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Truenat®

SARS CoV-2

Chip-based Real Time PCR Test for COVID-19

1. INTENDED USE

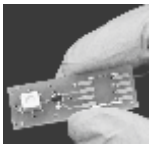
Truenat® SARS CoV-2 (REF 601420005 / 601420020 / 601420025 / 601420050 / 601420100 / 601420200) is a Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the semi-quantitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swab specimens and aids in confirmation of COVID-19. The test detects the *RdRp* gene of the virus and is recommended as a follow on test for confirmation of **positive results with other gene targets of the virus.** Samples testing positive by **Truenat® Beta CoV** can be confirmed using **Truenat® SARS CoV-2. Truenat® SARS CoV-2** runs on **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® SARS CoV-2** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

SARS CoV-2 is the causative agent for corona virus disease 2019 or COVID-19 in Humans. SARS CoV-2 is a Beta Corona Virus, one of the four genera of Corona Viruses. Coronaviruses are enveloped non-segmented positive sense RNAviruses belonging to the family coronaviridae and the order Nidovirales and broadly distributed in humans and other mammals. The common signs of COVID-19 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.

Early and correct identification of infection with SARS CoV-2 is important for effective isolation, treatment and case management of COVID-19. In line with WHO recommendations, molecular diagnostics are currently the method of choice for such virus detection and differentiation. However, molecular tests for COVID-19 have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days.

The **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis of and monitoring of COVID-19. This is enabled by making the real time PCR technology rapid, simple, robust and user friendly, thereby offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated **Truelab®** Real Time micro PCR Analyzers and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.



Truenat® SARS CoV-2 is a disposable, room temperature stable, Chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried RT-PCR reagents in microtube for performing Real Time RT-PCR test for viral infection and runs on the **Truelab®** Real Time micro PCR Analyzer. It requires only six (6) µL of purified RNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information. The **Truenat® SARS CoV-2** chip also stores information of used test to prevent any accidental re-use of the chip.

NOTE :Truelab®/ Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® SARS CoV-2 works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on Taqman chemistry. The RNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit and assayed using **Truenat® Beta CoV** test (refer **Truenat® Beta CoV** packinsert). If the sample tests positive for Beta CoV, Six (6) µL of the same extracted RNA from the Beta CoV positive sample is dispensed into the reaction well of the **Truenat® SARS CoV-2** chip and the test is inserted in the **Truelab®** Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® SARS CoV-2** Chip-based Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, SARS CoV-2 "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, semi-quantitative result is also displayed on the screen. Based on the detection of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed using the **Truelab®** micro PCR printer or transferred to the lab computer /or any remote computer via Wifi network or 3G/GPRS network. Upto 20,000 results in **Truelab® Uno Dx/Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this kit is *RdRp* gene and human *RNase P*. Detection of the human *RNase P* gene serves as a full process internal positive control (IPC) for proper swab collection, nucleic acid extraction and PCR.

5. CONTENTS OF THE Truenat® SARS CoV-2 KIT

A. Individually sealed pouches, each containing a
   1. **Truenat® SARS CoV-2** micro PCR chip.
   2. Microtube with freeze dried RT-PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

REF	601420005	601420020	601420025	601420050	601420100	601420200
Σ	5T	20T	25T	50T	100T	200T

6. STORAGE AND STABILITY

Truenat® SARS CoV-2 is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Universal Sample Pre-treatment Pack and **Trueprep® AUTO** Transport Medium for Swab Specimen Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures upto 45°C. Do not freeze.

7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF623010001 / 633010001 / 643010001 / 653010001) consisting of,

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001/603042001)
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 603021001/603022001/603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat®** Positive Control Kit - Panel I (REF 801010008), Powder free disposable gloves, waste disposal container with lid.

8. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Truenat® SARS CoV-2 requires purified nucleic acids from oropharyngeal or nasopharyngeal swabs specimen that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit and that have tested positive by **Truenat® Beta CoV** (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package inserts of **Trueprep® AUTO** Transport Medium for Swab Specimen Pack, **Trueprep® AUTO** Universal Sample Pre-treatment Pack, **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit and **Truenat® Beta CoV** for details).

9. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the User Manuals and package inserts of all the components of the **Truelab® Real Time micro PCR System** before use.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

10. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g. from Sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the **Truenat® SARS CoV-2** micro PCR chip, microtube and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.

11. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat®** assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truenat®** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat®** assay should be interpreted in the context of other clinical and laboratory findings.

12. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared Sodium hypochlorite [10 times dilution of 5% Sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves should be disposed off as potentially bio-hazardous waste e.g. in a biohazard waste container.

13. TEST PROCEDURE

(Please also refer the **Truelab®** Real Time Quantitiative micro PCR Analyzer user manual)

1. Switch on the **Truelab®** Analyzer .
2. Select User and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "SARS CoV-2" to be run from the Profiles Screen on the Analyzer screen. For **Truelab® Duo/Quattro**, select the Bay (Idle1/2) for **Duo** and (Idle1/2/3/4) for **Quattro** from the Status Screen to view the Profiles Screen. Select the test profile for "SARS CoV-2" to be run from the Profiles Screen on the Analyzer screen.
4. Enter the patient details as prompted in the **Truelab®** Analyzer screen.
5. Press Start Reaction.
6. For **Truelab® Uno Dx,** Press the eject button to open the chip tray. For **Truelab® Duo/Quattro**, the chip tray opens automatically on tapping the "Start Reaction" button.
7. Open a pouch of **Truenat® SARS CoV-2** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat® SARS CoV-2** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
9. Place the microtube containing freeze dried RT-PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation **after ensuring that white pellet of dried PCR reagents remains at the bottom of the microtube.** Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 16). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the Elute Collection Tube into the microtube. Allow it to stand for 30-60 seconds to get a clear solution. ⚠ Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the **Truenat® SARS CoV-2** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the micro tip as per the section on "Disposal and Destruction" (Section 16).
10. For **Truelab® Uno Dx,** slide the chip tray containing the **Truenat® SARS CoV-2** Chip-based Real Time PCR test loaded with the sample into the **Truelab®** Analyzer. Press Done on the "Please Load Sample" Alert message. For **Truelab® Duo/Quattro**, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.
11. Read the result from the screen.
12. After the reaction is completed, for **Truelab® Uno Dx,** push the Eject button to eject the chip tray. For **Truelab® Duo/Quattro**, tap the "Open/Close Tray" button to eject the chip tray.
13. Take out the **Truenat® SARS CoV-2** Chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 16).
14. Turn on **Truelab®** micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to the **Truelab®** Analyzer manual).
15. Switch off the **Truelab®** Analyzer.

14. RESULTS & INTERPRETATIONS

Two amplification curves are displayed on the **Truelab® Real Time micro PCR Analyzer** screen to indicate the progress of the test. Both the target and the internal positive control (IPC)* curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Cycle threshold (Ct) will depend on the number of target nucleic acids in the sample. The curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the viral load as "HIGH (Ct<20)","MEDIUM (20≤Ct<25)", "LOW (25≤Ct<30)" or " VERY LOW (Ct ≥ 30)" for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage. *While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

15. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab®** Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. **Truenat®** Positive Control Kit - Panel I (REF 801010008), The containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances: ● Whenever a new shipment of test kits is received. ● When opening a new test kit lot. ● If the temperature of the storage area falls outside of 2-30° C. ● By each new user prior to performing testing on clinical specimen.

16. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat® SARS CoV-2** chip, microtube, microtube cap, pipette tips etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

17. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity:

Evaluation of analytical sensitivity of **Truenat® SARS CoV-2** assay, in comparison to TaqMan SARS CoV-2 qRT-PCR VRDL assay was performed. Sample with low Ct value (ID 613) was used for this study. An aliquot of VTM of sample ID 613 was extracted using **Trueprep® AUTO** Sample Prep Device (As per manufacturer protocol). RNA was diluted 10 fold and six dilutions were made from **Trueprep® AUTO** elute. These dilution series were run on **Truenat® SARS CoV-2** chips as well as Taq Man SARS CoV-2 qRTPCR systems in parallel. Observed Ct values are given in below table. Both **Truenat®** assays detected up to dilution 10^{-5}(D5) from undiluted sample, with valid Ct value.

Dilutions	Truenat® Beta CoV		Truenat® SARS CoV -2		SARS CoV 2 real-time PCR		
	E gene	Rnase P	RdRp	Rnase P	E Gene	RdRp	Rnase P
613 Neat	15.6	21.14	14.2	21.2	25.75	27.43	27.99
613 D1	19.33	24.5	18	24.43	26.4	28.54	30.11
613 D2	23.6	28.29	20	27.56	29.7	31.8	34.12
613 D3	27	31.4	24.33	31	34.41	36.95	37.60
613 D4	29.8	ND	27.75	ND	37.01	39.32	39.51
613 D5	32.5	ND	30.57	ND	ND	ND	ND
613 D6	ND	ND	ND	ND	ND	ND	ND

Linearity & PCR Efficiency:
Using the dilution series from **Trueprep® AUTO** elutes run on **Truenat® SARS CoV-2**, log linear curve was plotted to check the linearity of Ct values on **Truenat® SARS CoV-2** test.

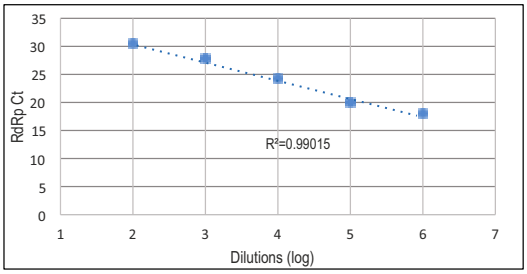
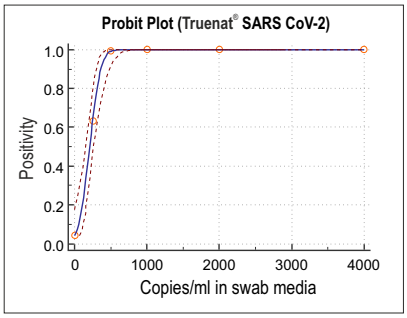


Figure 1: Linearity and PCR efficiency on **Truenat® SARS CoV-2**. Y axis indicate Ct values and X axis is arbitrary log numbers indicating dilutions.
Conclusion: Slope of the curve is -3.289. Assay was observed to be linear over the range of dilutions tested and PCR efficiency was found to be 99.57 %.

Limit of Detection (LoD):
The limit of detection (LoD) was estimated using AccuPlex™ SARS - CoV-2 Verification Panel (Seracare, 0505-0129), as per CLSI EP-17A guidelines. Briefly, six dilutions of quantified material was done in **Trueprep® AUTO** Transport Medium for Swab Specimen Pack. Each dilution was extracted 24 times using **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and tested on the **Truenat® SARS CoV-2** chip. Probit regression was used to estimate LoD at 95% positivity, as indicated below.



LoD was estimated to be 407 genome copies/ml in transport medium for swab specimen, with 95% CI interval of 335-553.

Precision:
To evaluate repeatability of the assay, three clinical elutes representing High, Medium and Low Ct values {Sample IDs: 383 (Ct: 26.71), 1263 (Ct: 20.75), 885 (Ct: 14.5)} were run on devices used in this evaluation. Following table depicts the Precision analysis. Ct values for RdRp are given, with observed standard deviation and % CV.

	Truenat® SARS CoV-2 RdRp (Ct)		
Equipment ID	**ID:383**	**ID:1263**	**ID:885**
TLDU0401	26.71	20.75	14.67
TLDU1308	26.14	21	14.5
TLDU1306	26.2	21.17	15
TLQU0001	26.14	20.75	14.5
Mean	**26.30**	**20.92**	**14.67**
STDEV	**0.28**	**0.21**	**0.24**
%CV	**1.1%**	**1.0%**	**1.6%**

Conclusion: The test was found to be reproducible with percent coefficient of variation in Ct values significantly less than 10%, across devices.

Specificity and cross reactivity:
Specificity of the test was evaluated using a panel of clinical samples, including COVID-19 negatives and other respiratory disease positive ones. The panel included; H1N1 (15 samples: 5 positive and 10 Negative), Severe Acute Respiratory Illness (SARI) (4 samples), and also negative blood samples (3 from SARS CoV-2 negative cases) and confirmed COVID-19 negatives (20 swab samples) were used. *RdRp* target was not detected in any of above specimens, indicating specificity and no cross reactivity to other common respiratory pathogens.

Clinical evaluation:
Clinical evaluation of **Truenat® SARS CoV-2** was performed at the State VRDL lab at Bangalore Medical College. Totally, 30 confirmed SARS CoV-2 positive and 45 confirmed negative swab samples were tested.

		SARS CoV 2 real-time PCR (n=75)		
		Positive	Negative	Total
Truenat® SARS CoV-2	Positive	30 [TP]	0 [FP]	30
	Negative	0 [FN]	45 [TN]	45
	Total	30	45	75

TP=True positive, TN= True negative, FP = False positive, FN = False negative

All positive and negatives were correctly detected on **Truenat® SARS CoV-2** assay indicating 100% sensitivity, specificity and 100% overall concordance to reference gold standard assay.

18. REFERENCES

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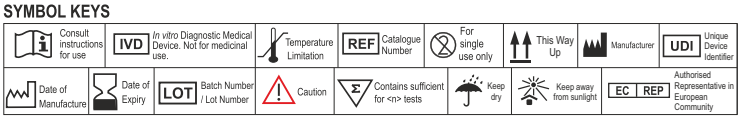
SYMBOL KEYS



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