CE

Truenat®

COVID-19

Chip-based Real Time Duplex PCR Test for COVID-19

1. INTENDED USE

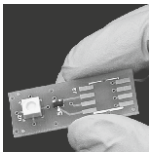
Truenat® COVID-19 (REF 601430005 / 601430020 / 601430025 / 601430050 / 601430100 / 601430200) is a Chip-based Real Time Duplex Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi-quantitative detection of SARS CoV-2 RNA in human oropharyngeal and nasopharyngeal swab specimen and aids in detection and confirmation of SARS CoV-2 infection and diagnosis of COVID-19. The test detects the *E* and *Orf1a* genes of the virus. **Truenat® COVID-19** runs on **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® COVID-19** 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 RNA viruses 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 Kits 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® COVID-19** is a disposable, room temperature stable, Chip-based Real Time Duplex 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® COVID-19** chip also stores information of used test to prevent any accidental re-use of the chip.



NOTE : Truelab® / Truenat® / Trueprep® / Truepet® / STABILYSE® 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® COVID-19 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. The **Truenat® COVID-19** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried PCR reagents, including reverse transcriptase (RT) and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® COVID-19** 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® COVID-19** 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, *E* and *Orf1a* gene "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 *E* and *Orf1a* 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® COVID-19 KIT

A. Individually sealed pouches, each containing a
   1. **Truenat® COVID-19** micro PCR chip.
   2. Microtube with freeze dried RT PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch.

B. Package Insert.

REF	601430005	601430020	601430025	601430050	601430100	601430200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep® AUTO Universal Sample Pre-treatment Pack (for Extraction with Trueprep® AUTO/AUTO v2)

A. Lysis Buffer (contains lysis cum transport medium).
B. Disposable transfer pipette (graduated).
C. Package Insert.

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
Σ	5T	20T	25T	50T	100T	200T

7. CONTENTS OF THE Trueprep® AUTO Transport Medium for Swab Specimen Pack (for Extraction with Trueprep® AUTO/AUTO v2)

A. Transport Medium for Swab specimen tubes (contains transport medium).
B. Package Insert.

REF	60206TS05	60206TS20	60206TS25	60206TS50	60206TS100	60206TS200
Σ	5T	20T	25T	50T	100T	200T

8. CONTENTS OF THE STABILYSE® Prep Free pack (for Rapid PCR Protocol)

A. Collection and Lysis Medium Tube
B. Package Insert.

REF	90101PF05	90101PF20	90101PF25	90101PF50	90101PF100	90101PF200
Σ	5T	20T	25T	50T	100T	200T

9. STORAGE AND STABILITY

Truenat® COVID-19 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, **Trueprep® AUTO** Transport Medium for Swab Specimen Pack and **STABILYSE®** Prep Free Pack are 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.

10. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF 623010001 / 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 (REF603021001/603022001/603023001).
3. **Truelab**® micro PCR Printer (REF 603050001).
4. **Truepet**® SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab**® Microtube Stand (REF 603070001).
6. Dry Bath (for Rapid PCR protocol)

Also required additionally are: **Trueprep**® **AUTO** Universal Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep**® **AUTO** Transport Medium for Swab Specimen Pack (REF60206TS05 / REF60206TS20 / REF60206TS25 / REF60206TS50 / REF60206TS100 / REF60206TS200), **STABILYSE**® Prep Free Pack (REF 90101PF05 / 90101PF20 / 90101PF25 / 90101PF50 / 90101PF100 / 90101PF200), **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.

11A.SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Swab specimen:

Oropharyngeal or nasopharyngeal swab specimen must be collected as per standard procedures using a standard nylon flocked swab. Insert the swab with specimen into the Transport Medium for Swab Specimen Tube provided and mix well by repeatedly twirling the swab in the buffer solution. Gently break the handle of the nylon swab at the break point, leaving the swab containing the specimen in the Transport Medium for Swab Specimen Tube. Tightly close the cap of the Transport Medium for Swab Specimen Tube (Refer to the package insert of **Trueprep**® **AUTO** Transport Medium for Swab Specimen Pack for further details). ⚠ Dispose off the remaining part of the swab after use, as per the section on "Disposal and Destruction" (Section 19).

Sample Storage and Transportation:

Transport Medium for Swab Specimen decontaminates the specimen and makes it ready for storage / transportation / extraction. The specimen in this form is stable for up to three (3) days at 40ºC and one(1) week at 30ºC.

Nucleic acid extraction:

Transfer 500 µL from the Transport Medium for Swab Specimen Tube into the Lysis Buffer Tube for oropharyngeal or nasopharyngeal swabs for further procedure (Refer to the package insert of **Trueprep**® **AUTO** Universal Sample Pre-treatment pack for further details) with the **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit (Refer to the User Manual of **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep**® **AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit for details). ⚠ Dispose off the Transport Medium for Swab Specimen Tube with cap, lysis buffer tube with cap and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 19).

11B.SPECIMEN PREPARATION FOR RAPID PCR PROTOCOL

Swab specimen:

Oropharyngeal or nasopharyngeal swab specimen swab specimen must be collected as per standard procedures using a standard nylon flocked swab. Insert the swab with specimen into the provided Collection and Lysis Medium Tube and mix well by repeatedly twirling the swab in the buffer solution. ⚠ Dispose off the swab after use, as per the section on "Disposal and Destruction" (Section 19). Tightly close the cap of the tube.

Sample Storage and Transportation:

The media is used as a medium for sample collection and lysis for Prep Free Swab Specimen. The specimen in this form is stable for up to two (2) days at 30ºC and one(1) week at 2ºC - 8ºC.

Sample processing : Set the dry bath at 104°C. Once the temperature reaches to the set temperature [+/- 2°C] place the solution in the Collection and Lysis Medium Tube in dry bath and incubate for 5 minutes at 104°C. Remove the tube from the dry bath and allow to stand at Room Temperature for at least 3 minutes (Refer to the package insert of **STABILYSE**® Prep Free pack for further details). Proceed for the further test procedure as per the Section 16. ⚠ Dispose off Collection and Lysis Medium Tube with cap after use, as per the section on "Disposal and Destruction" (Section 19).

12. 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, package inserts and Material Safety Data Sheets (MSDS) 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.

13. 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**® **COVID-19** 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.

14. 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.

15 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.

16. 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 "COVID-19" 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 "COVID-19" 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**® **COVID-19** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat**® **COVID-19** 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 19). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the Elute Collection Tube (for Extraction with **Trueprep**® **AUTO/AUTO v2**)/Collection and Lysis Medium Tube (for Rapid PCR protocol) 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**® **COVID-19** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the microtip as per the section on "Disposal and Destruction" (Section 19).
10. For **Truelab**® **Uno Dx,** slide the chip tray containing the **Truenat**® **COVID-19** 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® COVID-19** Chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 19).
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.

17. RESULTS & INTERPRETATIONS

Three 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 time taken (Ct) of the specimen will depend on the number of virus copies 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.

Detection Channel			Result Interpretation	Action
Orf1a	*E*	*RNase P*		
+	+	+/-	SARS CoV-2 POSITIVE	Report Positive
+	-	+/-	SARS CoV-2 POSITIVE	Report Positive
-	+	+/-	SARS CoV-2 PRESUMPTIVE POSITIVE	Repeat after 48-72 hours
-	-	+	SARS CoV-2 NEGATIVE	Report Negative
-	-	-	INVALID	Collect new swab and repeat

18. 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.

19. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as remaining part of the swab, Transport Medium for Swab Specimen Tube with cap, lysis buffer tube with cap, transfer pipettes, Collection and Lysis Medium Tube (used for Rapid PCR Protocol), **Truenat® COVID-19** 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.

20. SPECIFIC PERFORMANCE CHARACTERISTICS

Performance parameters

1. Analytical Sensitivity:

Evaluation of analytical sensitivity of **Truenat® COVID-19** assay, in comparison to TaqMan SARS CoV-2 qRT-PCR VRDL assay was performed. Sample with low Ct value (ID 54049) was used for this study. An aliquot of VTM of sample ID 54049 was extracted using **Trueprep® AUTO** Universal Cartridge Based 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® COVID-19** chips as well as Taq Man SARS CoV-2 qRTPCR systems in parallel. Observed Ct values are given in below table.

	Truenat® COVID-19			Taqman SARS CoV2 [VRDL]		
Sample ID	*Orf1a*	*E* Gene	RNAseP	*Orf1a*	*E* Gene	RNAseP
54049 D1	19.67	19.17	27.40	28.71	24.12	28.39
54049 D1	20.14	19.11	27.33			
54049 D1	19.60	19.00	27.33			
54049 D2	23.17	22.17	31.29	32.32	28.57	38.47
54049 D2	23.29	22.40	31.14			
54049 D2	23.20	22.33	30.60			
54049 D3	26.29	25.80	33.15	37.29	31.69	ND
54049 D3	26.29	26.50	33.17			
54049 D3	26.00	25.80	33.80			
54049 D4	29.67	29.14	ND	ND	34.06	ND
54049 D4	30.00	29.20	ND			
54049 D4	30.83	29.50	ND			
54049 D5	33.20	31.50	ND	ND	ND	ND
54049 D5	32.28	32.33	ND			
54049 D5	33.00	32.13	ND			
54049 D6	ND	ND	ND	ND	ND	ND
54049 D6	ND	ND	ND			
54049 D6	ND	ND	ND			

Conclusion: Truenat® assay detected up to dilution 10^{-5}(D5) from undiluted sample, with valid Ct value.

2. Linearity & PCR Efficiency:

Using the dilution series from **Trueprep® AUTO** elutes run on **Truenat® COVID-19**, log linear curve was plotted to check the linearity of Ct values on **Truenat® COVID-19** test.

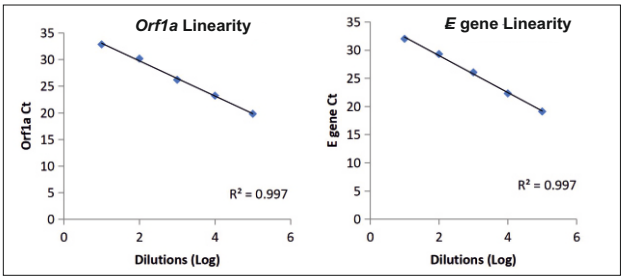


Figure 1: Linearity and PCR efficiency on **Truenat® COVID-19**. Y axis indicate Ct values and X axis is arbitrary log numbers indicating dilutions.

Conclusion: Slope of the curve is *Orf1a* (-3.303) and *E* gene (-3.278). Assay was observed to be linear over the range of dilutions tested and PCR efficiency was found to be 100.8 % for *Orf1a* and 101.87% for *E* gene.

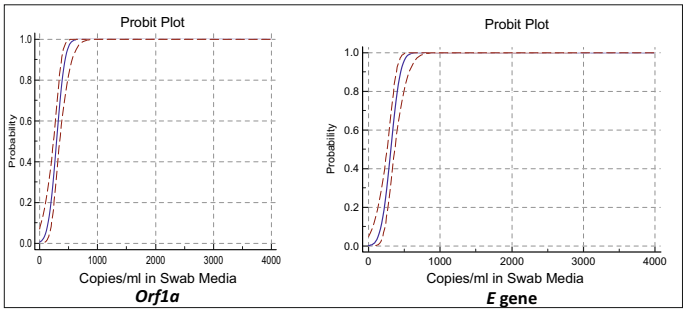
3. Analytical Sensitivity test with samples of low viral load:

Evaluation of analytical sensitivity of **Truenat® COVID-19** multiplexed PoC test, in comparison to TaqMan SARS-CoV-2 rRT-PCR VRDL assay was performed. Five Samples with low viral load (low viral copy number) for *Orf1a / E* gene was used for this study. An aliquot of virus transport medium (VTM) containing sample was extracted using **Trueprep® AUTO** and 12 replicates of each sample was run on **Truenat® COVID-19** and compared against TaqMan SARS CoV-2 qRT-PCR VRDL assay.

Conclusion: All replicates of 5 low viral load samples were detected by **Truenat® COVID-19**, *Orf1a* assay.

4. Limit of Detection (LoD):

The limit of detection (LoD) was estimated using AccuPlex™ SARS-CoV-2 Verification Panel (Seracare, 0505-0168), as per CLSI EP-17A guidelines. Briefly, six dilutions of quantified material were 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® COVID-19** chip. Probit regression was used to estimate LoD at 95% positivity, as indicated below.



LoD of *Orf1a* and *E* gene was estimated to be 480 and 487 genome copies/mL respectively and in transport medium for swab specimen, with 95% CI interval of 410 - 628 and 419-631 respectively.

5. Precision:

To evaluate reproducibility and repeatability of the assay, ten clinical elutes representing High, Medium and Low Ct values {Sample IDs:54053(Ct:20.75), 54716(Ct:25.8),54724(Ct:14.71),54745(Ct:29.43),54803(Ct:14.11),54897 (Ct:22.83),54918(Ct:24.06),54935(Ct:20.43),55055(Ct:14.60),55113(Ct:24.29} were run on devices used in this evaluation. Following table depicts the Precision analysis. Ct values for *E* gene and *Orf1a* are given, with observed standard deviation and % CV.

	ID: 54053		ID: 54716		ID: 54724		ID: 54745		ID: 54803	
Device ID	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene
TLDU1306	27.50	26.33	25.80	24.50	14.71	13.67	29.43	29.00	14.11	12.60
TLDU0431	28.13	26.43	26.80	25.00	14.30	13.50	30.14	29.17	13.50	12.60
TLDU0381	28.14	26.22	26.35	24.60	14.50	13.43	31.50	29.33	14.17	12.57
TLDU0366	26.75	25.80	26.00	24.43	14.17	13.33	28.67	29.00	13.86	12.60
Mean	27.63	26.20	26.24	24.63	14.42	13.48	29.94	29.13	13.91	12.59
STDEV	0.659	0.277	0.439	0.255	0.236	0.143	1.204	0.158	0.305	0.015
%CV	2.38	1.06	1.67	1.03	1.64	1.06	4.02	0.54	2.19	0.12

	ID: 54897		ID: 54918		ID: 54935		ID: 55055		ID: 55113	
Device ID	*Orf1a*	*E* Gene	*Orf1a*	*E*Gene	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene	*Orf1a*	*E* Gene
TLDU1306	22.83	22.27	24.06	21.29	20.43	19.43	14.60	13.60	24.29	23.00
TLDU0431	23.13	22.14	22.40	21.00	20.17	19.50	15.00	13.83	24.10	23.13
TLDU0381	23.00	22.00	23.60	21.00	21.25	19.80	15.14	13.50	24.71	23.14
TLDU0366	22.71	21.80	23.00	20.80	20.60	19.60	14.67	13.43	24.43	23.13
Mean	22.92	22.05	23.27	21.02	20.61	19.58	14.85	13.59	24.38	23.10
STDEV	0.185	0.201	0.722	0.202	0.460	0.161	0.259	0.175	0.257	0.067
%CV	0.81	0.91	3.10	0.96	2.23	0.82	1.74	1.28	1.05	0.29

Conclusion: The test was found to be reproducible with percent coefficient of variation less than 5% which is well below the accepted 10%, across samples and between devices.

6. Clinical Sensitivity:

Clinical sensitivity was tested by running 40 confirmed positives samples of SARS-COV-2; representing high [12], medium [13] and low Ct [15] value samples for testing and comparison with both the systems using three lots of **Truenat® COVID-19**. All positive samples were detected by all three lots of **Truenat® COVID-19** assay.

7. 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 (38 samples: 8 positive and 30 Negative), Severe Acute Respiratory Illness (SARI) (30 samples), and also blood samples (3 from SARS CoV-2 positive cases and 3 from SARS CoV-2 negative cases) and confirmed COVID-19 negatives (30 swab samples) were used.

E gene and *Orf1a* was not detected in any of above specimens, indicating specificity and no cross reactivity to other common respiratory pathogens.

8. Clinical evaluation:

Clinical evaluation of **Truenat® COVID-19** was performed at the State VRDL lab at Bangalore Medical College. Totally, 155 confirmed SARS CoV-2 positives and 323 confirmed RT-PCR negative samples were tested. Comparison of results from Real Time PCR (BioRad CFX96) and **Truenat® COVID-19** is summarized as follows:

		SARS CoV 2 real-time PCR (n=478)		
		Positive	Negative	Total
Truenat® COVID-19	Positive	155	0	155
	Negative	0	323	323
	Total	155	323	478

All positive and negatives were correctly detected on **Truenat® COVID-19** assay indicating 100% sensitivity, specificity and 100% overall concordance to reference gold standard assay.

21. REFERENCES

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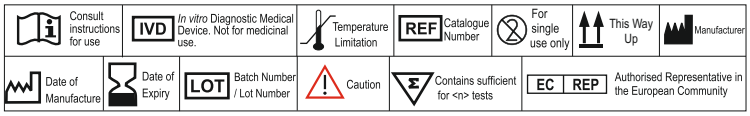
SYMBOL KEYS



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