TATA MD CHECK RT-PCR OmiSure

(Instructions for Use)

For *in-vitro* diagnostic use only

REF SPCOK035-100
SPCOK035-500

Σ 100Rxn
500Rxn

CE IVD -20°C

INTENDED USE

Tata MD CHECK RT-PCR OmiSure is an *in-vitro* diagnostic Real time RT-PCR qualitative assay for the detection of SARS-CoV-2 in human respiratory (nasopharyngeal / oropharyngeal) specimens. The kit can be used for identification of the Omicron (B.1.1.529, BA.1 and BA.2) SARS-CoV-2 Variant of Concern using extracted RNA from specimen collected in compatible viral transport media .

BACKGROUND

Coronavirus is a virus that causes an infection in the nose, sinuses, or upper respiratory tract. Most coronaviruses are not dangerous. In early 2020, after a December 2019 outbreak in China, the World Health Organization (WHO) identified SARS-CoV-2 as a new type of coronavirus. The outbreak quickly spread around the world.

SARS-CoV-2 is one of seven types of coronaviruses, including the ones that cause severe diseases like Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The other coronaviruses cause most of the colds that affect us during the year but are not a serious threat for otherwise healthy people.

COVID-19, caused by SARS-CoV-2, can trigger a respiratory tract infection, and affect the upper respiratory tract (sinuses, nose, and throat) or lower respiratory tract (windpipe and lungs). It spreads the same way as other coronaviruses i.e., mainly through person-to-person contact. Infections range from mild to severe. Standard recommendations are followed to prevent the spread of infection. The standard method for diagnosis of SARS-CoV-2 is by Real Time Reverse Transcription PCR (rRT-PCR).

Like other coronaviruses, the SARS-CoV-2 virus has been undergoing continuous mutations leading to multiple Variants of Concern (VOCs) such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and most recently Omicron (B.1.1.529). As per the advisory from WHO on 26 November 2021, Omicron VOC poses an increased risk and infections have increased steeply across the globe. Screening for Omicron variant will ensure timely detection, intervention and control the spread of this variant.

PRODUCT DESCRIPTION

Tata MD CHECK RT-PCR OmiSure is an *in-vitro* diagnostic Real time RT-PCR qualitative assay for the specific detection of SARS-CoV-2 in human respiratory (nasopharyngeal/oropharyngeal) specimens and for identification of the Omicron (B.1.1.529) SARS-CoV-2 variant.

This kit uses one-step RT-qPCR with a hydrolysis probe chemistry that uses 5' nuclease activity of Taq DNA polymerase and enables detection of PCR product which gets amplified at every step during PCR cycles by producing fluorescence. It is a multiplex assay kit where four targets (three SARS-CoV-2 and one human internal control) can be detected in a single tube reaction. The Tata MD CHECK RT-PCR OmiSure primer and probe sets are designed using a combination of S-gene dropout or S-gene target failure (SGTF) and S-gene mutation amplification (SGMA) specific to the Omicron variant, and RdRP-gene of SARS-CoV-2 along with human internal control RNase P in a single tube assay.

The kit identifies the Omicron variant through a combination of SGTF and SGMA in the S-gene region specific to the Omicron variant. The S-gene target sequences of the assay are designed to discriminate between the mutations present in the other variants and the Omicron variant. The assay is designed such that presence of Omicron variant will result in SGTF in FAM channel and SGMA in the HEX channel, indicative of presence of Omicron VOC. Further the presence of variant proof RdRP target region in the CY5 channel will allow for specific detection / confirmation of presence of the SARS-CoV-2 virus agnostic of any variant.

Our S gene target failure (SGTF) is designed in a unique region that is absent in Omicron (including its sub lineages BA.1 and BA.2) resulting in -ve signal in FAM channel. However, we have ensured that this region is present in all the other variants such as Alpha, Beta, Gamma or Delta thereby resulting in +ve signal in FAM channel.

The kit is suitable for all Real-Time PCR instruments which are equipped with minimum of four measurement channels (FAM, HEX / VIC, Texas Red / ROX and CY5).

KIT CONTENTS

Component	Vial Label	No. of vial for 100 Rxn	Volume per vial 100 Rxn	No. of vial for 500 Rxn	Volume per vial 500 Rxn
2X Master Mix	MM	2	625 µL	10	625 µL
20X Primer and Probe Mix	PPMx	1	125 µL	5	125 µL
Nuclease Free Water	H_2O	1	1250 µL	2	1250 µL
Positive Control	PC	1	100 µL	1	250 µL

STORAGE AND HANDLING

- Store all kit reagents at -20°C
- Do not repeatedly freeze-thaw reagents as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C
- Kit components are stable until the expiration date indicated on the box when stored and handled as recommended. However, the kit performance has been found satisfactory upto four freeze-thaw cycles

MATERIALS REQUIRED BUT NOT PROVIDED

- Real-Time PCR instrument with at least four detection channels suitable for FAM, HEX/VIC, Texas Red/ROX and CY5 measurement
- RNA extraction kit
- Disposable gloves
- Adjustable pipettes (single, multichannel)
- Sterile nuclease-free pipette tips with filter
- Vortex mixer
- Centrifuge with a rotor for 1.5/ 2.0 mL tubes
- Sterile nuclease-free PCR tubes, strips or plates recommended by the Real-Time PCR instrument manufacturer
- Commonly used molecular diagnostics laboratory reagents, disinfectants, and consumables

PRECAUTIONS

- It is recommended that this product is used by personnel specially instructed and trained in real-time PCR and *in-vitro* diagnostic procedures
- Treat all the specimens as potentially infectious
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens
- Store positive and/or potentially positive material separated from all other components of the kit
- Keep separate areas for master mix and template preparation and work under biosafety cabinets
- Use aerosol barrier pipette tips and frequently change the gloves
- Do not open the reaction tubes/plates post-amplification, to avoid amplicon contamination
- Do not smoke, drink, or eat in areas where kit reagents and/or human specimens are being used
- Do not use kit components that have passed their expiration date
- Negative results do not exclude SARS-CoV-2 infection and should not be used as the sole basis for treatment

ASSAY WORKFLOW

The assay workflow comprises of following steps:

A. Specimen Collection, Transport, and Storage: Collect specimen in viral transport medium

B. RNA Extraction: Extraction and / or purification of RNA from the specimen

C. One-step RT-PCR: One-step reverse transcription and polymerase chain reaction in a Real-time PCR instrument

D. Data Analysis and Interpretation: Data is analyzed and interpreted using the software provided by your Real-time PCR instrument manufacturer

Collect NP/OP specimen

RNA extraction

One-step RT-PCR

Analysis and Interpretation

SAMPLE COLLECTION, TRANSPORTATION AND STORAGE

Collect human respiratory (nasopharyngeal / oropharyngeal) specimen in viral transport media and store them as per the manufacturer's instructions and standard laboratory procedures.

SAMPLE PREPARATION – EXTRACTED SAMPLES

Prepare samples from specimens as per the RNA extraction protocol provided by the manufacturer of the RNA extraction kit and standard laboratory procedures.

RT-PCR REACTION SET-UP

1. Thaw all kit components of the kit on ice
2. Mix contents gently or vortex, spin down for 5 seconds and use immediately
3. Prepare the Tata MD CHECK RT-PCR OmiSure reaction mix
   a. Calculate the quantity of reagents for the experiment including controls. The table below assumes 'n' reactions, along with an additional positive control and negative control

Component	Volume per sample / control	Volume for 46 sample plus 2 controls	Volume for 94 samples plus 2 controls
2X Master Mix	12.5 µL	600 µL	1200 µL
20X Primer and Probe Mix	1.25 µL	60 µL	120 µL
Nuclease Free Water	3.25 µL	156 µL	312 µL
Total	**17 µL**	**816 µL**	**1632 µL**

   b. Prepare the reaction mix in a 1.5 / 2.0 mL tube and spin it down
4. Set up the PCR reaction plate / tubes
   a. Dispense 17 µL reaction mix in each tube or well (of a strip / plate)
   b. Add 8 µL of nuclease free water in NTC well
   c. Carefully add 8 µL samples in the designated wells in template addition area
   d. Add 2 µL of PC + 6 µL of nuclease free water in a separate hood in PC well.
5. Seal the tubes/plate carefully, briefly spin down and process in a compatible real-time PCR instrument

RT-PCR INSTRUMENT SETUP AND RUN

1. Set up the Real-time PCR instrument for the following detection channels:

Target	REPORTER
S-Gene TF	FAM
S-Gene MA	HEX/VIC
RdRP Gene	CY5
Human RNase P	Texas Red/ROX*

***Do not use ROX reference dye. Select passive reference dye to "NONE" in the qRT-PCR instrument to obtain RNase P signal.**

2. Program the machine for the thermal cycling conditions below:

Step		Temp °C	Time	# Cycles	Detection
Reverse Transcr-iption	Reverse Transcr-iption	50	15 mins	1	Off
	Activation	95	2 mins	1	Off
PCR and Detection	Denatur-ation	95	15 sec	40	Off
	Anneal/ Extension	58	30 sec		On

3. Execute the run on the PCR instrument

DATA ANALYSIS AND RESULT INTERPRETATION

Data Analysis and Result Interpretations

1. After completion of the run, analyze the data as per the instrument manufacturer's instructions
2. Analyze the curves by adjusting the fluorescence background. Set the baseline above NTC values, only if required. Refer to troubleshooting section for details
3. Check that the controls have performed as expected
4. Determine Ct cutoff value for the samples and controls for each channel. See table below for guidance

Sample	C_t Cutoff Value for Positive	C_t Cutoff Value for Negative
Positive control (PC)	Ct ≤ 40	-
Negative control (NTC)	-	Ct > 40 or Undetermined
Clinical samples	Ct ≤ 40	Ct > 40 or Undetermined

Note: a) C_t Cutoff Values are uniform across all the targets and internal controls, b) C_t Cutoff values are based on optimization in Tata MD facilities and must be validated for your infrastructure

5. Interpret clinical sample results with the help of the table below:

S-Gene (FAM)	S-Gene MA (HEX/ VIC)	RdRP (CY5)	RNase P (Texas Red/ ROX)	Results Interpretation [1]
-	+	+/-	+/-	SARS-CoV-2 Detected; Indicates presence of Omicron
-	-	+	+/-	SARS-CoV-2 Detected; Indicates presence of Omicron
+	-	+/-	+/-	SARS-CoV-2 Detected; Omicron not present
+	+	+	+/-	SARS-CoV-2 Detected; Indicates presence of Omicron
-	-	-	+	SARS-CoV-2 not Detected
-	-	-	-	Invalid results. Repeat the RNA extraction and re-run the test

[1] Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

LIMITATIONS

- Trained and skilled personnel who are familiar with this technology must handle the instrument and assay
- The generated results must be interpreted in conjunction with other clinical or laboratory findings
- Samples must be collected, transported, and stored using appropriate procedures to maintain integrity of the samples
- The RT-PCR OmiSure kit advanced performance was established using nasopharyngeal and oropharyngeal swab samples only. Other human respiratory specimen types should only be tested after further validation
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated
- It is the user's responsibility to validate system performance for any procedures used in their laboratory
- RNA extraction can be performed by commercially qualified kits only
- Exclusion of negative results will be completely dependent on type of infection (acute and chronic), and on appropriate specimen collection time and absence of inhibitors
- The presence of PCR associated contaminants may cause invalid results with this product. Good laboratory practices are recommended to avoid contamination and false results
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated
- Laboratories are required to report all test results to the appropriate public health authorities

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The LoD study established the lowest concentration of the SARS-CoV-2 virus that can be detected by the assay. The study results showed that the LoD of the TATA MD CHECK RT-PCR OmiSure for all the genes is 40 copies / reaction.

Clinical Performance

A total of 88 SARS-CoV-2 positive (including 40 Omicron positive samples) and 85 SARS-CoV-2 negative samples were used for clinical performance study of OmiSure at ICMR - National Institute of Virology (NIV), Pune. The study demonstrated 100% sensitivity and 99.25% specificity for Omicron identification and 100% sensitivity and 100% specificity for broader SARS-CoV-2 detection.

ICMR report

RT-PCR Result for (Omicron Positive)				
		Positive	Negative	Total
TATA MD CHECK RT-PCR OmiSure	Positive	40	1 (other variant)	41
	Negative	0	132 (Negative, Delta and other variant)	132
	Total	40	133	173

Parameter	Estimate (%)	95% CI
Sensitivity	100.00%	91.1% to 100%
Specificity	99.25%	95.8% to 99.9%

RT-PCR Result for (SARS-CoV-2 Positive)				
		Positive	Negative	Total
TATA MD CHECK RT-PCR OmiSure	Positive	88	0	88
	Negative	0	85	85
	Total	88	85	173

Parameter	Estimate (%)	95% CI
Sensitivity	100.00%	95.7% to 100%
Specificity	100.00%	95.7% to 100%

TROUBLESHOOTING

Positive control showed no amplification

Causes- Degradation

- Ensure that all reagents were stored as per manufacturer's instructions
- Ensure that all the components have been added to the reaction
- Check the thermal cycling program of the real-time PCR instrument
- Repeat the entire experiment with a known positive sample to rule out degradation of positive control

Negative controls are positive

Causes - Cross-contamination

- Repeat the experiment with fresh reagents
- Follow good laboratory practices while handling samples, kit components and consumables to avoid contamination issues

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have Ct values lower than the baseline value)

- Check the channel settings
- Analyze the curves by adjusting the fluorescence background
- Set the baseline above NTC values
- Inhibitory substances could be interfering with the reaction. Choose another RNA isolation kit and repeat the assay
- In some PCR machines the S gene target failure (SGTF) signals for FAM channel may be shallow, not sigmoidal in shape and run parallel to the baseline. In such circumstances it is recommended that the threshold for this channel alone needs to be re-adjusted manually. Therefore, any interpretation solely based on Ct values without adjusting the threshold, can lead to erroneous interpretation of results by users for S gene target failure.

An amplification signal is detected in the early cycles/No signal

Causes- High viral/human RNA load

- Dilute the extracted sample to increase the Ct value
- Use less starting material for extraction
- Dilute the sample 1:10 and re-run the assay

DOCUMENTATION AND SUPPORT

For product feedback, please contact us at feedback@tatamd.com
For technical support, contact at labsupport@tatamd.com

For additional documentation and product information, visit- www.tatamd.com

For a reference of the symbols used in the product labels and documentation, visit- https://www.tatamd.com/home/assets/Symbols.pdf

The general terms and conditions of sale and information about limited product warranty are available at- https://tatamd.com/documents/terms-conditions.html

Version History

Revision	Date	Description
00	07-01-2022	First release of IFU for Tata MD CHECK RT-PCR OmiSure
01	27-01-2022	Added more clarity on Data Analysis and Result Interpretation

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