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1. **PURPOSE**

To provide instructions for performing chip based RT-PCR) for the detection of SARS-CoV-2 target genes using Truenat in the RNA isolated from the clinical specimens.

2. INTRODUCTION

This SOP describes Truenat method to detect SARS-CoV-2 from the clinical specimen. Truenat is highly sensitive and, has very low limit of detection). The procedure is carried out after viral RNA has been extracted from ICMR approved specimen types, ideally in a BSL 2 equivalent facility. Adherence to good laboratory practices is critical for quality test results.

3. PRINCIPLE

SARS-CoV-2 is detected based on the presence of viral RNA in patient specimens. RNA isolated from the specimen is converted to complementary DNA (cDNA). cDNA is then used to amplify viral target genes by PCR. Real time detection of amplified target during PCR is achieved by using fluorescent probes in the PCR reaction and detection of fluorescence signals by the instrument. Presence or absence of fluorescence signal/s specific to the viral target gene/s is the indicator of the presence of the virus in the specimen.

During PCR reaction, primers and probes bind to their specific target regions on cDNA template at a suitable annealing temperature. Taq DNA polymerase (Taq) binds to the 3'end of the primer and starts its polymerase activity. When Taq encounters the probe bound to the cDNA, it uses its exonuclease activity to degrade the bound probe and continues to synthesize the complementary DNA. When degraded, the fluorophore in the probe is released from the Quencher and emits Fluorescence. As amplification cycles progress more copies of the target DNA are generated to which free probes bind and subsequently get degraded by Taq DNA polymerase. Accumulation of free fluorophore in the reaction mix is detected by the real time PCR machine and is shown as gradual increase in fluorescence intensity. Therefore, presence of target in reaction tube will lead to detectable increase in fluorescence intensity.

In a sample with no SARS-CoV-2 specific cDNA, probes have no target to bind to and are unable to emit fluorescence, as they are inhibited by the quencher in the intact probe.

PCR primer-probe combinations can be designed in such a way that one can detect signal to multiple targets in one reaction. While designing such combinations one needs to make sure that the instrument specification matches with fluorophore excitation & emission wavelengths.

Ct Value: The number of cycles at which the detected fluorescence signal exceeds background levels is called the threshold cycle (Ct). Lower Ct value implies high levels of target RNA in the patient sample. Conversely, high Ct value implies low levels of target RNA in the patient sample (4).As a rule of thumb, Ct values above 38 are not considered valid.

4. PERSONNEL QUALIFICATIONS & RESPONSIBILITIES

The lab personnel performing this procedure must have:

- Knowledge of the principle of the procedure being used
- Expertise in micro pipetting skills
- Knowledge of good laboratory practices
- Understanding of organization of workflow in a COVID - PCR lab
- Knowledge of contamination control methods
- Understanding of the importance of laboratory results for patient management

Responsibilities

- It is the responsibility of the lab personnel to correctly understand and perform this procedure.
- All users of this procedure who do not understand it or are unable to carry it out as described are responsible for seeking advice from their supervisor.

5. EQUIPMENT & MATERIALS

5.1 Truelab® Real Time micro PCR Workstation consisting of Trueprep® AUTO/AUTO v2 Sample Prep Device, 2. Truelab® Uno Dx/Truelab® Duo/Truelab® Quattro Real Time micro PCR Analyzer . 3. Truelab micro PCR Printer 4. Truepet® SPA fixed volume precision micropipette - 6 µl. 5. Truelab® Microtube Stand .

5.2 Also required additionally are: Trueprep® AUTO Universal Sample Pre-treatment Pack ,Trueprep® AUTO Transport Medium for Swab Specimen Pack, Trueprep® AUTO Universal Cartridge Based Sample Prep Kit

5.3 or Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit

5.4 Truenat™ Universal Control Kit , Powder free disposable gloves, waste disposal container with lid.

5.5 Autoclave

5.6 Biosafety Cabinet (Optional)

5.7 Refrigerators

5.7.1 -70/ -80°C, -20°C freezers

5.7.2 4°C Refrigerators

5.7.3 Benchtop minifuge

5.8 Reagents & Disinfectants

5.8.1 RNAse Zap

5.8.2 Truenat Covid -19 RT- PCR Kit that contains

- Trueprep Auto Universal Extraction Kit
- Truenat Covid 19 duplex Detection Kit
- Universal Control Kit Panel 1
- **Should be stored at the temperature recommended by the manufacturer and always protected from light. The reagents should be aliquoted to avoid repeated freeze thaw.**

5.8.3 Nuclease free Water

5.8.4 Reagent grade Ethanol and Isopropanol

5.8.5 Absolute alcohol

5.8.6 Sodium Hypochlorite

5.8.7 Biohazard bags Red &Yellow

5.9 Personal Protecting Equipment (PPE)

5.9.1 Lab gowns
5.9.2 Surgical/medical masks/
5.9.3 N95 Mask
5.9.4 Gloves
5.9.5 Shoe Covers
5.9.6 Hair Covers

6. PROCEDURE

7.

6.1 Testing

1. Switch on the Truelab™Analyzer
2. Select User and enter password.
3. Select the test profile for “H1N1” to be run from the TM Profiles Screen on the Analyzer screen.
4. Enter the patient details i.e. Patient Name, Patient ID and Age in the Truelab™Analyzer screen.
5. Press Start Reaction.
6. Press the eject button to open the chip tray.
7. Open a pouch of Truenat™ Covid 19 and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Label the tube with the patient ID using a marker pen on the microtube.
9. 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.
10. Place the microtube containing freeze dried RT PCR reagents in the microtube TM 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 in 1% hypochlorite solution. Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the Elute Collected Tube into the microtube. Allow it to stand for 30-60 seconds to get a clear solution.
11. **Note:-** Do not mix it by tapping, shaking or by reverse pipetting.
12. 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 well

surface and not to spill elute on the outside of the well. Dispose off the microtip in 1% hypochlorite solution.

13. Slide the chip tray containing the Truenat™ Covid 19 chipTM based Real Time PCR test loaded with the sample into the TruelabAnalyzerTM. Press Done on the "Please Load Sample" Alert message
14. Read the result from the screen.
15. After the reaction is completed, for Truelab Uno Dx, push the Eject button to TM eject the chip tray.
16. Take out the Truenat™ Covid 19 chip-based Real Time PCR test at end of the test and dispose it off IN 1% hypochlorite.
17. 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.
18. Switch off the Truelab™Analyzer or repeat steps 3 - 16 to run another sample.

6.2 Targets

Target
ORF1ab
E gene
RNaseP

Detection of the Positive control in fluorescence channel for E and Orf 1 gene. Detection of the internal control (IC) in fluorescence channel for RNase P.

6.3 Data Collection.

- After completion of the run, save the result file. Ensure a proper backup of the data.
- Discard the chip in the amplification room in a waste bin lined with red biohazard bag.

7 QUALITY CONTROL

To ensure that the Truelab™ Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The Universal Control kit (REF 601100008) containing Positive Control and Negative Control are to be used as control material.

Run 1 positive and 1 negative control under the following circumstances:

- After every 20 tests
- When opening a new test kit lot
- When asked by authorized signatory for any other reason.

8 RESULT & INTERPRETATION

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”, “MEDIUM”,“LOW” or “ VERY LOW” 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.

The negative control reactions for probe/primer sets should not exhibit fluorescence growth curves (Orf 1 ab and E gene that cross the threshold line.

If a false positive occurs with one or more of the primers and probe non template control (NTC) reactions, sample contamination may have occurred.

The positive control reactions for each probe/primer reactions should give following Ct values:

Positive control	**Expected Ct values**
ORF1ab	≤32

E gene	≤32
RNase P	≤32

All clinical samples should exhibit RNase P reaction curves that cross the threshold line at or before 32cycles.

Failure to detect RNase P in any of the clinical samples may indicate:

(a) Improper extraction of nucleic acid from clinical materials resulting in loss of RNA

(b) Carryover of RT-PCR inhibitors from clinical specimens

(c) Improper assay set up and execution

9. 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.

10. WASTE MANAGEMENT, CONTAMINATION CONTROL AND OTHER SAFETY PRECAUTIONS

a. Waste management:

Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents and require use of standard precautions.

Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures.

The waste should be collected in bins lined with red bags, removed periodically from each room and autoclaved.

i. Always properly clean work area after completion of tasks
ii. Establish a regular (e.g. weekly) and thorough laboratory cleaning protocols (floors, doors, walls)

b. Contamination Control

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 containing sample

11. REFERENCES

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b) Diagnostic testing for SARS-CoV-2: interim guidance document WHO, 11 September 2020: https://apps.who.int/iris/handle/10665/334254
c) Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase. Holland PM, Abramson RD, Watson R, Gelfand DH. Proc Natl Acad Sci U S A. 1991 Aug 15;88(16):7276-80. doi: 10.1073/pnas.88.16.7276. PMID:1871133;PMCID: PMC52277. https://pubmed.ncbi.nlm.nih.gov/1871133/
d) Phases of Real-Time PCR: https://www.labce.com/spg1913034_phases_of_real_time_pcr.aspx
e) Trunat™ Operating Manual

SOP CHANGE HISTORY

New version # / date	Old version # / date	No. of changes	Description of changes	Source of change request