsure Direct Multiplex RT PCR Kit with extraction free technology	CVDSRD122101A	12/2021	11/2022	102/CKTL/2021 Dated: 27/01/2022

Yours faithfully

(Dr. Gauri Misra)
Scientist Grade-II & Head SRRDU

NATIONAL INSTITUTE OF BIOLOGICALS

(Ministry of Health & Family Welfare),
Government of India,
A-32, SECTOR-62,NOIDA-201307(UP),

TC-7725

File No.N.13-101/2021-22-SRRD/CKTL

COVID-19 KIT TESTING LABORATORY
CERTIFICATE OF ANALYSIS

Sample Received From:	TRIVITRON HEALTH CARE PVT. LTD., Visakhapatnam, Andhra Pradesh, Letter No.- NIL, Dated- 11/01/2022
Date of Sample Receipt:	13/01/2022
Sample Forwarding No.	22749
Name of Product	COVIDsure Direct Multiplex RT PCR Kit with extraction free technology
Name of Manufacturer:	M/s TRIVITRON HEALTHCARE PVT. LTD., Visakhapatnam, AP
Batch/Lot No:	CVDSRD122101A
Manufacturing Date:	12/2021
Expiry Date:	11/2022
Start Date of Analysis:	24/01/2022
Analytical Report No:	102/CKTL/2021

S.No	Test(s) Conducted	Acceptance criteria*	Result	Remarks
1	Sensitivity	>=94.7 %	100%	Complies
2	Specificity	>=98.8 %	100%	Complies
CONCLUSION: The Batch No. CVDSRD122101A of product COVIDsure Direct Multiplex RT PCR Kit with extraction free technology tested as per manufacturer's specifications and found **Standard Quality** with respect to above test(s)				
Special Remarks,if any:NIL				

* Acceptance criteria is applied according to ICMR SOPs/manufacturer's specifications.

Disclaimers

1. NIB validation process does not approve / disapprove the kit design
2. NIB validation process does not certify user friendliness of the kit / assay
3. Validation of a kit by NIB is not an assurance that the kit specifications would be included in the tendering process.

In addition to the above (1, 2, 3), the following disclaimer/Limitation needs to be included in all validation reports by NIB, Noida for SYBR green based real-time PCR assay

Interpretation of the SYBR green based test results requires expertise and experience which may not be available in many routine diagnostic laboratories involved in COVID-19 testing.

Note: This report is exclusively for COVIDsure Direct Multiplex RT PCR Kit with extraction free technology manufactured by M/s TRIVITRON HEALTHCARE PVT. LTD., Visakhapatnam, AP .The company shall not use or publish information or report for advertising or promotional purposes.

Date:27/01/2022

Laboratory Head/Authorized Signatory

TC772520 22000169F

Name: Dr. Gauri Misra
Designation:Scientist Gr-II & Head

The results apply to the sample as received