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Reaching Impact, Saturation, and Epidemic Control (RISE)

Learning Resource Package for SARS-CoV-2 diagnostics

Guidelines on testing methodologies, testing strategies, and setting up of SARS-CoV-2 testing laboratories

August 2022

Executive Summary

The first cases of novel coronavirus disease 2019 (COVID-19) were reported by the World Health Organization (WHO) in December 2019. Since then, there have been almost 270 million reported cases of COVID-19 and more than 5 million deaths due to this disease. COVID-19 is caused by a virus known as SARS-CoV-2. The COVID-19 pandemic represents one of the most severe public health catastrophes the world has seen.

In the early stages of the pandemic there were few tools at our disposal to tackle the spread of the virus. The only control measures were non-pharmaceutical interventions (NPIs), such as social distancing, wearing of face masks and, most extreme of all, lockdowns. While such measures can reduce the transmission of COVID-19, they also have detrimental effects on mental health, children's education, and a country's economy.

We now have a wider range of tools at our disposal. There has been some progress in treatment of COVID-19 with the availability of new and repurposed drugs. Importantly, several effective vaccines have been developed, which indicates that there may be light at the end of the pandemic tunnel. However, despite the medical and technological advances that have been made, their rollout globally has been uneven, and there are considerable issues in terms of access, especially to vaccines. Therefore, diagnostics remain a key weapon in the fight against COVID-19.

Diagnostics are important for several reasons. The laboratory confirmation of cases rapidly provides information to enable appropriate clinical care and public health measures such as isolation or quarantine in a timely manner. Diagnostics are essential to enable effective disease surveillance, monitor disease trends over time and assess the effectiveness of any control interventions that may be used.

There are three major types of diagnostics that can be particularly useful during a pandemic situation. The first type are molecular tests, such as nucleic acid amplification tests (NAATs) and polymerase chain reaction (PCR) tests, which are used to detect viral nucleic acid. The second type detect viral proteins; these include lateral flow assays, also referred to as antigen rapid diagnostic tests. Finally, there are tests that detect antibodies produced by an individual's immune system in response to a viral infection. This wide range of diagnostic tools requires a commensurately wide range of infrastructure, equipment and highly trained staff.

The Learning Resource Package (LRP) presented here covers all steps of the diagnostic process. It begins with the training presentations for the molecular testing protocols, sampling process, biosafety aspects and troubleshooting exercises. This is followed by the trainers' scripts and manual, standard operating procedures for few protocols and finally the training evaluation methodology.

This LRP is a comprehensive resource for all laboratory technicians, scientists, microbiologists and pathologists involved in the diagnostic laboratory ecosystem.

Acknowledgments

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We are grateful to the team at FIND, the global alliance for diagnostics, for insights derived from its vast experience in laboratory management. Further, this volume would not have been possible without the support of JHPIEGO, a non-profit organization for international health affiliated with Johns Hopkins University.

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Intended Audience(s)

This LRP is intended for members of committees appointed for developing or updating a SARS-CoV-2 testing facility, and other relevant stakeholders who influence SARS-CoV-2 testing related activities, such as laboratory personnel, laboratory managers, administrators, program managers and healthcare workers working in SARS-CoV-2 diagnostics.

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List of Abbreviations

BMW	Biomedical Waste Management
BSC	Biosafety Cabinet
BSL	Biosafety Level
CAPA	Corrective and Preventive Action
CBNAAT	Cartridge-Based Nucleic Acid Amplification Test
CBWTF	Common Bio-medical Waste Treatment and Disposal Facility
CDC	Centre for Disease Control
cDNA	Complementary DNA
CLIA	Chemiluminescent Immunoassay
CPCB	Central Pollution Control Board
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CsCl	Cesium Chloride
CSF	Cerebro Spinal Fluid
Ct	Cycle Threshold
DEPC	Diethyl Pyrocarbonate
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
E gene	Envelope Gene
ELISA	Enzyme-Linked Immunosorbent Assay
EQA	External Quality Assurance
GCE	Genome Copy Equivalent
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMPP	Good Microbiological Practices and Procedures
HoCl	Hypo Chlorite
IATA	International Air Transport Authority
IC	Internal Control
ICMR	Indian Council of Medical Research
ICP	Internal Control Procedures

ILC	Inter-Laboratory Comparison
ILI	Influenza-Like Illness
INSACOG	Indian SARS-Cov-2 Genomics Consortium
KPI	Key Performance Indicators
LAMP	Loop-Mediated Isothermal Amplification
LFIA	Lateral Flow Immunoassay
LIMS	Laboratory Information Management System
LoD	Limit Of Detection
MIQE	Minimum Information for Publication of quantitative Real time PCR
MoHFW	Ministry of Health and Family Welfare
MSDS	Material Safety Data Sheet
N gene	Nucleocapsid Gene
NAAT	Nucleic Acid Amplification Test
NABL	National Accreditation Board for Testing and Calibration Laboratories
NACO	National AIDS Control Organisation
NFW	Nuclease Free Water
NIV	National Institute of Virology
NTC	No Template Control
ORF	Open Reading Frame
PBS	Phosphate Buffered Saline
PCC	Probe Check Control
PCR	Polymerase Chain Reaction
PFU	Plaque-Forming Unit
POCT	Point-Of-Care Testing
PPE	Personal Protective Equipment
QC	Quality Control
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
RAT	Rapid Antigen Test
RBD	Receptor Binding Domain
RdRp	RNA-dependent RNA Polymerase
RFU	Relative Fluorescence Unit
RGSL	Regional Genome Sequencing Laboratory

RNA	Ribonucleic Acid
RT-LAMP	Reverse-Transcription-Loop-Mediated Isothermal Amplification
RT-PCR	Real-Time/Reverse Transcription-Polymerase Chain Reaction
S gene	Spike Gene
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SDS	Sodium Dodecyl Sulphate
SGMA	Spike Gene Mutation Amplification
SGTF	Spike Gene Target Failure
SOP	Standard Operating Procedure
SPC	Sample Processing Control
SRF	Specimen Referral Form
SWM	Solid Waste Management
TAT	Turn Around Time
TE	Tris EDTA
TRF	Test Requisition Form
URT	Upper Respiratory Tract
UV	Ultraviolet
VLM	Viral Lysis Medium
VOC	Variant of Concern
VTM	Viral Transport Medium
WHO	World Health Organization

Table of Contents

Donning and Doffing of PPE

WHY PPE?

- Laboratory and hospital staff are at very high risk of getting infected with SARS-CoV-2 virus, which spreads through droplet as well as aerosol mode and is highly contagious from person to person.
- To protect the healthcare worker from exposure to potential infectious agents and prevent any form of injury, PPE is a must .
- Donning PPE is the main risk mitigation activity.

PPE – Required for the Staff

- Full sleeves laboratory coats must be worn at all times in a laboratory
- Appropriate disposable gloves must be worn for all procedures –for any contact with blood, other body fluids or other potentially infectious materials.
- Safety glasses or goggles, face shields (visors) or other protective devices- protect the eyes and face from splashes- impacting objects or artificial ultraviolet radiation.
- Footwear must be worn in the laboratory - design that minimizes slips and trips - reduces the likelihood of injury from falling objects and exposure to biological agents.
- Respiratory protection – N95 Mask

PPE requirement in COVID - 19 Laboratory

Activity	Recommendation	Safety level
Specimen Collection	Full PPE (Single use, overall with sterile gloves, Eye & face protection and N95 respirator)	NA
Transport, Receipt	Lab coat, N95 Respirator and Gloves	NA
Specimen opening and RNA extraction	Single use gown, with sterile gloves, N95 respirator, eye protection	BSL- 2 (Negative Pressure)
Master mix, PCR setup	Laboratory coat/apron, face mask , sterile gloves	NA (PCR Hood, Positive Pressure)

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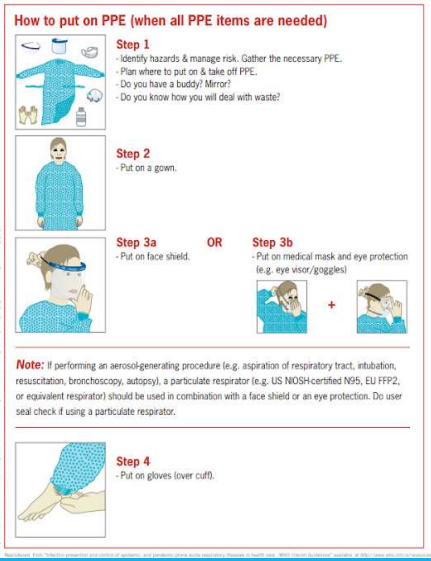
Donning of PPE-Steps….I

- Remove all personal belongings: mobile phone, watch, bangles, jewelry, pen, etc. and store them safely in a locker
- Drink water and use the washroom prior to donning PPE
- Put on scrubs or work uniform
- Wash hands thoroughly
- Go to the donning room.
- Choose the right size of PPE
- Perform hand hygiene

Donning of PPE – Steps Involved

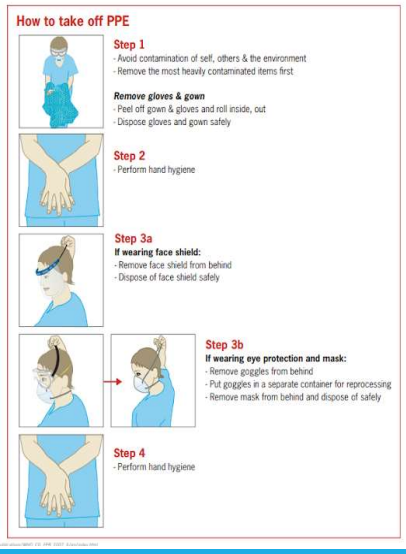


1. Put on gloves.
2. Put on the Coverall gown starting from below, followed by arms.
3. Pull the coverall up, close fully up to neck.
4. Wear shoe cover, if it is separate. Some cover-all come with foot covers.
5. Put on the N95 mask. No need to wear two masks or add a surgical mask. Check fitness by blowing air. There should be no leak.
6. Pull the head cover & fix it.
7. Put on goggles or face shield.
8. Put on the plastic apron (if it is part of the kit)
9. Put on second set of gloves. Ensure that gloves cover the gown sleeve.
10. Perform hand hygiene.

Doffing of PPE- 1

- Doffing room has two chairs- clean and dirty.
- Perform hand hygiene over gloves.
- Sit on the dirty chair.

Doffing of PPE – Steps Involved



1. Remove the plastic apron- discard -red bag (if its part of kit)
2. Perform hand hygiene
3. Remove foot cover and discard.
4. Perform hand hygiene.
5. Remove the outer pair of gloves and discard.
6. Perform hand hygiene on inner gloves .
7. Remove Face shield.
8. Perform hand hygiene.
9. Remove coverall by sliding hood and then rolling from inside out and then discard.
10. Remove and discard inner gloves.
11. Wear new pair.
12. Remove and discard N95 mask, without touching the outer surface.
13. Move to clean chair. Disinfect the outer side of shoes with wipes.
14. Remove gloves.
15. Perform hand hygiene.

Potential adverse effect of PPE

- Dry Skin
- Headache
- Dyspnoea
- Sweating
- Itching

Donning and Doffing of PPE - Video

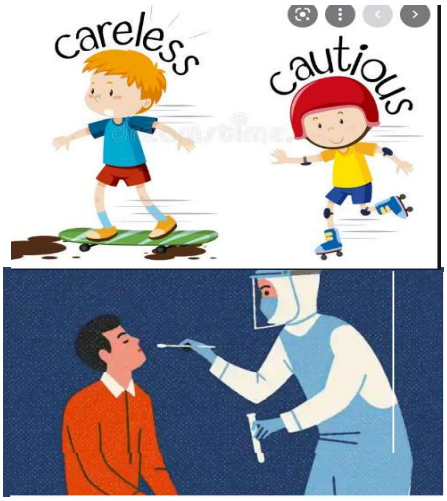
https://www.youtube.com/watch?v=KXuecIFu1PU

Specimen collection, Packaging storage and transport

Specimen Collection

Specimen Collection

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious diseases



What materials are needed for sample collection

Step	Supplies
Safety materials	Appropriate PPE, waste disposal and disinfectants
Sample materials	Swabs, transport medium, suitable collection tools and containers
Suitable labelling materials	Marker pens or barcodes
Triple packaging materials	Absorbent material, plastic bag, sturdy outer container, racks, cooler box, thermometer
Documentation	COVID-19 test specimen referral form, Excel line list with SRF ID and Patient demographic details-Electronic Data
Transportation	Regular schedule and method to move the samples to the laboratory, personnel trained in sample handling

PPE recommended during specimen collection

- Long-sleeved isolation gown that fully cover your torso and extend from your neck to your knees and cover your arms to the end of your wrists.
- Head coverings, shoe covers or dedicated shoes
- N95 respirator that is secured properly and fits your face
- Eye protection (e.g. goggles or disposable full-face shield) that can be adjusted to fit your face
- Clean non-sterile disposable gloves that cover the wrist area of your isolation gown

Specimens recommended for COVID-19 diagnosis

According to the WHO interim guidance document (Sep11, 2020) on diagnostic testing for SARS-CoV-2:

Upper respiratory specimens -Early-stage infections (Most of the suspected cases)

Lower respiratory specimens - Negative URT with a strong clinical suspicion of COVID-19

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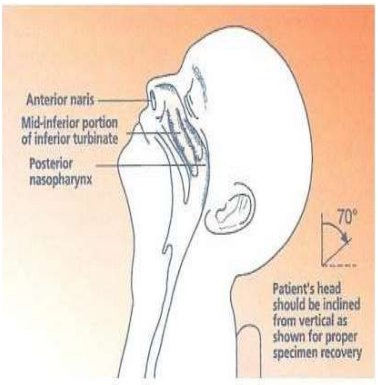
Upper respiratory tract specimens	• **Nasopharyngeal swab (preferred)** • **Oropharyngeal swab**
Lower respiratory tract specimens	• Bronchoalveolar lavage • Tracheal aspirate • Sputum

Collection of Nasopharyngeal swab

Materials

Sterile Dacron/Nylon flocked swab

Viral Transport Medium (3 ml sterile VTM)



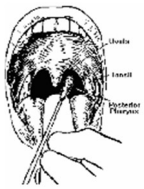
Procedure

- Tilt patient's head back 70 degrees
- Insert swab into nostril (Swab should reach depth to distance from nostrils to outer opening of the ear
- Leave swab in place for several seconds to absorb secretions
- Slowly remove swab while rotating it
- Place tip of swab into VTM and snap/cut off the applicator stick.

Collection of Oropharyngeal swab

Materials:

- Sterile Dacron/Nylon flocked swab
- Viral Transport Medium (3 ml sterile VTM)



Procedure:

- head Hold the tongue out of the way with a tongue depressor.
- Use a sweeping motion to swab posterior pharyngeal wall and tonsillar pillars
- Avoid swabbing soft palate and do not touch the tongue with swab tip.
- Put the swab in VTM.
- https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf

Other specimens

Bronchoalveolar lavage, tracheal aspirate, pleural fluid, lung biopsy (generally performed by a physician in the hospital setting)

- Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Sputum (collected under the guidance of a trained healthcare professional

- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

What should you do to become familiar with sample collection?

- **Watch:**
  - Video: How to collect oropharyngeal and nasopharyngeal specimens for the diagnosis of COVID-19 YouTube
  - Video on how to take a combined nose and throat swab for COVID-19

Review the CDC guidelines for sputum collection, bronchoalveolar lavage and tracheal aspirate collection

General guidelines to be followed during specimen collection

- Consider all specimens as **POTENTIALLY HAZARDOUS / INFECTIOUS.**
- Handle all specimens with gloves in a secure manner.
- Label each specimen container with the patient's unique identification (ID) number (e.g. medical record number), unique specimen ID (SRF ID), specimen type, and the date and time the specimen was collected
- Do not contaminate the outside of the specimen container.
- Do not handle laboratory requisition forms with gloves

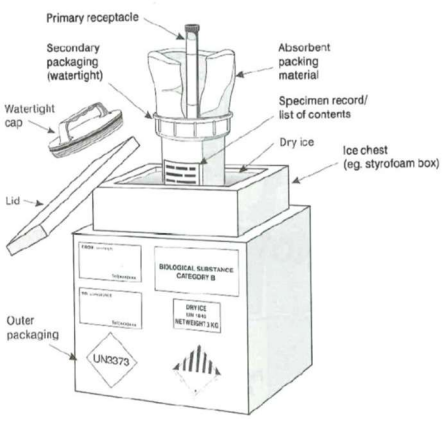
Storage

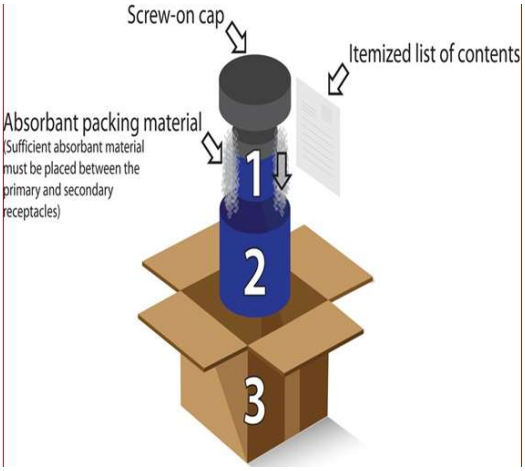
Specimen storage and transport

Specimen type	Collection materials	Storage & transport
	Molecular testing	
Nasopharyngeal and oropharyngeal swab	Dacron swab in viral transport medium in a sterile leak-proof container	Refrigerate at 2–8 °C up to 5 days
Sputum (deep cough)	Sterile leak-proof container	Refrigerate and ship at 2–8 °C up to 48 hours, if >48 hours freeze at −70 °C and ship on dry ice
Bronchoalveolar lavage	2–3 ml in sterile leak-proof container	Refrigerate and ship at 2–8 °C up to 48 hours, if >48 hours freeze at −70 °C and ship on dry ice
Endotracheal or nasopharyngeal aspirate	2–3 ml in sterile leak-proof container	Refrigerate and ship at 2–8 °C up to 48 hours, if >48 hours freeze at −70 °C and ship on dry ice

Packing & Transport

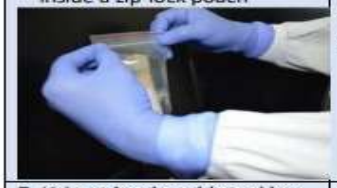
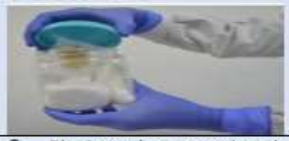
Triple Packaging





Specimen packaging

Procedure for Specimen Packaging and Transport

1. Use PPE while handling specimen 	2. Seal the neck of the sample vials using parafilm 	3. Cover the sample vials using absorbent material 	4. Arrange primary container (vial) in secondary container 
5. Placing the centrifuge tube inside a zip-lock pouch 	6. Placing the zip-lock pouch inside a sturdy plastic container and seal the neck of the container 	***Note:*** *Sample vials can also be placed inside a zip-lock pouch, covered in absorbent material and secured by heat-sealing or rubber bands. Then, the zip-lock pouch should be placed inside another plastic pouch and secured*	7. Using a thermocol box as an outer container and placing the secondary container within it, surrounded by hard-frozen gel packs 
7. Using a hard card-board box as an outer container and placing the secondary container and the gel packs 	8. Placing the completed Specimen Referral Form (available on www.niv.co.in) and request letter inside a leak-proof, zip-lock pouch 	9. Securing the zip-lock pouch with the Specimen Referral Form on the outer container 	10. Attaching the labels: • Senders' address, contact number; Consignee's address /contact number; • Biological substance-Category B; • 'UN 3373'; Orientation label, Handle with care 

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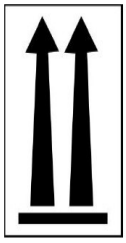
Labelling of Package

- Sender's, name, address and telephone number
- Whom to contact in case of emergency with telephonenumber
- Receiver's name, address and telephone number
- UN number eg.3373
- Temperature storage requirements
- Arrow mark to indicate upright direction

Label name:	Infectious substance
Minimum dimensions:	100 × 100 mm
(for small packages:	50 × 50 mm)
No. of labels per package:	1
Colour:	Black and white

The words "INFECTIOUS SUBSTANCE" shall be shown. The statement "In case of damage or leakage immediately notify a Public Health Authority" is required in some countries.



Label name:	Orientation label
Minimum dimensions:	Standard A7: 74 × 105 mm
No. per package:	2 on opposite sides
Colour:	Black and white or red and white

The words "THIS SIDE UP" or "THIS END UP" may also be displayed on the top cover of the package.

Reaching Impact, Saturation, and Epidemic Control (RISE)

Guidelines followed for sample packaging & transport

- **Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)**
- **IATA (International Air Transport Authority)guidelines**

CATEGORY A

Any infectious substance that could generated disability, life threatening or fatal disease in healthy humans or animals

Proper shipping name: UN 2814 "INFECTIOUS SUBSTANCE, AFFECTING HUMANS"

UN 2900 "INFECTIOUS SUBSTANCE, AFFECTING ANIMALS ONLY"

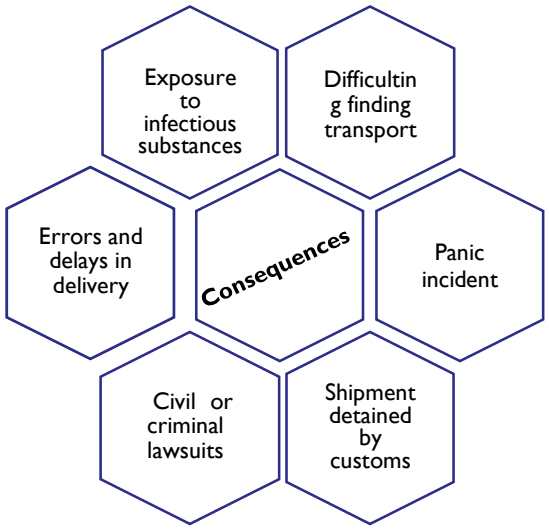
CATEGORY B

Any biological substance that does not meet the criteria for Category A.

Proper shipping name : UN 3373: "BIOLOGICAL SUBSTANCE, CATEGORY B"

A 3291: "CLINICAL WASTE, UNSPECIFIED

Consequences of improper packaging



Registration

Specimen Registration Process

The specimens received will be registered in the ICMR Portal using the RTPCR App as demonstrated in the video below:

https://youtu.be/CXTmT6JIXII

COVID-19 Sample collection management system :

https://covid19cc.nic.in/icmr/Citizen/RPTPCRSampleReport.aspx

Receipt at the laboratory

Protocol for receiving Specimen at LAB

1. The specimen transport box is opened in a Bio Safety Level 2 (BSL-2) equivalent laboratory inside a certified biosafety cabinet (BSC)

2. The specimen must pass acceptance criteria in order to be eligible for receiving by the laboratory and for further processing.

Specimen acceptance criteria

1. Specimen is properly labeled with unique identification number and date
2. Specimen label matches (unique identification number and date) with the request form/ online details received
3. Specimen is received at recommended temperature (2-8°C)

Specimen rejection criteria

1. Viral transport media (VTM) tube content has leaked, No cold chain maintenance
2. Specimen Referral Form is soiled with leaked content
3. Unlabeled specimen, Mismatched CRF

3. Specimen must be processed within the recommended time frame when stored at 2-8°C and should be aliquoted and stored at -80°C for long-term storage.

Aliquoting

Protocol for Specimen Aliquoting

1. Bring in the other required items on your checklist e.g. biohazard bags for solid waste and container for liquid waste. Bring in a container with appropriate amount of 1% sodium hypochlorite for rinsing the Pasteur pipettes and VTM tubes.
2. Open the specimen transport box and take out all the VTM tubes containing the specimen and place on a stand.
3. Disinfect the inner side of the specimen box with freshly prepared 1% sodium hypochlorite solution and take it out of the BSC.
4. Arrange the VTM tubes as per the order in the specimen reception log and verify the specimen labels
5. Apply the specimen acceptance and rejection criteria
6. Vortex the VTM tubes for 30 seconds to ensure release of specimen from the swab into the VTM.

Protocol for Specimen Aliquoting

7. Specimen must be processed within the recommended time frame when stored at 2-8°C Transfer the aliquots to -70/-80°C freezer for long-term storage.

8. After completing the work tie the biohazard bag inside the BSC and bring it out of the BSC. Place it in the biohazard bin. The waste should be handed over to the autoclave team.

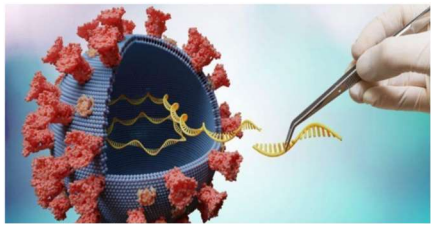
9. Leave the BSL-2 lab and doff the PPE.

Samples for WGS

Need for Genomic Surveillance

Sequencing and analyzing the genomic data of virus is required :

- ***To fully understand the spread and evolution of the SARS CoV-2 virus, and***
- ***To tackle its future spread***



Analysis of SARS-CoV-2 genome sequences would allow

- ***To study the evolution of the virus***
- ***Assess whether mutations influence transmission, clinical outcomes, severity.***
- ***Impact interventions such as public health intervention measures and vaccines.***

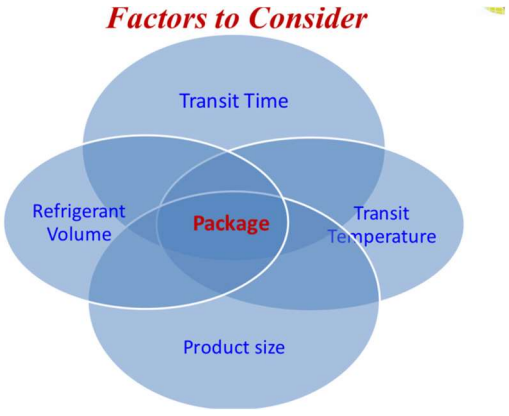
Specimen Collection, Packaging and Transport Guidelines for Genome Sequencing

Sample collection:

- Within country from clinics
- Travelers tested at the airports. All positive samples from airport-based tests should be sequenced

Data sheet: A brief socio-demographic, clinical and travel information (not more than 15 items) containing sheet should accompany all samples collected at the above two sites.

Roles and Responsibilities: The laboratory in-charge will collect, package & transport SARS- CoV-2 positive samples.



Contd..

- After carrying out the RT-PCR test the remaining samples (within 72 hours of collection, stored at 2-8°), which are RT-PCR positive will be transported in VTM with cool pack (4-8 degree) or in ice.
- Only those samples which are positive for SARS-CoV-2 by RT PCR preferably with a Ct value of 25 or less should be packaged & transported.
- Alternatively, remaining RNA samples may be stored and aliquoted in the 1.5 ml micro-centrifuge tubes followed by proper labelling and sealing with the parafilm (stored at -70degree). RNA placed together in plastic/ cardboard cryobox and packed in the thermocol box with dry ice should be shipped to the respective RGSL for sequencing.

Line listed excel to accompany specimens/RNA to RGSL

Name of the COVID-19 positive sample referral lab/health care facility:										
Date:										
Sr. No	SRF ID	Name	Age	Gender	Address	Patient Mobile	Type of Specimen	Date of collection of sample	Ct Value of all target genes detected by RTPCR Test for SARS-CoV-2	Status (Symptomatic / Asymptomatic)

INSACOG : https://ihip.nhp.gov.in/idsp/#!/facilityinfo

References

- Centers for Disease Control and Prevention. Preparation of viral transport medium. Accessed 5 June 2020. https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf
- Centers for Disease Control and Prevention. Interim guidelines for collecting, handling, and testing clinical specimens for COVID-19. 22 May 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
- Centers for Disease Control and Prevention. Interim laboratory biosafety guidelines for the handling and transport of samples associated with the novel coronavirus 2019 (2019-nCoV). 11 May 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

आज़ादी का अमृत महोत्सव

RNA extraction

USAID FROM THE AMERICAN PEOPLE

RISE Reaching Impact, Saturation, and Epidemic Control

Purpose

- To release nucleic acid from the cell for use in other procedures
- Must be pure and free from contamination with protein, carbohydrate, lipids or other nucleic acids.

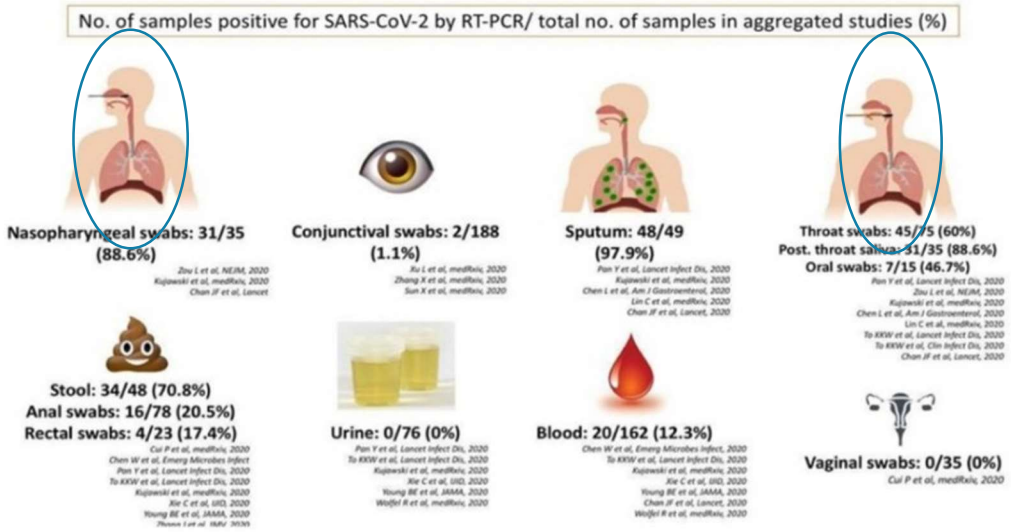
Applications

- Amplification methods (PCR, qRT-PCR/RealTime RT PCR)
- Restriction enzyme digest
- Hybridization methods (Southern & Northern blot)
- Molecular cloning
- Sequencing

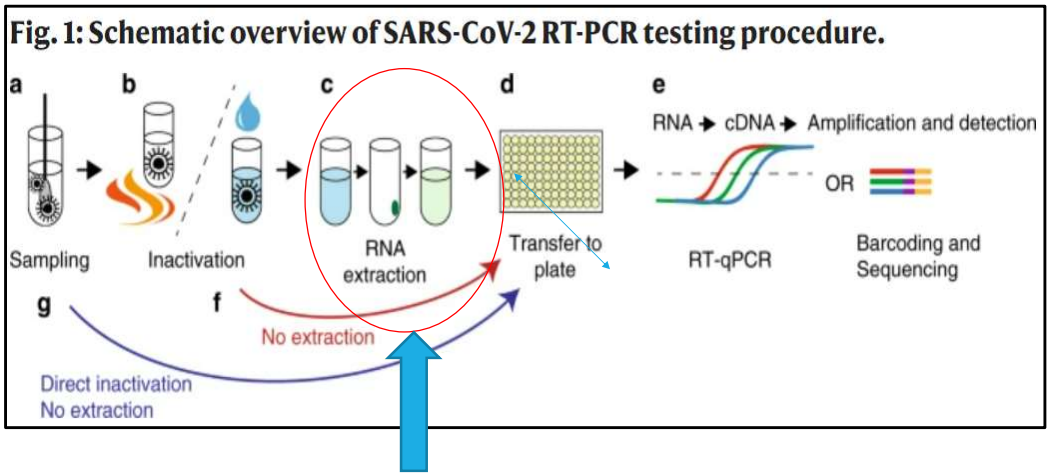
Nucleic Acid Preparation Sample Source?

- Whole blood
- Buffy coat
- Serum or plasma
- Bone material
- Buccal cells
- Cultured cells
- **Pretreat to make nucleated cells available**
  - Whole blood
  - Tissue samples
  - Microorganisms
  - Urine
- Amniocytes or amniotic fluid
- Dried blood spots
- Fresh or frozen tissue (biopsy material)
- Sputum, urine, CSF, or other body fluids
- Fixed or paraffin-embedded tissue
- **Need sufficient sample for adequate yield**

COVID-19 Shedding



Real time RT-PCR Workflow



Principles for Handling of All Clinical Specimens

- Observe universal precautions for biohazards.
- Use protective gowns, gloves, face and eye shields.
- Decontaminate all spills (5-10%) and work areas with (1%) bleach.
- Dispose of all waste in appropriate biologic waste containers.
- **Use gloves. Your RNA depends on it!**

Precautions for Working with RNA in the Clinical Laboratory

- RNA is not a stable molecule!
- A proper work flow should be maintained.
- It is easily degraded by RNase enzymes.
- Use sterile, disposable plastic ware (tubes, filter tips) marked "For RNA Use Only".
- Always wear gloves and frequently change
- Working in a hood is a good practice, cleaning work station before and after work.
- Take extra precautions to avoid carry over contaminations.
- Treat liquids with DEPC, except Tris-based buffers

Lab Supplies

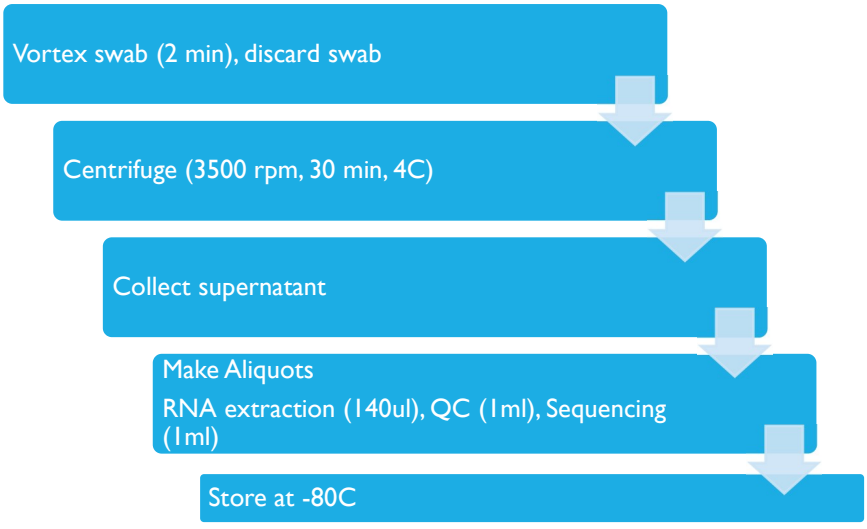
Reagents and materials required:

- Gloves
- Lab coat
- RNaseZap (Sigma, # R2020-250ML)
- Nuclease-free (autoclaved) 1.5 ml microcentrifuge tubes
- QiaAmp Viral RNA mini kit (Qiagen, #52904)
- 95-100% Ethanol, microbiology grade
- 70% Ethanol for cleaning surfaces
- PBS, RNase-free

Equipment required:

- -20°C freezer
- Micropipettors
- Bucket with ice
- Class II biological safety cabinet (BSC) with UV light
- Microcentrifuge
- Micropipettors and sterile pipette tips with aerosol-resistant filters
- Vortex
- Waterbath at 37°C

Sample Preparation



Basic steps for DNA/RNA extraction

1. Cell disruption or cell lysis, to expose the DNA/RNA within.
    - **Chemical**
    - **enzymatic**
    - **Mechanical**
2. Removing membrane lipids by adding a detergent. The detergent molecules are able to pull apart the membranes.
3. Precipitation of DNA/RNA: by alcohol

Precipitation

- The first part of precipitation uses phenol/chloroform to remove the proteins from the DNA
    - Phenol denatures proteins and dissolves denatured proteins.
    - Chloroform is also a protein denaturant
- The second part of DNA precipitation is the addition of salts
    - The salts interrupt the hydrogen bonds between the water and DNA molecules.
- The DNA is then precipitated from the protein in a subsequent step with isopropanol or ethanol
    - In the presence of cations, ethanol induces a structural change in DNA molecules that causes them to aggregate and precipitate out of solution.
- The DNA is pelleted by spinning with a centrifuge and the supernatant removed.

Washing and Resuspension

Washing:

The precipitated DNA is loaded with acetate salts. It is "washed" with a 70% ethanol solution to remove salts and other water soluble impurities.

Resuspension:

The clean DNA is now resuspended in a buffer to ensure stability and long term storage.

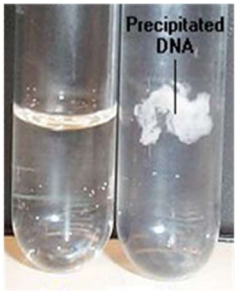
The most commonly used buffer for resuspension is called 1xTE

NA Isolation Methods: Liquid Phase Organic Extraction

- **Phenol (50): chloroform/isoamyl alcohol (50:49:1)**
- Lysed samples mixed with above; two layers are formed.
  - Proteins remain at interface.
  - DNA is removed with top aqueous layer.
  - DNA is precipitated with alcohol and rehydrated.
- **Disadvantages:**
  - Slow, labor-intensive, toxic (phenol, chloroform)
  - Fume hood required; disposal of hazardous materials required

Inorganic Isolation Methods

- Also called "salting out".
- Uses low pH and high salt condition to selectively precipitate proteins.
- DNA is left in solution (picture 1).
- Precipitate out DNA with isoproproanol (picture 2 and 3)



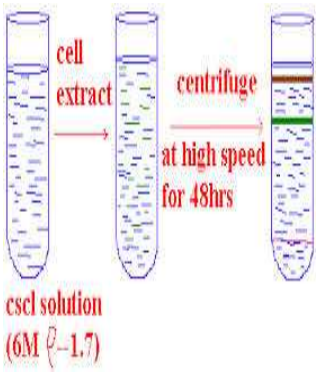
Pic. 1 Pic. 2

Pic. 3

RNA Isolation Methods: Cesium Chloride Gradient

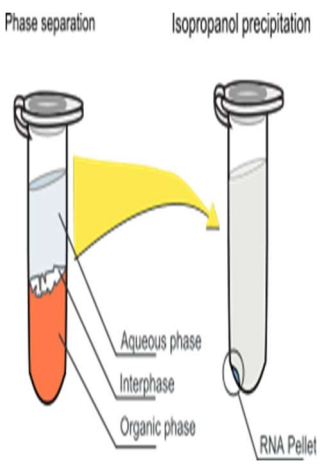
- Used mainly to get clean RNA for Northern blots
- Homogenize cells in guanidinium isothiocyanate and β-mercaptoethanol solution.
- Add to CsCl gradient and centrifuge for 12–20 hours; RNA will be at the bottom

  of tube.
- Re-dissolve in TE/SDS buffer.
- Precipitate RNA with salt and ethanol, then rehydrate.
- Advantage: high quality
- Disadvantages: extremely time-consuming, hazardous materials disposal issues



RNA Isolation Methods Guanidinium - based Organic Isolation

- Phenol/guanidinium solution (Trizol) disrupts cells, solubilizes cell components, but maintains integrity of RNA.
- Add chloroform, mix, and centrifuge.
- Proteins/DNA remain at interface.
- RNA is removed with aqueous top layer.
- RNA is precipitated with alcohol and rehydrated.
- Advantage: faster than CsCl method
- Disadvantages: fume hood required, hazardous waste disposal issues

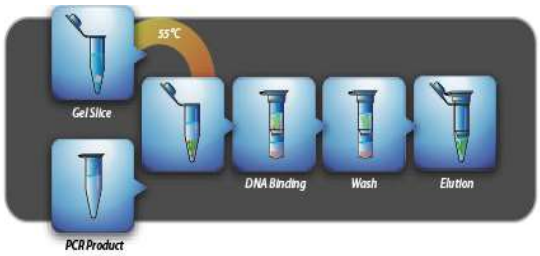


NA Isolation Methods:Solid Phase Procedures

- Uses solid support columns, magnetic beads, or chelating agents
- Solid support columns: Fibrous or silica matrices bind DNA allowing separation from other contaminants.
- Magnetic beads: DNA binds to beads; beads are separated from other contaminants with magnet.
- Chelating resins
- **Advantages:**
  - Fast and easy, no precipitation required

Solid Phase Isolation

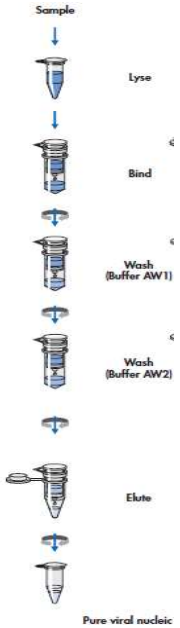
- More rapid and effective
- Use solid matrix to bind the DNA.
- Wash away contaminants.
- Elute DNA from column



QIAamp Viral RNA Mini kit, QIAGEN

Workflow

- Lysis buffer: Buffer AVL+ Carrier RNA
- Ethanol (96-100%) to enhance and influence the binding of nucleic acids to silica
- Wash buffer1: Buffer AW1(conc.) + Ethanol
  To remove the protein and colored contaminants
- Wash buffer2: Buffer AW2(conc.) + Ethanol
  To remove the salts - *Dry spin column*
- Elution buffer: AVE or DNase, RNase free H_2O
- Store at ≤ -70°C



Components and their storage

Reagent	Preparation of working solution			Storage	Stability
QIAamp Viral RNA Mini kit	-			Room temperature (15-25 °C)	Expiry Date of Kit
Carrier RNA- buffer AVE [final concentration of Carrier RNA: 1 µg/µl]	Buffer AVE 310 µl + Carrier RNA (Lyophilize: 310 µg)			Aliquot and store at -20 °C Note: Do not freeze-thaw > 3 times	Expiry Date of Kit
Buffer AVL– Carrier RNA solution (prepared fresh)	No. of sample	Vol. Buffer AVL (ml)	Vol. Carrier RNA-AVE (ul)	2-8°C Note: • Precipitated buffer AVL-carrier RNA should be incubate at 80°C, ≤ 5 min before use. • Do not warm the solution > 6 times	48 hours
	1	0.56	5.6		
	N	0.56 x N	5.6 x N		
Buffer AW1	AW1 conc. (ml)	Ethanol (ml)	Final volume (ml)	Room temperature (15-25 °C)	Expiry Date of Kit
	98	130	228		
Buffer AW1	AW2 conc. (ml)	Ethanol (ml)	Final volume (ml)	Room temperature (15-25 °C)	Expiry Date of Kit
	66	160	226		

Magnetic based separation (Magmax RNA isolation kit)

1. Disrupt samples in a guanidinium thiocyanate based solution (lysis buffer)
2. Paramagnetic beads with a nucleic acid binding surface are added to the sample to bind NA
3. The beads/nucleic acids are captured on magnets, and proteins and other contaminants are washed away.
4. The beads are then washed again to remove residual binding solution. NA are eluted in a small volume of elution buffer.

Advantage

- Recovery of total NA
- High quality and purity
- Short time of procedure
- Can be automated
- Less Sample required

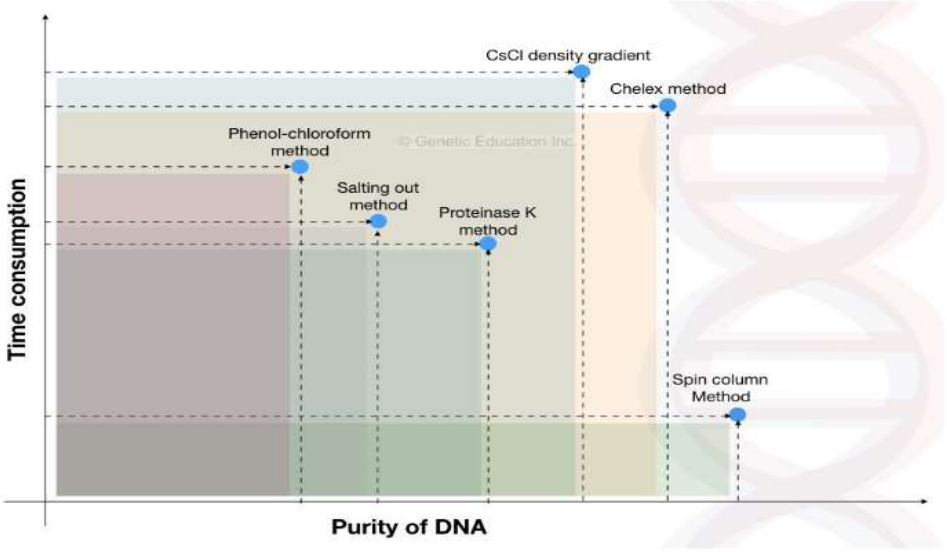
RNAses

- RNases are naturally occurring enzymes which are small proteins that can renature and become active.
- Degrade RNA
- Common laboratory contaminant (from bacterial and human sources)
- Also released from cellular compartments during isolation of RNA from biological samples
- MUST be eliminated or inactivated BEFORE isolation. Can be difficult to inactivate
- CRITICAL to have a separate RNAse free area of lab

Protecting Against RNAse

- Wear gloves all times
- Use RNase-free tubes and pipette tips
- Use dedicated, RNase-free, chemicals
- Pre-treat materials with extended heat (180 C for several hours), wash with DEPC-treated water, NaOH or H2O2
- Supplement reactions with RNase inhibitors

Purity vs time



Determining Purity

- Sample absorbances are determined on the spectrophotometer at 260nm and 280nm
- The absorbance wavelength is directly proportional to the concentration of nucleic acid.
- The OD at 260nm should be 1.6-2.00 times more than the absorbance at 280nm.
- Divide the OD at 260nm by the OD at 280nm to get the ratio.
- If the 260nm/280nm ratio is less than 1.6 for DNA, 2.0-2.3 for RNA this indicates contamination, usually with protein.
- DNA -If the OD ratio is higher than 2.0 it may be contaminated with RNA.
- Ratio of the readings : O.D.260/O.D.280 is a measure of purity.
- Pure preparations of DNA and RNA have O.D 260/280 of 1.8 and 2.0 respectively.

Troubleshooting Nucleic Acid Preparation Methods

Problem: No or low nucleic acid yield.

- Make sure that ample time was allowed for resuspension or rehydration of sample.
- Repeat isolation from any remaining original sample (adjust procedure for possible low cell number or poorly handled starting material).
- Concentrate dilute nucleic acid using ethanol precipitation.

Problem: Poor nucleic acid quality

- Can be assessed on the basis of extraction control (MOCK)
- If sample is degraded, repeat isolation from remaining original sample, if possible.
- If sample is contaminated with proteins or other substances, clean it up by re-isolating (improvement depends on the extraction procedure used).

Open System: Real time RT-PCR Testing For COVID-19 Detection

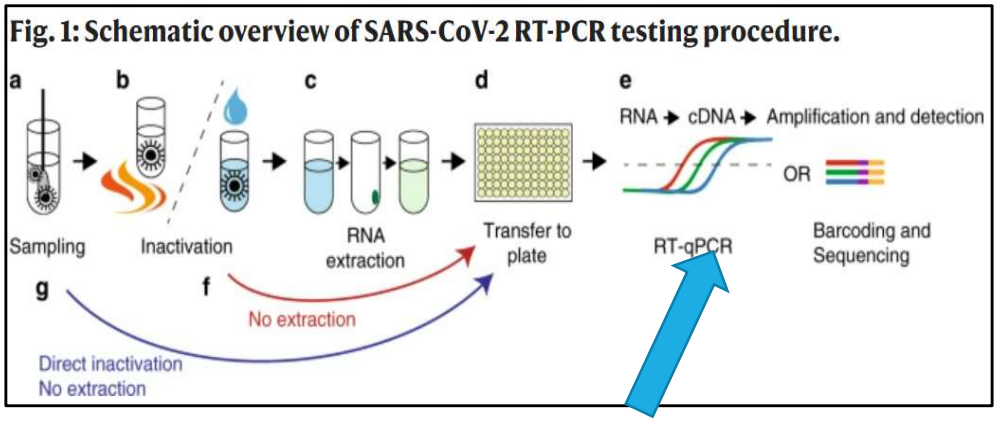
Training Objectives

- Background – Virus and Genome
- Types of Samples and Collection Technique
- Diagnosis and ICMR Guidelines
- Real time RT-PCR Principle and Targets
- Technical Training
- MM preparation and PCR Set Up
- Quality Control Procedures
- Interpretation of Results
- Dos and Don'ts

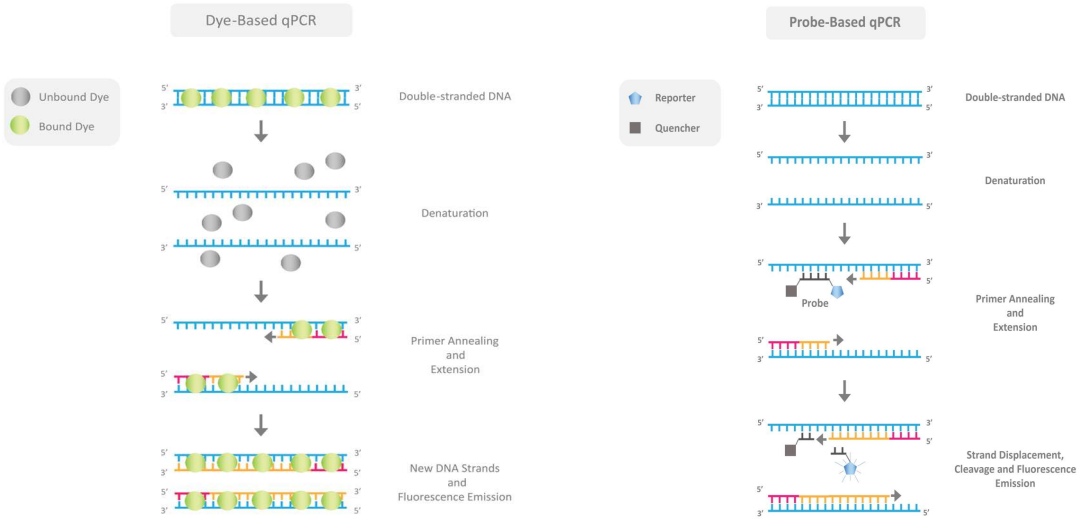
Intended Use of real time RT-PCR Assay

- Gold standard test for detecting cases of COVID-19
- Specialized laboratory setup with specific biosafety and biosecurity precautions
- Average time taken is around 4-5 hours from receipt of sample to getting the result
- Advantages:
  - Accuracy of detection
  - Ability to run large no. of samples in a single run (For example 90 samples in a 96 well block machine)
  - **Real time RT-PCR as the confirmatory test for diagnosis of SARS-CoV-2**

Real time RT-PCR Workflow

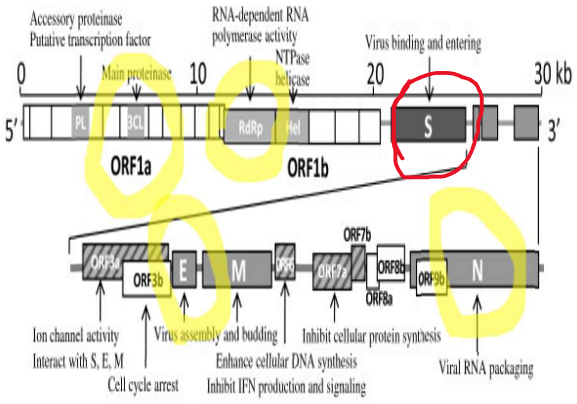


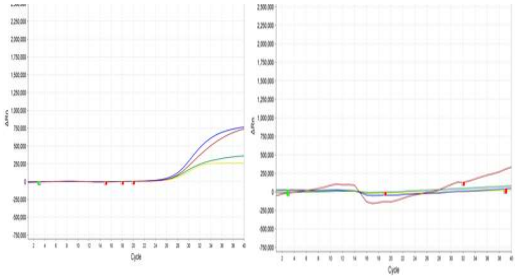
What is real time RT-PCR assay



SARS-CoV-2: Genome - Principle and Targets for real time RT-PCR

Targets for primers/probes

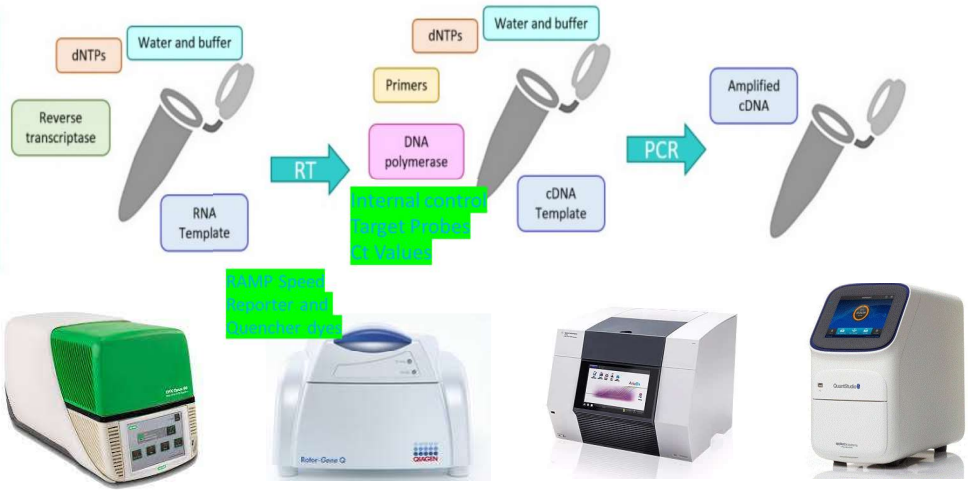




Result Interpretation

S.No	SPECIMEN	FAM Ct value	ROX Ct value	HEX/VIC Ct value
1	Positive	<35	<35	<35
2	Negative	>35	>35	>35

Real time RT-PCR – Open Systems

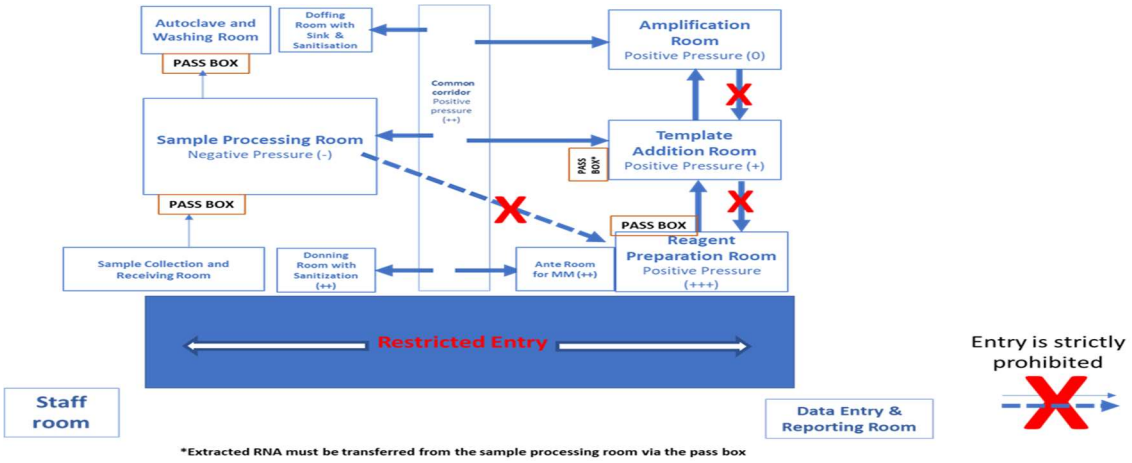


Disclaimer

Selection of real time RT-PCR kits is based on:

- Preference of the lab based
- Standardization in the lab
- Ease of use
- Cost
- Assay time
- Availability of the kit

Setting Up PCR – Laboratory Facility



CoviPath™ COVID-19 RT-PCR Kit

Multiplex real-time RT-PCR test for the qualitative detection of SARS-CoV-2

CoviPath™ COVID-19 RT-PCR Kit includes the following components:

- CoviPath™ COVID-19 Assay Multiplex – Multiplexed assay that contains the following:
  - Two primer/probe sets specific to different SARS-CoV-2 genomic regions (ORF1ab and N gene)
  - One primer/probe set targeting the RNase P gene. The RNase P gene serves as an internal positive control to monitor the sample source.
- CoviPath™ COVID-19 Control – RNA control that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assay.
- CoviPath™ 1-Step Multiplex Master Mix (No ROX™)

Component	Storage	Shelf life[1]
CoviPath™ COVID-19 Assay Multiplex (ORF1ab, N gene, RNase P gene)	–30°C to –20°C	12 months
CoviPath™ COVID-19 Control	–30°C to –20°C	12 months
CoviPath™ 1-Step Multiplex Master Mix (No ROX™)	–30°C to –20°C	12 months

The CoviPath™ COVID-19 RT-PCR Kit is available in the following kit sizes.
200 reactions (Cat. No. A50780)
500 reactions (Cat. No. A52000)

Real time RT- PCR Reaction

- Negative Control for real time RT-PCR

  A known negative sample is used during extraction

  The purified negative control is used as the negative control for real time RT-PCR

- Positive Control Preparation

  Dilution 1 - 95µl of TE buffer + 5ul of Covipath COVID-19 control (mix & centrifuge)

  Dilution 2 - 75ul of dilution buffer + 25ul of dilution 1 (mix & centrifuge).

  Dilution 2 will be used as a template in real time RT-PCR.

- No template control (NTC)

  NFW to be added for NTC

- Unknown Sample – Extracted RNA

Real time RT- PCR Reaction

Preparation of Master mix

Master mix contents	96 well		384 well	
	1X	N reaction	1X	N reaction
Multiplex Master Mix	6.25µl	6.25*N	5 µl	5*N
RT PCR assay multiplex	1.25 µl	1.25*N	1 µl	1*N
NFW	7.5 µl	7.5*N	4 µl	4*N
Total reaction volume	15 µl		10 µl	

PCR Conditions

STAGE	TEMPERATURE	TIME	CYCLES
1	25°	2 MIN	1
2	53°	10 MIN	1
3	95°	2 MIN	1
4	95°	3 SEC	40
	60°	30 SEC (Data collection)	

- A minimum of one Negative Control and one Positive Control must be present for each run.

Real time RT- PCR Reaction

Fluorescent Channels

Reporter/Quencher	Target
FAM/None	ORF1ab
VIC/None	N gene
JUN/None	RNaseP

Quality control

- Detection of the Positive control in fluorescence channel FAM and VIC.
- Detection of the internal control (IC) in fluorescence channel JUN in NTC.

Note:

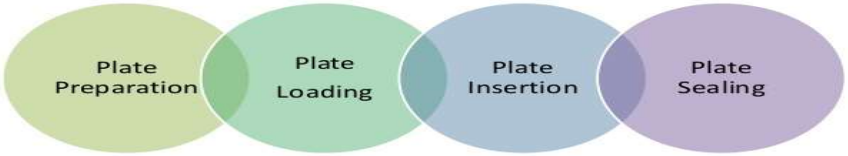
Set the threshold above the maximum level of No template control curve (random noise curve), then analyze the results.

Setting up of real time RT-PCR

- ✓ Biosafety cabinet: Wipe with 1% Hypochlorite solution and leave for 10 minutes followed by 70% ethanol and leave for 10 minutes
- ✓ Commercial products are also available for example: DNAse Zap, RNAse erase etc.
- ✓ Switch on UV and leave for 10 minutes
- ✓ Take out intended reagents and thaw
- ✓ Make sure the enzymes are kept in cool rack
- ✓ Keep all work sheets

Real time RT-PCR set up

- 48-well plates, 96-well plates, 384-well plates, 3072-through-hole plates, etc are available.
- It includes following **four steps**:



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Quality Control Procedures

- ✓ **Positive and negative test controls** must be included to accurately interpret patient test results
- ✓ **Positive control** (provided in the kit) – used to monitor the real time RT-PCR reaction setup and reagent integrity
- ✓ **Negative Control** – a known negative sample is used to monitor cross contamination during RNA extraction
- ✓ **No Template Control (NTC)** – NFW is used and used to monitor contamination during PCR reaction set up
- ✓ Known positive and known negative samples with varying Ct values must be included in every batch in the real time RT-PCR step as ICP

External Controls should be performed:

- A known positive sample and a known negative sample must be included each time a new lot of kit is received.
- Each time a new operator is performing the test (i.e., operator who has not performed the test recently).
- When problems (storage, operator, instrument, or other) are suspected or identified.
- If otherwise required by the labs standard QC procedures.

Result Summary

- The negative control reactions for probe/primer sets should not exhibit fluorescence growth curves (FAM and VIC) 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: (Varies with the kit)

Channels	Positive control	Expected Ct values
FAM	ORF1ab	≤37
VIC	N gene	≤37
JUN	RNase P	≤35

All clinical samples should exhibit RNase P reaction curves that cross the threshold line at or before 35 cycles.

- 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

Result Summary – Criteria for Reporting and Re-test

ORF1ab	N gene	RNase P	Status	Result[1]	Action
Negative	Negative	Negative	Invalid	NA	Repeat the test. If the repeat result remains invalid, consider collecting a new specimen.
Negative	Negative	Positive	Valid	SARS-CoV-2 not detected	Report results.
Positive	Negative	Positive	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Negative	Positive	Positive	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Positive	Positive	Positive	Valid	SARS-CoV-2 detected	Report results.
At least one positive		Negative	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.

COVID variants – Of Concern

- Viruses, like SARS-CoV-2, change over time and will continue to change the more they circulate. Sometimes, variants of the virus may develop.
- A variant is where the virus contains at least one new change to the original virus.(mutations)
- Mutations that contribute to virus adaption and fitness do occur, they tend to be in the minority compared with tolerated low-effect or no-effect 'neutral' amino acid changes
- The spike protein mediates attachment of the virus to host cell-surface receptors and fusion between virus and cell membranes
- It is also the principal target of neutralizing antibodies generated following infection by SARS-CoV-2, and is the SARS-CoV-2 component of both mRNA and adenovirus-based vaccines licensed for use and others awaiting regulatory approval.
- The mutations that affect the antigenicity of the spike protein are of particular importance and are the ones circulating now.

COVID variants

Variants of concern (VOC)

- ✓ Increase in transmissibility or detrimental change in COVID-19 epidemiology;

OR

- ✓ Increase in virulence or change in clinical disease presentation;

OR

- ✓ Decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics.

Key Coronavirus variants

WHO name	Scientific name	Country where first documented
Alpha	B.1.1.7	Kent, UK
Beta	B.1.351	South Africa
Gamma	P.1	Brazil
Delta	B.1.617.2	India
Omicron	B.1.1.529	Multiple countries

RT- PCR Kits for Detection

भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग, स्वास्थ्य और परिवार
कल्याण मंत्रालय, भारत सरकार

Indian Council of Medical Research
Department of Health Research, Ministry of Health
and Family Welfare, Government of India

Date: 15.01.2022

ICMR APPROVED REAL TIME PCR (RT-PCR) ASSAYS FOR DETECTION OF OMICRON VARIANT OF SARS-COV-2

S. No	Name of company	Name of the kit	*Lot no. / Batch No.
1.	TATA Medical & Diagnostics Ltd., Mumbai (Maharashtra), India	TATA MD CHECK RT-PCR OmiSure	K035-V001
2.	Kriya Medical Technologies Pvt. Ltd., Chennai (TamilNadu), India	KRIVIDA Novus SARS-CoV-2 RT-PCR kit- Omicron detection kit	OD060122/01

TATA RT PCR Omisure - Omicron detection kit

- Distinguishes Omicron from Delta, wild-type and all other VOCs of SARS-CoV-2
- 3 gene targets, single tube, fully multiplexed assay
- Simultaneous S-gene Target Failure (SGTF) and S-Gene Mutation Amplification (SGMA).
- 100% Sensitivity, 99.25% Specificity, validated at ICMR-NIV

Sample	Ct value for positive	Ct value for negative
PC	Ct <=40	-
NC	-	Ct > 40 or undetermined
Clin. samples	Ct <=40	Ct > 40 or undetermined
Ct values are uniform across all the targets and internal controls. Ct values must be validated for your infrastructure		

S-Gene TF	S-Gene MA	RdRp	RNase P	Result interpretation
-	+	+/-	+/-	SARS-CoV-2 Detected; indicates presence of Omicron
+	-	+/-	+/-	SARS-CoV-2 Detected; Omicron not present
-	-	+	+/-	SARS-CoV-2 Detected; Probable Omicron present
+	+	+	+/-	SARS-CoV-2 Detected; Probable mixed infection
-	-	-	+	SARS-CoV-2 not Detected
-	-	-	-	Invalid results. Repeat RNA extraction and re-run the test

Interpretation of QC Failures:

No signal is detected in all the fluorescence channels:

- No result can be concluded
- Assay must be repeated from extraction/ fresh sample has to be obtained

No signal with positive controls in fluorescence channel FAM/VIC:

- Incorrect selection of fluorescence channel
- In correct Temperature profile
- Incorrect configuration of real time RT-PCR
- Improper storage condition

Weak or no signal of the internal control in fluorescence channel JUN:

- Real time RT-PCR conditions did not comply with the protocol
- The of real time RT-PCR was inhibited

Limitations & Interfering substances

- Performance of the test has only been established in the following specimens (such as nasopharyngeal or oropharyngeal swabs, nasal and mid turbinate nasal swabs, nasopharyngeal aspirate, tracheal aspirates, and bronchoalveolar lavage/wash).
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or Immuno-suppressant drugs have not been evaluated.
- The COVID-19 real time RT-PCR Kit cannot rule out diseases caused by other bacterial or viral pathogens.

Kit Performance Specifications

- **Sensitivity:**

  The LoD of 10 GCE/reaction (genome copy equivalent) is calculated from a starting concentration of 250 GCE/mL of specimen. (Varies from kit to kit)

- **Specificity:**

  In silico analysis of forty-three organisms was performed. The in silico analysis indicates that significant amplification of non-target sequences that result in cross reactivity or potentially interfere with detection of SARS-CoV-2 is not likely to occur.

Do's and Don't's

- Use high quality and pure RNA template
- Proper storage of RNA
- Aliquot the reagents and controls and store them at proper temperature
- Use the optimal template amount as instructed
- Mix all your reaction components properly
- Perform controls reactions
    i. No template control
    ii. Positive control
    iii. Negative/Internal control

Do's and Don't's

- Double check your cycle settings before your run
- Prevent contaminations
    i. use aerosol resistant filter pipette tips
    ii. work in designated, separate areas
    iii. use separate pipettes for pre and post amplification steps
    iv. wear nitrile gloves and change them often
    v. open the tubes only inside the cabinets
    vi. place the tubes in ice box
    vii. return them immediately after the reaction is set up

Maintenance of Real time PCR machines

- Ensure the instrument is dust free.
- Clean the heated cover and sample wells once a month or as needed.
- Calibration is done once in two years as per the manufacturer recommendation.

Waste Management and other Safety Measurements

- Used tips and PCR plates must be discarded in 1% sodium hypochlorite
- Sodium hypochlorite is drained from the liner and sent for effluent treatment
- While the treated tips and plates are discarded in the appropriate discard bags

ILQC – ICMR QC Lab

- All labs will have to send 5 random positive and 5 random negative samples per month to QC labs (Biannually)
- Samples can be aliquoted and stored for a week before the allotted date
- Samples with a ct value between 25 to 35 for positive cases and undetermined ct values for the negative samples should be selected
- Make sure they are not freeze thawed more than once and stored in cryo vials with appropriate labels
- https://covidqcqa.icmr.org.in/login.php

आज़ादी का अमृत महोत्सव

Interpretation of real-time PCR Results & Ensuring Quality Results

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RISE Reaching Impact, Saturation, and Epidemic Control

Concepts covered

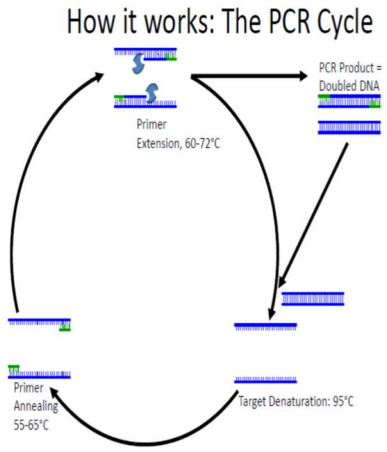
- Basic concepts of PCR and qPCR (real-time PCR)– what is the difference
- Reporting of qPCR results
- Ct Values and Interpretation
- Multiplexing in qPCR assays
- Types of controls for PCR reactions
- Quality control for PCR
  - Quality control during extraction
  - Quality control PCR and Post-PCR- clean room
- Minimizing contamination in a molecular biology laboratory
- Validating a real-time PCR assay and MIQE guidelines

Terms- Similar but not quite the same!

- qPCR
- RT-PCR
- Real-time RT-PCR

What are we performing for SARS CoV2 detection?

PCR



The Power of PCR

In each cycle of PCR, the amount of targeted DNA doubles

Cycles	DNA copies
0	1
1	2
2	4
3	8
...	...
25	33,554,432
n	2^n

Conventional PCR vs Real-time PCR

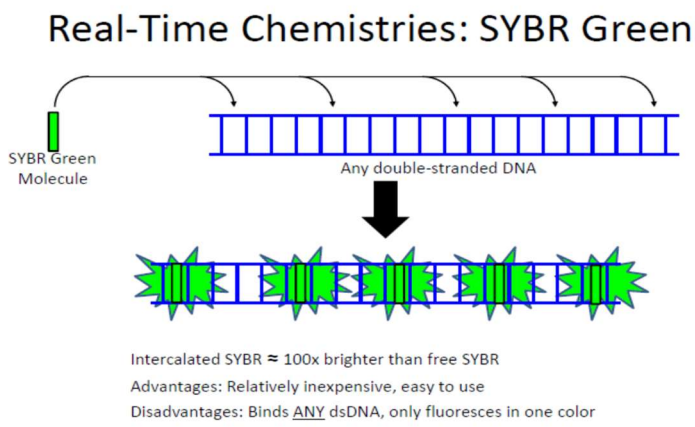
Conventional PCR

- End-point detection
- Detection after PCR by gel electrophoresis, hybridization etc
- Not quantitative

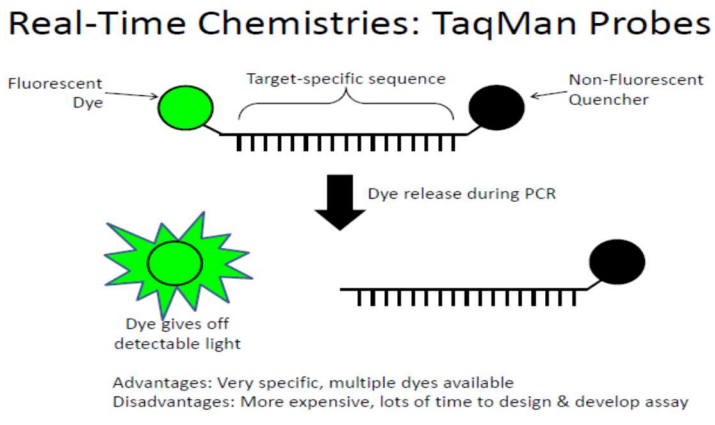
Real-time PCR

- Continuous measurement
- Log phase quantitation
- Can be semi-quantitative or quantitative if proper standards used

Real-Time PCR Chemistries

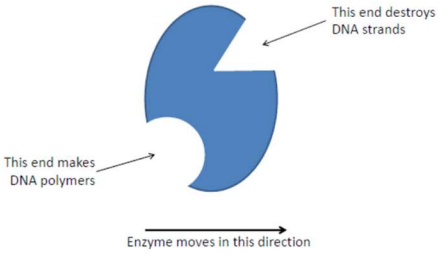


Real-Time PCR Chemistries

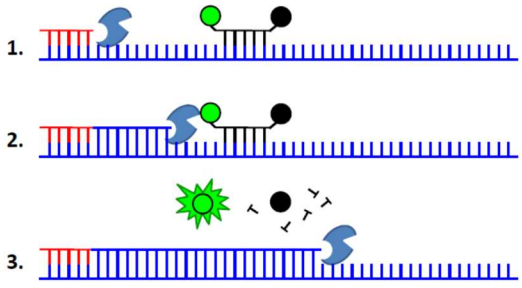


Real-Time PCR Chemistries

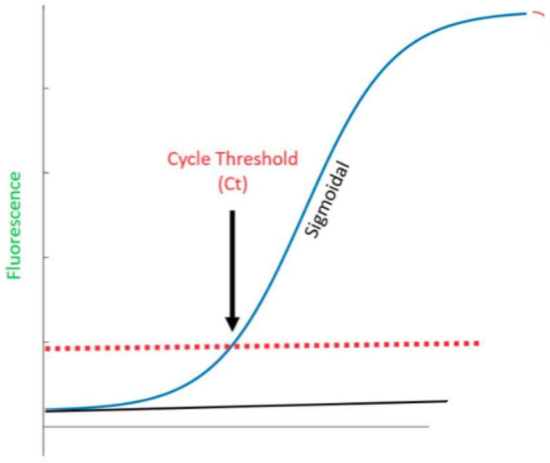
TaqMan Assay – How it works:
Taq Polymerase Has Two Enzyme Functions



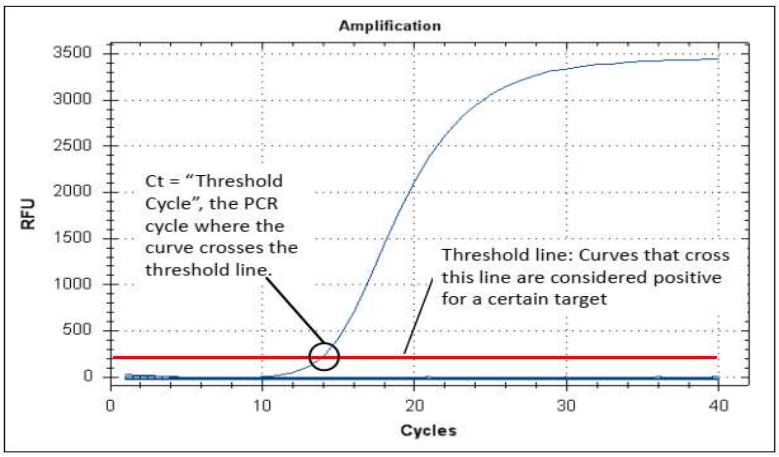
TaqMan Assay – How it works:
Dye Release During DNA Polymerization



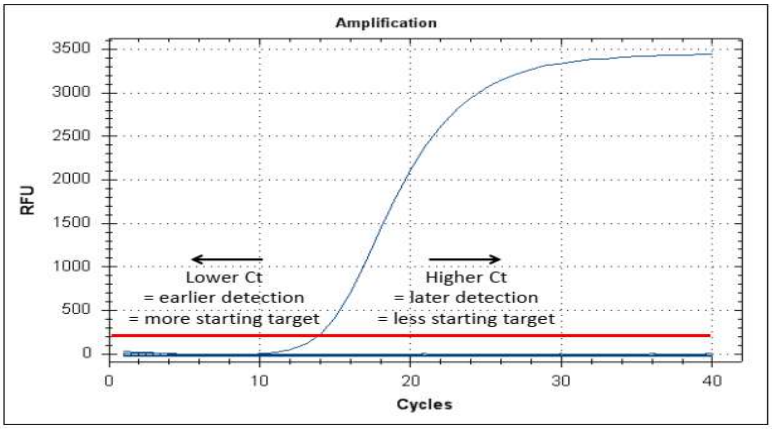
Ct Values and Interpretation



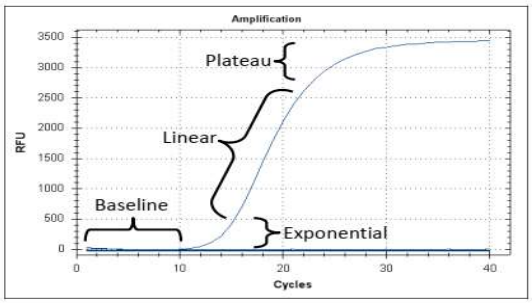
Data: The Amplification Curve



Data: The Amplification Curve



Data: The Amplification Curve



Linear view

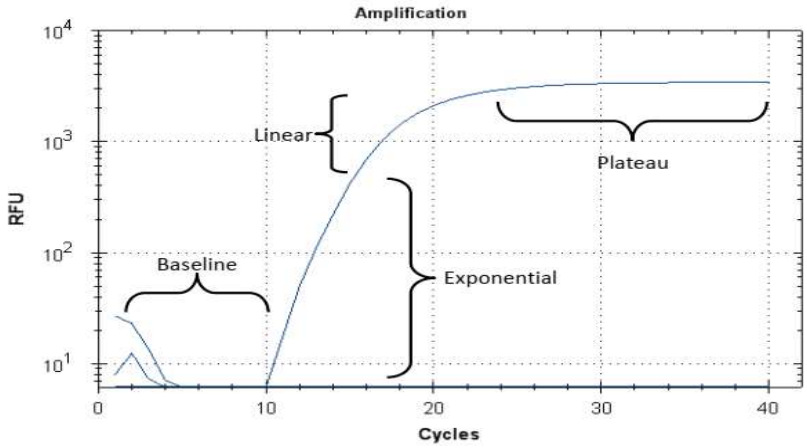
Baseline: amplification is occuring, but there is no detectable fluorescence

Exponential: Fluorescence now detectable, and rising at an exponential rate

Linear: Fluorescence rising at a linear rate

Plateau: Fluorescence has reached its maximum level in this reaction

Parts of the Amplification Curve: Log View



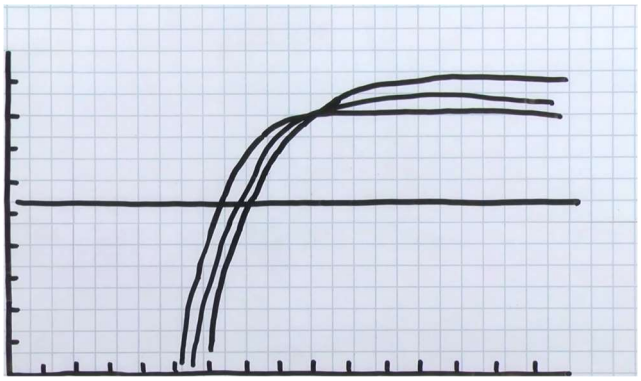
Cycle Threshold Value

- The increase in dye signal is in direct proportion to the number of PCR products. By collecting the fluorescent signal –during the exponential phase of the reaction-we are able to obtain quantitative information on the starting amount of the DNA target

- To simplify, Ct value is the cycle number at which the fluorescent signal of the reaction crosses the threshold. The Ct value is inversely related to the starting amount of target DNA/cDNA

Cycle Threshold Value

Let's examine this representative qPCR amplification plot. A threshold line is drawn through the plot in the exponential phase of the curve.



Threshold Level Selection

Method: Set to the middle of the exponential phase of amplification of the (+) control

Pros:

- More consistency
- Less affected by random noise in the reaction

Cons:

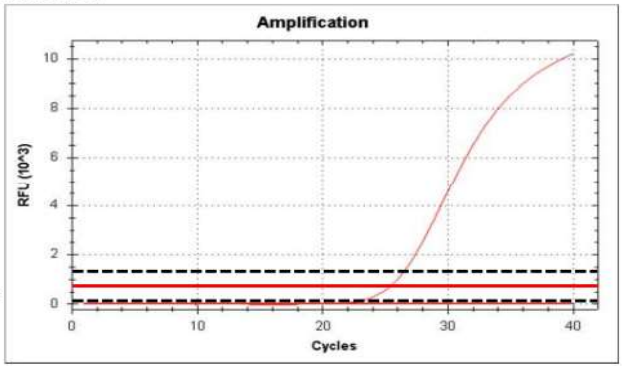
- Higher reported Ct vs. "as low as possible" method
- Still somewhat subjective

Threshold Level Selection – Example 1

Threshold setting: "50% Method"

Step 1:
Visually determine the "lower" and "upper" bound of the exponential phase of amplification

Step 2:
Set your threshold at halfway between the upper and lower bounds



SOP Alteration: Threshold Level Selection

Method 2: Set the threshold as low as the background noise will allow

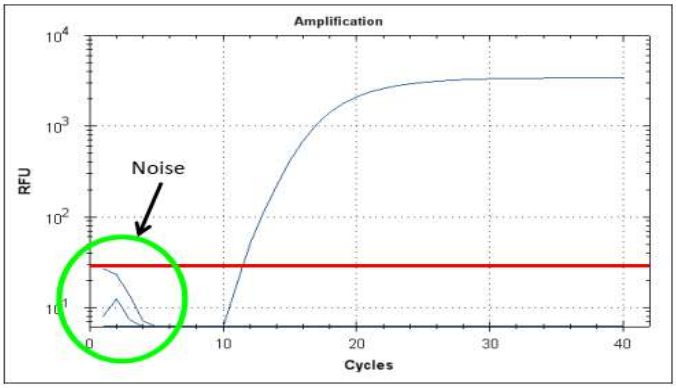
Pros:

- Allows for lowest possible Ct values per experiment

Cons:

- Ct values change with with high "noise" levels
- Ct values of controls can be inconsistent from lab to lab

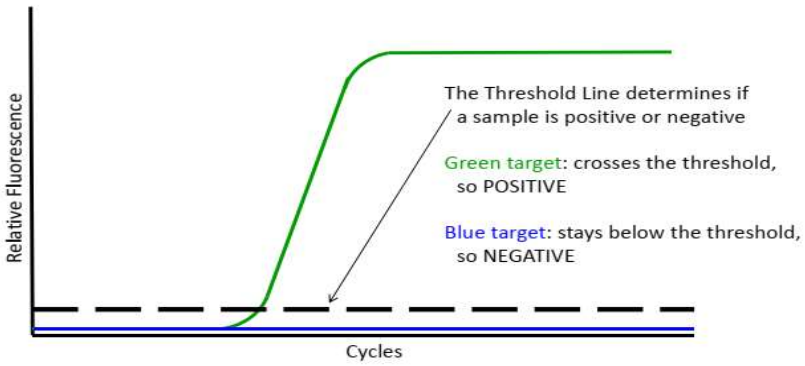
Threshold Level Selection – Example 2



Step 1:
In log view mode, visually identify the highest level of noise amongst all your samples

Step 2:
Set your threshold line just above the highest level of noise.

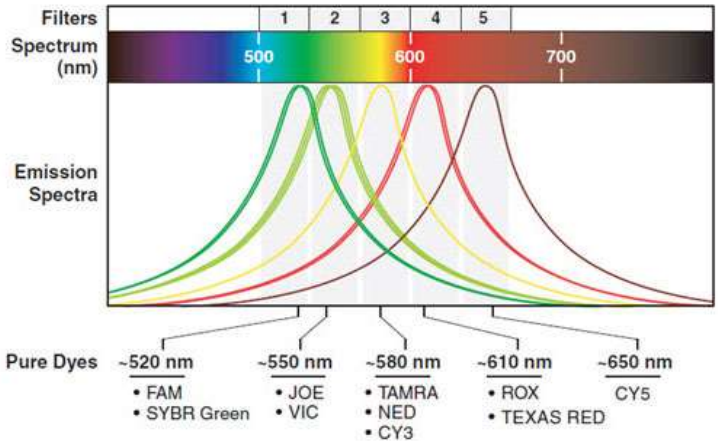
Data Interpretation: Positives and Negatives



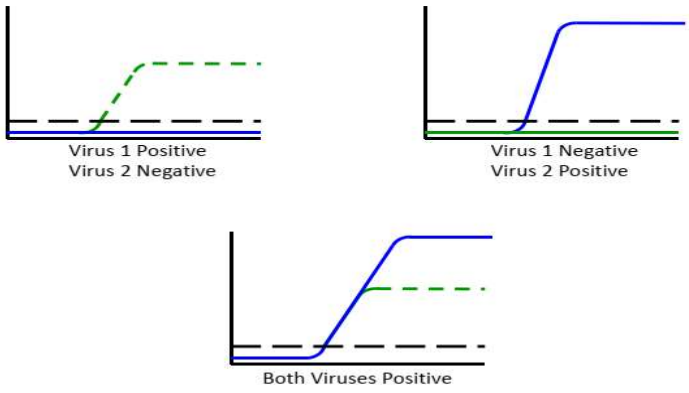
Multiplexing in molecular assays

- In recent years, the trend has been towards creating molecular assays which can detect multiple pathogens in a single assay
- Multiplex PCR denotes the amplification of multiple targets in a single reaction.
  - Gel electrophoresis and analysis of amplicons with different base pairs
  - Sequence specific probes
  - Melting characteristics of amplicons

Multiplexing qPCR assays requires careful choosing of fluorophores and quenchers



Data Interpretation: Multiple Targets



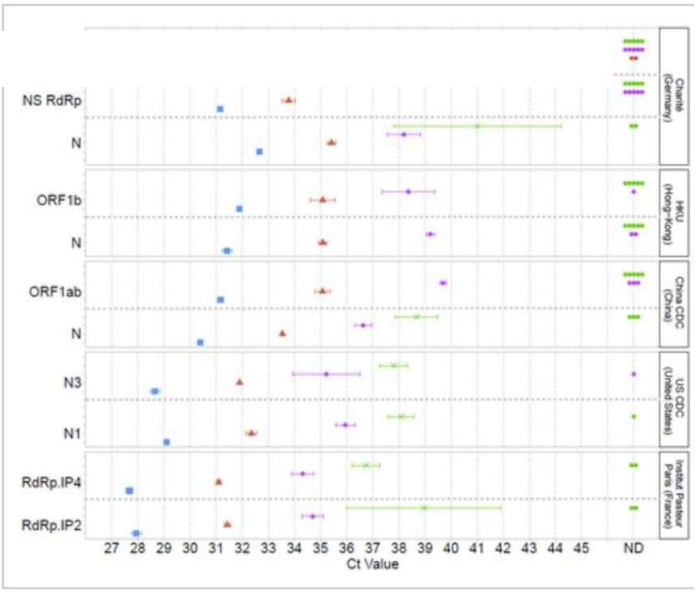
Data Interpretation with a Positive Control

- Negative sample:
  A sample is considered negative for a particular target if the data generated meet two criteria:

  A. The amplification curve for this target does not cross the threshold line.

  B. The extraction/amplification control in the well is positive. *If this second criterion is not met, the result is considered* "equivocal" *for this target rather than negative.*

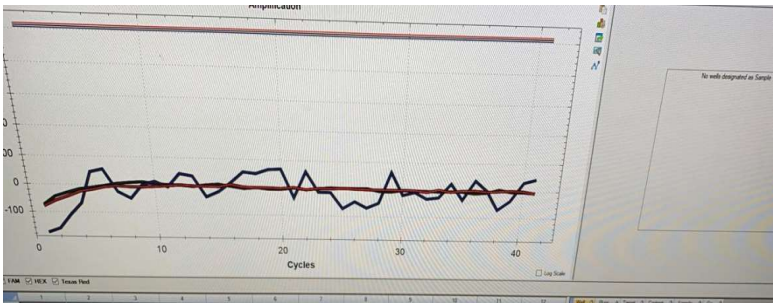
VARIOUS GENE TARGETS USED BY GLOBAL REFERRAL LABS PUBLISHED BY WHO



Types of Controls in PCR and qPCR

- No template control
- Negative Control vs No Template Control
- Positive Control
- Extraction controls
  - Internal extraction controls- E.g. Human RNAse P gene, actin gene in Influenza qPCR, SARS CoV2 qPCR
  - Spiked extraction controls- E.g. PhHV and MS2 phage for stool specimens

WHEN TO RETEST?



1. Invalid result
2. Internal control fails to amplify in absence of gene targets for SARS-CoV-2
3. This could mean
   a. Unsatisfactory extraction/reverse transcription/amplification and detection steps.
   b. Poor specimen quality (collection/transport/storage)

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

a) Improper extraction of nucleic acid from clinical materials resulting in loss of RNA or carry-over of RT-PCR inhibitors from clinical specimens

b) Absence of enough human cellular material in sample to enable detection

c) Improper assay set up and execution

d) Reagent or equipment malfunction

RETESTING

Repeat test and if still invalid repeat testing with a properly collected fresh specimen

When to Retest?

- Presumptive Positive –One of the genes is positive (Usually only the screening gene) and the other negative. This may also be seen in early or late stages of infection when the viral load is low and due to different LODs of different gene targets
- Additional testing may be necessary to differentiate SARS-CoV-2 from other Sarbecovirus for better clinical management/epidemiological decisions

False negative real-time RT-PCR: Possible causes

Pre-analytical:

- Sample collection: Inadequate
- Improper transport
- Inappropriate site of sampling for the stage of disease

Analytical:

- Intrinsic limitation of qRT-PCR or specific kit: Low sensitivity
- Laboratory testing issues

How to control contamination in a molecular biology laboratory

- Laboratory design
- Laboratory practices
- Chemical and enzymatic controls

Laboratory design

Mechanical barriers to prevent contamination

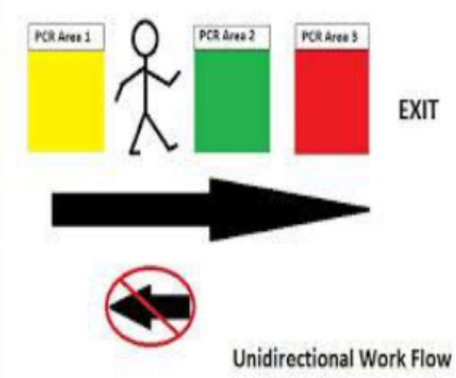
Separation of pre and post amplification work areas

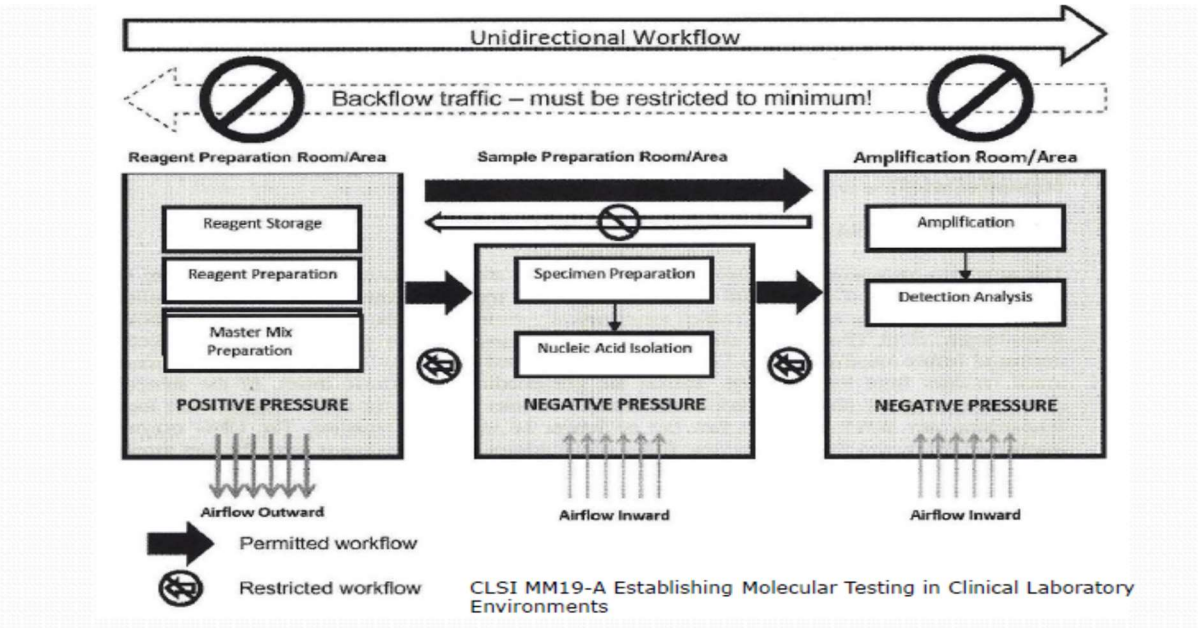
- Area 1- Reagent preparation
- Area 2- Nucleic acid extraction
- Area 3- Amplification, Product detection , plasmid dilution

Physical separation if not substantial distance

Unidirectional workflow

- For both personnel as well as specimens
- Amplification product free area to areas with amplification
- Separate PPE for each area
- AT LEAST FOR THE REAGENT PREPARATION AREA
- Avoid or limit reverse direction





Workflow in a PCR laboratory

A unidirectional workflow should be used to reduce the potential for contamination.

Reagent Preparation Room
PCR master mix preparation and aliquoting room

↓

Sample Preparation Room
(a) Processing of sample (b) Isolation of nucleic acids
(c) Addition of sample to master mix aliquot

↓

Amplification and Product Visualization Room
(a) PCR thermal cycling (b) Confirmation
i. gel electrophoresis (conventional PCR)
ii. visualization (real-time PCR)

Best laboratory practices to be followed for molecular assays

- Separate work areas for nucleic acid extraction, mastermix preparation, PCR set up and post-amplification procedures (if applicable)
  - No sharing of PPE in these different areas (especially "clean room")
- Always use filter pipette tips
- Positive displacement pipettes
- Latex powdered gloves should not be used during PCR assays
- Prefer nitrile gloves

Best practices to be followed for molecular assays

- Make sure all surfaces and equipment are cleaned before and after use
  - 5-10% bleach (Sodium hypochlorite)/DNA ZAP solution etc
  - Followed by wiping with 70% ethanol
- Change gloves frequently to avoid cross-over contamination in assays
- Multiple aliquots of nuclease free water- single use only
- Multiple aliquots of working concentrations of primer/probe
- Always include a blank (no template control, NTC)

Real-time PCR machines – gamut of choices

Figure 3.1: Representation of optical detection system layout

The real-time machine not only monitors DNA synthesis during the PCR, it also determines the melting point of the product at the end of the amplification reactions.

Thermocyclers and Real-time PCR machines

- Uninterrupted power supply a must for real-time PCR machines
- Dye calibrations must be done biannually or annually as required
- Temperature, LASER performance, alignment, safety devices should be checked and optical systems calibrated annually
- Assays being performed on different real-time PCR machines must be validated for each separate instrument

Quality control around equipment and consumables

Equipment	Calibrated	Maintenance			
		Daily	Weekly	Monthly	Annual
Pipettes					
Laminar Flow Hood					
Centrifuge					
Heating Block					
Waterbath					
Thermocycler					
Scale					
Plate Reader					
Sequencer					
Fridge/Freezers					

Table 1. Common equipment used in a molecular laboratory and the maintenance and calibration required

Validation of PCR and Real-time PCR assays

Clinical Chemistry 55:4
611–622 (2009)

Special Report

The MIQE Guidelines: *M*inimum *I*nformation for Publication of *Q*uantitative Real-Time PCR *E*xperiments

Stephen A. Bustin,[1*] Vladimir Benes,[2] Jeremy A. Garson,[3,4] Jan Hellemans,[5] Jim Huggett,[6] Mikael Kubista,[7,8] Reinhold Mueller,[9] Tania Nolan,[10] Michael W. Pfaffl,[11] Gregory L. Shipley,[12] Jo Vandesompele,[5] and Carl T. Wittwer[13,14]

PCR assay performance

1) PCR efficiency
2) Linear dynamic range
3) Limit of detection (LOD)
4) Precision
   - Intraassay variation
   - Inter-assay variation

Linearity

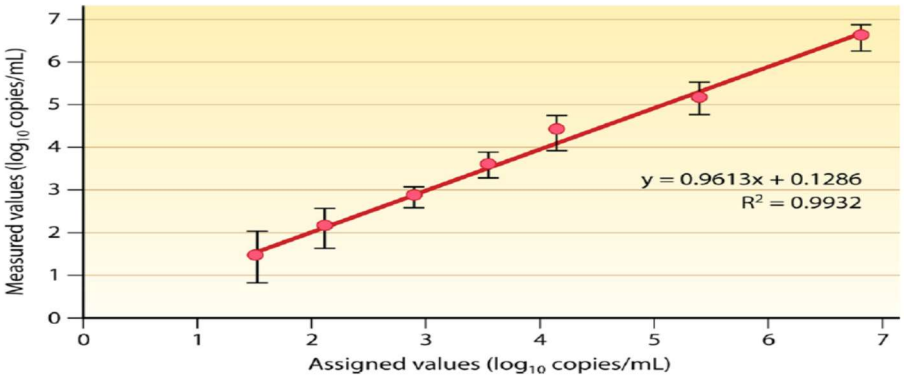
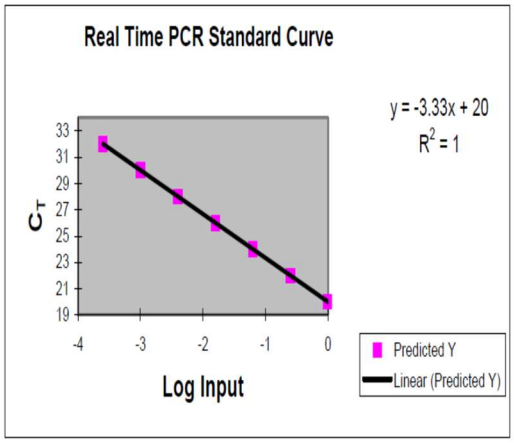


FIG. 1. Plot of results from a linearity experiment to determine reportable range. Seven concentrations of analyte prepared by dilution of a high-concentration standard were tested in triplicate. Assigned

PCR efficiency

- PCR amplification efficiency is the rate at which PCR amplicon is generated, commonly expressed as a percentage.
- Slope of the standard curve of a quantitative PCR is commonly used to estimate the PCR amplification efficiency of a real-time PCR reaction.

Real Time PCR Standard Curve

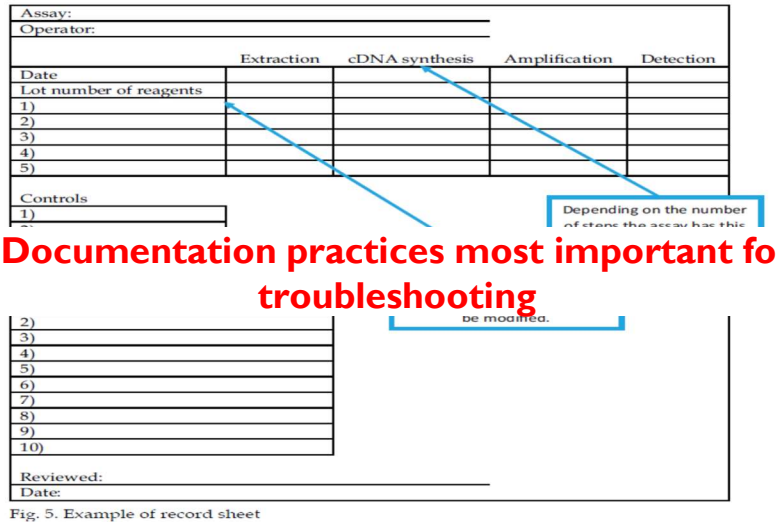


A calculation for estimating the efficiency (E) of a real-time PCR assay is:

$$E = (10^{-1/slope} - 1) \times 100$$

Figure 1: Real-Time PCR Standard Curve representing 100% PCR Efficiency

Worksheets/Record sheets for each molecular test being performed is important – especially when troubleshooting



Assay:
Operator:

	Extraction	cDNA synthesis	Amplification	Detection
Date				
Lot number of reagents				
1)				
2)				
3)				
4)				
5)				

Controls
1)

Depending on the number of steps the assay has this

Documentation practices most important for troubleshooting

be modified.

2)
3)
4)
5)
6)
7)
8)
9)
10)

Reviewed:
Date:

Fig. 5. Example of record sheet

आज़ादी का अमृत महोत्सव

Closed Systems for COVID-19 Detection (CBNAAT - GENE XPERT)

USAID FROM THE AMERICAN PEOPLE

RISE Reaching Impact, Saturation, and Epidemic Control

Training Objectives

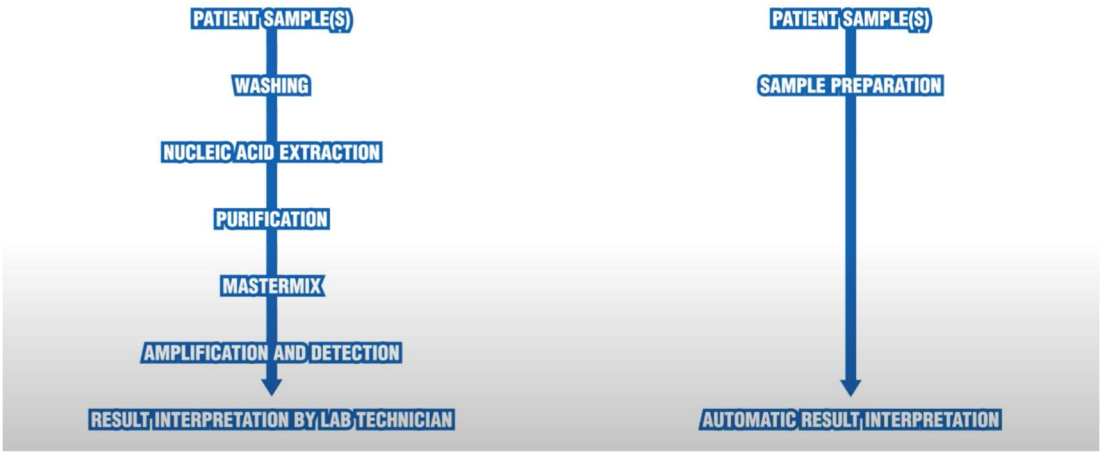
- Introduction
- Intended Use
- ICMR Advisory
- Technical Training
- Maintenance
- Quality control

Introduction

Introduction

- The Xpert Xpress SARS-CoV-2 test is a cartridge-based nucleic acid amplification test (CBNAAT) that is performed using the GeneXpert system.
- The GeneXpert system is a closed platform that simplifies molecular testing
- Fully integrated and automated (that is, specimen preparation, amplification and detection)
- Single run with a short turnaround time of 45 minutes

Conventional RT-PCR vs GeneXpert



Automated Xpert® Xpress SARS-CoV-2

The Cepheid Solution - Advantages

- Detection of SARS-CoV-2- single platform
- On-board internal controls for each sample
- Approximate Time to Result: 45 minutes
- Closed cartridge system minimizes risk of contamination
- On-demand results
- minimal training requirements
- Random access

Cepheid models

Instrument configuration	Number of modules	Capacity per 8 hour shift/day*
GeneXpert GX-IV	4	32
GeneXpert GX-XVI	16	128
GeneXpert GX-INF 48s	48	384
GeneXpert GX-INF 80	80	640

*The reference document provides the capacity of GeneXpert systems for 24 hours; however, for this document the capacities have been extrapolated to 8 hours per day.

Intended Use

Intended Use

- The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test
- Intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider.
- Testing is limited to laboratories that are ICMR approved for PCR or CBNAAT in India.

Targets

- N2 - Nucleocapsid
- E - envelope

These are genetically well characterized for SARS-CoV-2, and the combination of targets provides sensitive and specific detection.

ICMR Advisory

ICMR Advisory

- As per the ICMR Advisory, CBNAAT is considered to be the **first choice of RT-PCR** test for routine surveillance in hospital settings and in non-containment areas.
- For routine surveillance in containment zones and screening at points of entry, a rapid antigen test is the first choice and RT-PCR/CBNAAT/TrueNat are the **second choice of test**.
- A single positive CBNAAT is considered to be confirmatory, without requiring any repeat testing.
- ICMR recommends that any testing with the CBNAAT platforms for SARS-CoV-2 is carried under Biosafety Level 2 (BSL-2) conditions and with appropriate biosafety precautions.

ICMR Advisory contd..

- Laboratories that are already functional for SARS-CoV-2 testing with RT-PCR and that have the **appropriate BSL-2 setup** may initiate testing using CBNAAT platforms for SARS-CoV-2 without any further approval from ICMR.
- In addition, private laboratories that intend to initiate testing using CBNAAT should have **NABL-accreditation** for the molecular detection of RNA viruses using either RT-PCR or a specific CBNAAT platform.
- All those laboratories that apply can reach out to ICMR with a copy of their NABL application, and ICMR will provide **expedited approval for CBNAAT** subject to NABL approval.

Technical training

Specimen collection and Storage

- Viral Transport Medium containing nasopharyngeal swab/nasal swabs/aspirates

Room temperature up to 8 hrs	-----	5-30 degree C
Up to 7 days	-----	2-8 degree C
More than 7 days	-----	-70 degree C

- Proper storage conditions during specimen transport must be maintained to ensure the integrity of specimens.

Proper Cartridge Handling Techniques

Correct

- Do not touch the reaction tube
- Keep the cartridge upright
- Do not tilt after sample is added

Incorrect

Warnings and Precautions

- Do not shake the cartridge
- Do not use a cartridge... :
  - if it appears wet, has leaked with signs of precipitate, or if the lid seal appears to have been broken
  - if it appears damaged
  - that has been dropped after removing it from packaging
  - that has been shaken. Shaking or dropping the cartridge after opening the cartridge lid may yield indeterminate results.
  - that has a damaged reaction tube (bent, missing, cracked)
  - that has been used: each cartridge is single-use to process one test
- Do not reuse pipettes
- Do not reuse swabs

Xpert Xpress SARS-CoV-2 Cartridge Preparation

1

Take one Xpert cartridge for each sample.

2

Rapidly invert the tube 5 times.

3

Open the cartridge lid.

4

Using a clean 300 µL pipette (supplied), transfer 300 µL (one draw) of the sample to the cartridge.

5

Close the cartridge lid.

Start the test within 30 minutes of adding the sample to the cartridge

Starting the test

- Turn on the GeneXpert instrument- Log in - user name and password.
- Click Create Test (GeneXpert Dx).
- Type the Patient ID, make sure the Patient ID is typed correctly. Scan or type in the Sample ID.
- The Scan Cartridge dialog box appears. Scan the barcode on the Xpert Xpress SARS-CoV-2 Assay cartridge.
- The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- Click Start Test (GeneXpert Dx). Enter your password, if requested.
- Open the instrument module door with the blinking green light and load the cartridge.
- Close the door. The test starts and the green light stops blinking.

End Test

When the test is finished, the light turns off.

- Wait until the system releases the door lock before opening the module door and removing the cartridge.
- The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

Click the View Results icon to view results.

- Upon completion of the test, click the Report button of the View Results window to view and/or generate a PDF report file.

Cepheid videos

- Cepheid's Xpert® Xpress SARS-CoV-2 Test

  https://youtu.be/2s4KxC4M8Gw

- GeneXpert Xpert Cartridge, A Look Inside

  https://youtu.be/mIsBLmjus6Q

SARS-CoV-2 Positive

- The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) or just N2 gene is detected have a Ct within the valid range and endpoint above the minimum setting
- SPC: NA; SPC is ignored because coronavirus target amplification occurred
- Probe Check: PASS; all probe check results pass
- Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
- A positive result does not rule out bacterial infection or co-infection with other viruses.

SARS-CoV-2 PRESUMPTIVE POSITIVE

- The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present
- Sample should be retested.
- For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 for epidemiological purposes or clinical management.
- The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting
- SPC: NA; SPC is ignored because a target amplification has occurred.
- Probe Check: PASS; all probe check results pass

SARS-CoV-2 Negative

- The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected.
- The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting
- SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting
- Probe Check: PASS; all probe check results pass

Reasons to Repeat the Assay

- A PRESUMPTIVE POSITIVE result indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Only one of the SARS-CoV-2 nucleic acid target was detected (E gene) while the other SARS-CoV-2 nucleic acid target (N2 gene) was not detected.
- An INSTRUMENT ERROR result could be due to, but not limited to, the maximum pressure limits were exceeded.
- A NO RESULT- REPEAT TEST indicates that insufficient data were collected. For example, Probe Check Control failed or a power failure occurred.
- If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.

Instrument Error

- If you encounter an INSTRUMENT ERROR, touch CLEAR ERROR and follow the on-screen instructions.
- When the Home screen appears, follow the retest procedure.
- If another INSTRUMENT ERROR occurs upon retest, contact Technical Support for assistance.

No Result – Repeat test

- No RESULT indicates that insufficient data were collected.
  - Test was stopped with stop test button
  - Electrical failure
- Secure the power
- Repeat the test with a new cartridge

- If you encounter a NO RESULT - REPEAT TEST result, follow the retest procedure.
- If a NO RESULT- REPEAT TEST result occurs upon retest, contact Technical Support for assistance.

Invalid result

- SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined.
- SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting
- Probe Check - PASS; all probe check results pass

Possible causes:

- Improper sample collection or preparation
- Presence of interfering substances in the sample

Solution:

- Repeat the test with a new cartridge

Result interpretation

Result displayed	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+	+/-
	+	-	
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-
ERROR	NO RESULT	NO RESULT	NO RESULT
No Result	NO RESULT	NO RESULT	NO RESULT

Retest Procedure

1	2	3	4
Discard used cartridge Follow your institution's safety guidelines for disposal of cartridges	 Obtain the residual specimen, mix according to Package Insert If the leftover specimen volume is insufficient, or the retest continues to return an INSTRUMENT ERROR or NO RESULT, collect a new specimen.	 Obtain a new cartridge Label appropriately as retest on the new cartridge Process the specimen per the Package Insert	 Run the test on the System

Factors That Negatively Affect Results

- Improper specimen collection
- The performance of this assay with other specimen types or samples has not been evaluated.
- Inadequate numbers of organisms are present in the specimen.
- Improper transport or storage of collected specimen
- Storage and transport conditions are specimen specific
- Refer to the Package Insert for the appropriate handling instructions
- Improper testing procedure
- Modification to the testing procedures may alter the performance of the test
- Careful compliance with the package insert is necessary to avoid erroneous results

Limitations

- The performance characteristics of this test have been established with the specimen types listed in the Intended Use section only. The performance of this assay with other specimen types or samples has not been evaluated.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if an insufficient number of organisms are present in the specimen.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding, resulting in failure to detect the presence of the virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Performance characteristics

- Analytical sensitivity (limit of detection, LoD): 0.0050 and 0.0200 PFU/mL for the N2 target and E target, respectively
- Analytical reactivity (inclusivity):
  - For the E target, Xpert Xpress SARS-CoV-2 had a **100% match to all sequences**, with the exception of four sequences that had a single mismatch.
  - For the N2 target, Xpert Xpress SARS-CoV-2 had a **100% match to all sequences**, with the exception of two sequences that had a single mismatch.
- Analytical specificity (exclusivity): E primers and probes were found not to be specific for SARS-CoV-2 and will detect other human and bat SARS-coronaviruses. No potential unintended cross-reactivity with other organisms was predicted based on the in silico analysis.

Xpert® Xpress SARS-CoV-2 Kit Storage and Handling

- Store test kits at 2-28 C . Do not use expired cartridges.
- Each single-use cartridge is used to process one test. Do not reuse processed cartridges.
- Do not open a cartridge until ready to use.
  - Start the test within 30 minutes of adding the sample to the cartridge.
- To avoid cross contamination during sample handling steps, change gloves between samples

Waste Disposal

- 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.

Infrastructure Requirements

- 2 Small rooms/one room with partition one for Biosafety Cabinet (BSL-2 level) and one for the GeneXpert Machine
- Recommended RT – 15-30 degree C (Air conditioning to ensure temp. maintained)
- Separate rooms for donning/doffing/autoclave/staff reporting room
- Uninterruptible Power Supply/ Surge Protector
- Storage of cartridges and specimen reagent - 2-28 degree C
- Cartridges are stable if kept at 2 to 45°C for less than 6 weeks at 75% relative humidity
- An average household refrigerator can hold the supplies needed for 2 weeks in a laboratory performing 12 to 16 tests per day.

HR

- Minimum requirement for an 8 hour shift for running GeneXpert 4 x4 machine. This manpower will increase as the number of machines increase.
- 1 Microbiologist to authenticate the reports.
- 1-2 BSc/MSc (MLT/Microbiology) technicians to run the assays.
- 1-2 House keeping staff to do cleaning and proper waste disposal.
- Desired expertise of the laboratory staff:
- Good understanding of laboratory biosafety and biosecurity and trained in handling respiratory samples for viral diagnosis, RNA extraction and real-time PCR.
- Experience of work in virology and handling clinical specimens, especially respiratory samples.

Maintenance

Maintenance of equipment

- The instrument should be checked and cleaned periodically (10% bleach followed by 70% ethanol) as a precautionary measure.
- The equipment should be maintained clean and dust-free in a secure location when not in use.
- The equipment should be cleaned before and after use.
- In the case of spills, appropriate spill management procedures should be followed as per standard protocols.
- Daily, monthly, weekly, quarterly and annual maintenance should be performed and recorded as per the Maintenance Log.
- Regular Data back up- Archiving results
- Annual calibration or 2000 tests/module

Instrument Maintenance Log

GeneXpert® System Maintenance Log

Institution name and lab location

GeneXpert® Serial Number:

Installation Date:

Date of last Calibration:

Maintenance Month: Year:

Instructions: 1. Enter the name of your institution, GeneXpert Serial Number, last calibration date, installation date, and the current month and year above.
2. For each maintenance activity listed below check the box(es) under the day of the month that the activities were performed and enter your initials (2characters maximum) in the bottom row.
3. Save the file after entering the data. We recommend saving one file each month for a complete record of activities

Daily Maintenance	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean work area	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Keep module doors upright	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Discard used cartridges	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Weekly Maintenance																															
Reboot the GeneXpert Instrument	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Reboot the software and computer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Monthly Maintenance																															
Archive Runs ** (Chapter5)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Delete Runs **(Chapter5)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Disinfect GeneXpert Instrument surfaces* (Chapter10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Disinfect Cartridge Bay Interior* (Chapter 10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Disinfect Plunger Rod* (Chapter 10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Clean Fan Filters	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Calibration of the Instrument																															
Yearly or every 2000 runs per module, whichever is first (Chapter7)***	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
As Required																															
Print System Log Report (Chapter 10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Technician Initials (two letters)																															

Quality Control

Xpert® Xpress SARS-CoV-2 Cartridge Controls

- **Each Xpert cartridge is a self-contained test device.**
  A Sample Volume Adequacy Control (SVA), Sample Processing Control (SPC), and a Probe Check Control (PCC) are included in the cartridge.

- The SVA is present to:
  - verifies that the correct sample volume is added to the cartridge
- The SPC is present to:
  - verifies that sample processing is adequate
  - monitor for the presence of inhibitors in the PCR reaction
- The PCC verifies:
  - reagent rehydration
  - PCR tube filling in the cartridge
  - probe integrity
  - dye stability

Quality Control

- There are two in-built internal controls that run with every cartridge to check whether the instrument is working accurately.

- External positive and negative controls can also be run from time to time.
  - Each time a new lot of Xpert® Xpress SARS-CoV-2 Assay reagents is received.
  - Each time a new shipment of Xpert Xpress SARS-CoV-2 Assay reagents is received even if it is the same lot previously received.
  - Each time a new operator is performing the test
  - When problems (storage, operator, instrument, or other) are suspected or identified.
  - If otherwise required by your institution's standard QC procedures.

Quality Assurance

- There is no NABL approved EQAS program for Covid 19 testing in the country.
- ICMR- Interlaboratory comparison (ILC) of both Positive and Negative samples can be done with another NABL accredited Laboratory that has CBNAAT in its scope.
- ILC samples should be examined by personnel who routinely examine patient samples, using the same procedure as those used for patient samples.

Commercially Available External Controls

Vendor	Description	Configuration	Storage
AccuPlex™ SARS-CoV-2 Reference Material Kit (SeraCare) Catalog # 0505-0126	Positive Control	1.5ml	2-8°C or -20°C
	Negative Control	1.5ml	2-8°C or -20°C

- Many other vendors for quality control material are also available in addition to the one outlined above.
- External controls should be used in accordance with local, state accrediting organizations, as applicable

Closed System for COVID-19 Detection: TRUENAT (MOLBIO)

Truenat - Learning Objectives

1. Introduction
2. ICMR Advisory
3. Technical training
4. Maintenance
5. Quality control

Introduction

Introduction

- Molecular tests -restricted -require skilled staff and elaborate infrastructure
- TrueNat (Molbio) - Indigenously developed, chip-based, real-time PCR testing system
- Rapid, simple, robust and user-friendly test - semi-quantitative viral detection method
- **Short test duration of 1 hour**
- Combination of portable, lightweight, battery-operated
- "sample to result" capability, even in resource-limited settings

Advantages

- Enables decentralisation and near-patient diagnosis and monitoring and is useful even in resource-limited settings
  - portable, lightweight, battery-operated and fully automated
  - rapid, simple, easy to use, robust and user-friendly
  - "laboratory in a suitcase" and can be used in remote areas
  - room temperature-stable (2–30˚C)
- Considerably lower biosafety requirements
  - VLM inactivates the virus
  - closed nature of the TrueNat platform and minimal sample handling
- Automated reporting system and is GPRS/Bluetooth enabled

Capacity of Truelab Analyzers

Type of Truelab micro PCR analyzer	Tests/8 hours
Truelab Uno Dx	10–12 tests
Truelab Duo	20–24 tests
Truelab Quattro	40–48 tests

ICMR Advisory

ICMR Advisory

- **First choice of test** on a par with RT-PCR for routine surveillance in hospital settings and in non-containment zones.
- **Second choice** in routine surveillance in containment zones and screening at points of entry,
- A single TrueNat-positive test -**confirmatory**, with no repeat testing required.
- No re-testing is recommended prior to discharge from a COVID-19 facility
- No further RT-PCR-based confirmation required for samples that are confirmed TrueNat positive

ICMR recommendation:

- Two-step singleplex assay, comprising screening (E gene) and confirmatory (RdRP gene) assays
- Multiplex assay, comprising screening (E gene) and confirmatory (Orf1a gene) assays.

ICMR advisory contd..

- Testing is limited to laboratories that are **ICMR-approved for RT-PCR** or TrueNat testing in India.
- All private laboratories who intend to begin TrueNat-based testing for COVID-19 should be encouraged to immediately apply for **NABL accreditation**.
- ICMR will provide **expedited approval** for TrueNat subject to NABL approval, which can be submitted within a maximum time span of four weeks from the date of approval.

Technical training

Intended use

TrueNat COVID-19 diagnostic assays are intended for the detection of RNA from SARS-CoV-2, in either nasopharyngeal swab and/or oropharyngeal swab specimens and aid in the detection and confirmation of COVID-19

Sample collection, storage and transportation

- Oropharyngeal or nasopharyngeal swab specimens
- Trueprep Transport Medium for Swab Specimen is used as a medium for collection, decontamination and transport
- Transport medium for the swab specimen decontaminates the specimen and maintains the integrity of the nucleic acid
- Specimens collected in the transport medium are stable for up to 3 days at 40°C and 1 week at 30°C

Target genes

- envelope protein (*E*) gene
- RNA-dependent RNA polymerase *(RdRp)* gene
- open reading frame 1a (*Orf1a)* gene.

Internal positive control (IPC) - human RNase P serves as a full process

Types of assays

Assay 1: TrueNat Beta CoV E gene screening assay

All negative results are to be considered as "true negatives". All positive samples should be subject to confirmation by assay 2.

Assay 2: TrueNat SARS-CoV-2 RdRp gene confirmatory assay

All samples that test positive by this assay must be considered to be "true positives".

Assay 3: Multiplex assay COVID-19 -screening (E gene) and confirmatory (Orf1a) targets

All negatives are to be considered as "true negatives". All samples that test positive by this assay must be considered to be "true positives".

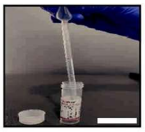
Principle

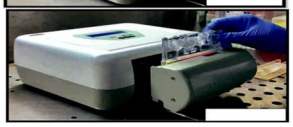
- Real-time reverse-transcription polymerase chain reaction (RT-PCR), based on **Taqman chemistry**
- The purified RNA is treated with the PCR reagents containing **the reverse transcriptase (RT)** enzyme, mixed well and allowed to stand for 30 to 60 seconds
- This mixture is added to the reaction well of the TrueNat chip and the test is inserted into the Truelab 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-labelled fluorescent probe in the TrueNat chip-based real-time PCR test to **release fluorophores in an exponential manner**.
- This is then captured by the built-in **opto-electronic sensor** and displayed as an amplification curve on the analyzer screen, on a real-time basis during the test run.
- **Ribonuclease P (RNase P),** a human constitutive gene, is used as an internal control in every assay.

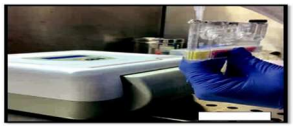
COVID-19

Sample Processing Protocol on Trueprep® AUTO

- Wear Personal Protective Equipment (PPE) as per ICMR guidelines
- Open the cap of the Transport Medium for Swab Specimen Tube
- Transfer 0.5 mL of swab sample into the LYSIS BUFFER Tube using 1 mL Transfer Pipette (Fig. 1)
- Discard* the Transfer pipette
- Remove the CARTRIDGE from the pouch,label it & place it on the Cartridge stand. Keep the ELUTE COLLECTION TUBE (ECT), ECT label and elute transfer pipette in the pouch for later use
- Transfer the entire content of the Lysis Buff er Tube to the SAMPLE CHAMBER (BLACK CAP) of cartridge using 3ml transfer pipette. Discard the pipette
- Switch "ON" the TRUEPREP® AUTO device and press "EJECT" button to open, gently pull out the door (cartridge holder) , insert and press 'START'
- The device will beep at the end of the process (20 min.) & cartridge holder will eject automatically
- Gently pull out cartridge holder, remove cartridge, place it on the cartridge stand
- Pierce the ELUTE CHAMBER with provided transfer pipette (Fig. 5), transfer the entire volume into Elute Collection Tube (ECT). Label the ECT tube with patient details Discard* the transfer pipette & Cartridge







Handling of Transfer Pipettes

Take care to remove Transfer pipettes as shown in (Fig. A)

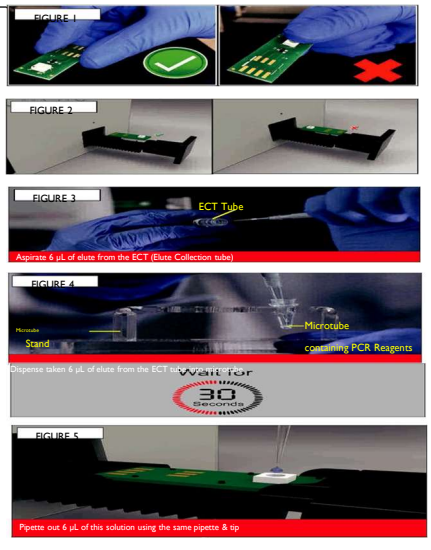
DO NOT insert hands into the package of transfer pipettes (Fig. B) as this could contaminate all the pipettes

Addition of swab sample to lysis buffer

Transferring liquified sample into cartridge

Truelab™ Real Time micro PCR Protocol

- Switch "ON" the TRUELAB™ ANALYZER. LED will glow in Green
- Select USER ID, enter password to Login. Select the Bay. Select test profile
- Press "START REACTION" on the screen. Chip tray opens. "Please Load Sample" will appear (Don't press "YES" until chip loading is complete)
- Open the chip pouch of TRUENAT™ Covid 19 and takeout the chip without touching white well portion. Place it on the chip tray by aligning with the holes & pins
- Place the microtube containing freeze dried RT PCR reagents (master mix) in the microtube stand. Fix 6 µL filter barrier tip provided in the pouch with the 6 µL TRUEPET® PIPETTE.
- Aspirate 6µL of the elute collected in the Elute Collection tube (Fig. 3) & dispense to microtube containing freeze dried RT PCR reagents . **Do not discard the pipette tip**
- INCUBATE FOR 30 SECONDS at room temperature - use the same pipette & tip to dispense the mastermix into the chip well Discard* the pipette tip
- Press YES on the screen. Chip tray closes and reaction will start.
- After completion of the test, .tap the "Open/Close Tray" button to eject the chip tray. Discard* the chip
- Press "PRINT" to print result page using Truelab micro PCR Printer



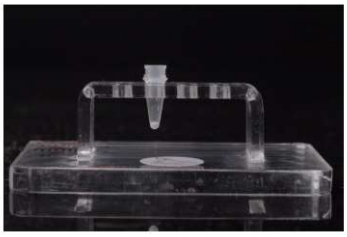
Placing Mastermix on the Microtube Stand

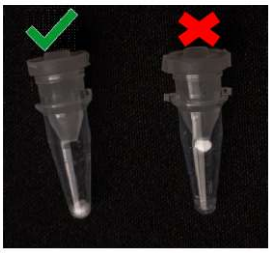
1. Master mix cake may sometimes get dislodged from the bottom while inserting the tip. So gently tap the tube to bring down the cake
2. Add the elute and wait for 30 seconds. Do not mix the contents

Placing Mastermix on the microtube stand

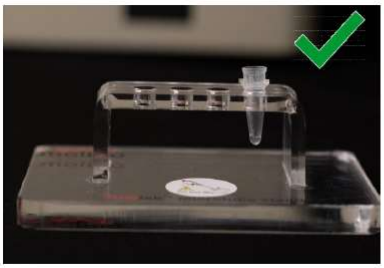
Always check for the presense of mastemix cake

Cake should be at the bottom of the tube

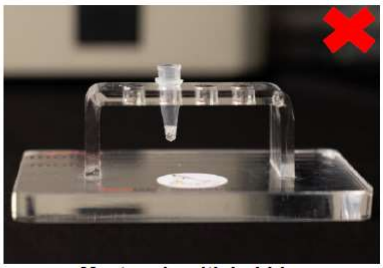




Checking Mastermix Solubility



Properly dissolved mastermix



Mastermix with bubbles

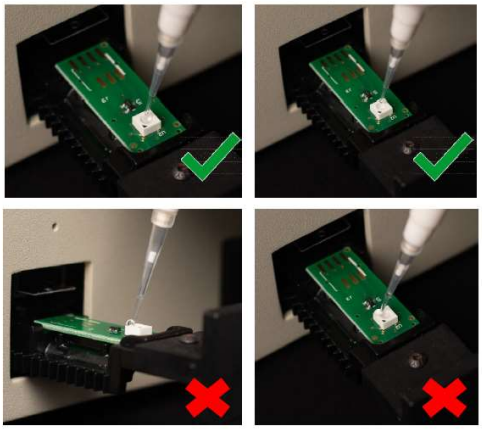
Pipette Handling

DO NOT place the pipette down on device/other surfaces. This could lead to contamination

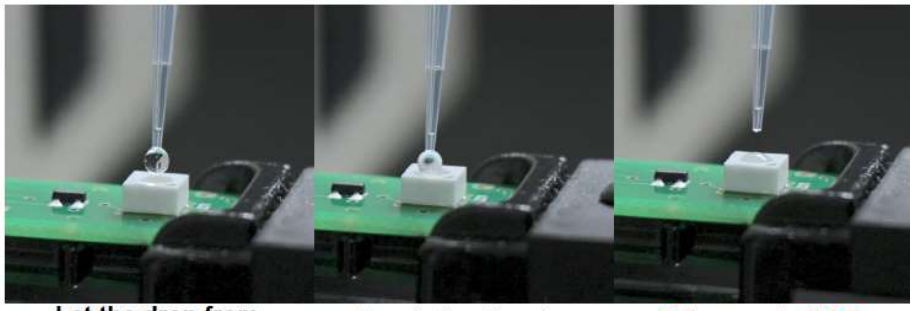
Place pipette properly back in stand after loading elute onto the chip

Loading of Eluate

Loading the elute onto the chip reaction well



Loading of Eluate Contd…



Let the drop from below the tip by pressing the pipette. Place directly over the well

Touch the drop to the well so that it gets released into the well

Take care to NOT pierce the well with the tip during loading

Interpretation of results

- Amplification curves **displayed on the Truelab** real-time micro PCR Analyzer screen
- The time taken identified by the **cycle threshold (Ct)** of the specimen will depend on the number of virus copies in the sample
- Both the target gene and the IPC RNase P curves show a steep, exponential path when the fluorescence crosses the threshold value in the case of **positive samples**
- The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of **negative samples**
- At the end of the test run, results are displayed as "**detected**" for a positive result and "**not detected**" for a negative result.
- TrueNat the cut off is 32
- In some specimens with a high target load, the IPC may not amplify, however, the test run is still considered valid. If the IPC curve remains horizontal in a negative sample, the test is considered invalid.

Result interpretation and reporting

TrueNat COVID-19 test results based on Ct values

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 to 72 hours
-	-	+	SARS- CoV-2 NEGATIVE	Report negative
-	-	-	INVALID	Collect new swab and repeat

- ICMR requires all laboratories to enter data via the ICMR portal (https://cvstatus.icmr.gov.in/login.php) to help in guiding national strategies

Truenat video

https://youtube/5kJUw7gJIAQ

Do's and Dont's

Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

- ✓ Place the device in a dust free environment
- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Replace the cartridge holder tray in case of a spillage in the device

- ✓ If the device is kept idle for more than 10 days, carry out the flushing protocol (Refer manual section 8.2.3) before resuming operation

- ✗ Do not leave a used cartridge inside the device. Remove the cartridge after extraction and collect the elute in the ECT
- ✗ Do not keep used material on the work bench and on the device

- ✗ Do not tilt or shake the device while connecting charger, during extraction or while removing cartridge from device
- ✗ Do not touch the nozzle of the liquefaction buffer bottle to the lysis buffer tube during sample preparation

Reaching Impact, Saturation, and Epidemic Control (RISE)

Do's and Dont's

Truelab® Uno Dx/Duo/Quattro Real Time Quantitative micro PCR Analyzer

- ✓ Place the analyzer on a flat surface in a dust free environment, away from direct sunlight and vibration causing instruments

- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Ensure that the elute is loaded in the center of the reaction well

- ✗ Do not move the analyzer when a test is in progress
- ✗ Do not spill water or any other solution on the surface of the analyzer
- ✗ Do not touch the white reaction well or the connector pads of the chip. Hold the chip by the edges only

- ✗ Do not leave a used chip inside the device
- ✗ Do not keep used material on the work bench and on the device

Reaching Impact, Saturation, and Epidemic Control (RISE)

Safety precautions

- For in vitro diagnostic use only.
- Bring all reagents and specimen to room temperature (20 – 30 degree C) before use.
- Do not use kit beyond expiry date.
- 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.
- All materials of human origin should be handled as though potentially infectious.
- Do not pipette any material by mouth.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
- Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

TrueNat device errors

True prep Auto v2 Universal Cartridge-based sample preparation device errors

- thick specimens
- cartridge not being detected
- problems with the reset card/QR code reader
- device heating plates not working
- damaged cartridge valve or pressure drop errors

Truelab Real-Time Quantitative micro PCR Analyzer errors

- expired chip
- thermal cycling error
- issues with chip memory
- aborted test run
- runtime error, or failed IPC run.
- If repeat testing does not resolve the errors, seek support from the manufacturer

Performance characteristics

Limit of detection (LoD)

TrueNat Beta CoV *E* gene - 486 genome copies/mL,

TrueNat SARS-CoV-2 *RdRp* gene - 407 genome copies/mL.

TrueNat COVID-19 Multiplex assay - 487 (E gene) and 480 genome copies/mL (Orf1A)

The sensitivity, specificity, positive predictive values and negative predictive values were shown to be 100%

(ICMR-NIV, Pune validation study).
https://pubmed.ncbi.nlm.nih.gov/33146156/

Biosafety and BMW

- The biological specimens, chips, transfer devices, cartridges and gloves used for TrueNat testing should be disposed of immediately
- Freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines
- A regular laboratory cleaning protocol should be maintained to prevent the occurrence of contamination.
- There are chances of spills, especially when staff are not adequately trained and at the same time are under immense pressure to deliver rapid results.
- 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 before continuing work.

Truenat SARS-CoV-2 Kit Storage and Stability

Truenat™ COVID-19 is stable for two (2) years from the date of manufacture if stored between 2-30 degree 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 degree C.

It is also stable for one (1) month at temperatures upto 45 degree C.

Infrastructure & HR

Infrastructure

- One room with a partition, or two rooms
- One for RNA extraction and one for the TrueNat PCR analyzer
- A biosafety cabinet (Class 2A/2B) is not mandatory but is desirable for carrying out RNA extraction.
- Additionally separate rooms are required for: Donning PPE/Doffing PPE/Autoclaves/Staff/reporting

HR

- Availability of the following minimum staff is required (for 8-hour shifts/day):
- Microbiologists: one or more with experience of work in molecular virology
- Technicians: 1-2 LTs as per the load of the laboratory
- Multitasking staff: one or more for washing/cleaning

Limitations of Assay

- Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site
- Although very rare, mutations within the highly conserved regions of the target genome where the TrueNat assay primers and/or probes bind may result in the under-quantitation of or a failure to detect the presence of the pathogen concerned
- 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 at all times and ensure careful adherence to the procedures specified in the package inserts, to avoid nucleic acid contamination from previous amplifications, positive controls or specimens.
- As with any diagnostic test, results from the TrueNat assay should be interpreted in the context of other clinical and laboratory findings.

Factors That Negatively Affect Results

Improper specimen collection

The performance of this assay with other specimen types or samples has not been evaluated.

Inadequate numbers of organisms are present in the specimen.

Improper transport or storage of collected specimen

Storage and transport conditions are specimen specific

Refer to the Package Insert for the appropriate handling instructions

Improper testing procedure

Modification to the testing procedures may alter the performance of the test

Careful compliance with the package insert is necessary to avoid erroneous results

Maintenance

Maintenance

- The maintenance of required for the devices is minimal.
- The devices should be maintained clean and dust-free in a secure location when not in use.
- The devices are must be cleaned before and after use
- In case of spills appropriate spill management procedures are must be followed as per standard protocols
- Daily, monthly and need-based maintenance shall should be performed and recorded as per the TrueNnat Preventive Maintenance Log
- **The fixed volume (6 microliter) pipette needs to be replaced every 6 months by the company for free of cost**

Maintenance Log

Truelab and Trueprep maintenance log																															Version: 1.1 Date:
Compiled by:									Reviewed by:																					Approved by:	
Month:																															
	Days of the month																														
Maintenance activity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Daily maintenance																															
Clean work area																															
Discard used chips and cartridges																															
Monthly maintenance																															
Disinfect instrument surfaces	Date: Initials:																														
Clean Truelab bays	Date: Initials:																														
Temperature calibration	Date: Initials:																														
Verification of the fixed 6µl pipette	Date: Initials:																														
As necessary																															
Flush protocol for the Trueprep instrument																															
Spillage tray or linear motion guide tray replacement																															
Slider glass replacement – indicate bay																															

Quality control

Quality control and QA

Ribonuclease P (RNase P), a human constitutive gene, is used as an IPC in every assay

Known positive and negative controls (TrueNat Universal Control kit) are run alongside samples from time to time.

- 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

External quality assurance (EQA)

No NABL-approved EQA programme for COVID-19 testing in India. Inter-laboratory comparison (ILC) of both positive and negative samples should be carried out at least once every 6 months, with another NABL-accredited laboratory test that has TrueNat in its scope.

आज़ादी का अमृत महोत्सव

Highthroughput laboratory for diagnosis and seroprevalance SARS-CoV-2

USAID FROM THE AMERICAN PEOPLE

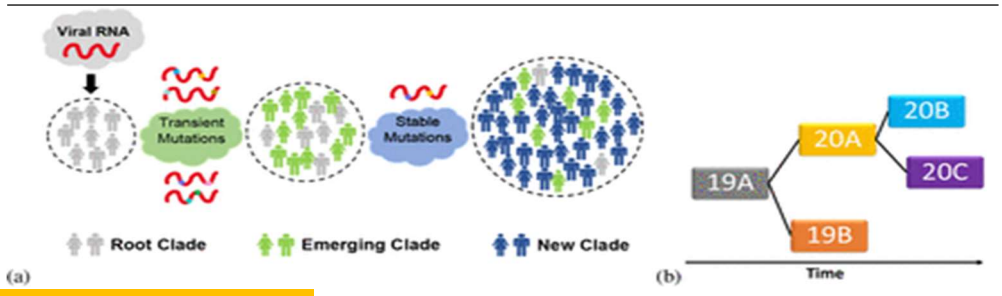
RISE Reaching Impact, Saturation, and Epidemic Control

Acknowledgement: This slide topic was developed & created by **Dr. Debdutta Bhattacharya, PhD,**
Scientist-D/Assistant Director, *ICMR-Regional Medical Research Centre*

Epidemiology of COVID-19

- **Agent** - Corona viruses belong to a large family of viruses, some causing illness in people and others that circulate among animals, including camels, cats, bats etc.
- The etiologic agent responsible for present outbreak of COVID-19 is SARS-CoV-2 which is a novel coronavirus.
- Transmission of coronaviruses can occur via respiratory secretions. Nosocomial transmission has been documented in COVID-19.
- Current estimates of the incubation period of 2019-nCoV range from **2-14 days with** median of 5 days.
- Most common symptoms include **fever, fatigue, dry cough and breathing difficulty**.
- Upper respiratory tract symptoms like sore throat, rhinorrhea, and gastrointestinal symptoms like diarrhea and nausea/ vomiting are seen in about 20% of cases.

Virus clade in view of SARS-CoV-2



In the case of SARS-CoV-2, clades are very interesting because they can be used to track how the virus bounced around the world, entering and re-entering various regions. The variations emerge one from another, and that information allows the added element of time to be added to the tracking.

Clades Mutations	Clades Mutations
19A	Root clade
19B	C8782T, T28144C
20A	C14408T, A23403G
20B	C14408T, A23403G, G28881A, G28882A, G28883C
20C	C14408T, A23403G, C1059T, G25563T

Background

- The 2019 novel coronavirus (2019-nCoV) is a new virus that causes respiratory illness in people
- On 31 December 2019, the WHO China Country Office was informed of cases of pneumonia unknown etiology (unknown cause) detected in Wuhan City, Hubei Province of China.
- The Chinese authorities identified a new type of coronavirus, which was isolated on 7 January 2020.
- On 13 January 2020, the Ministry of Public Health, Thailand reported the first imported case of lab-confirmed novel coronavirus (2019-nCoV) from Wuhan, Hubei Province, China.
- Early on, many of the patients from in Wuhan, China reportedly had some link to a large seafood and animal market, suggesting animal-to-person spread.
- However, a growing number of patients reportedly have not had exposure to animal markets, indicating human to human transmission.

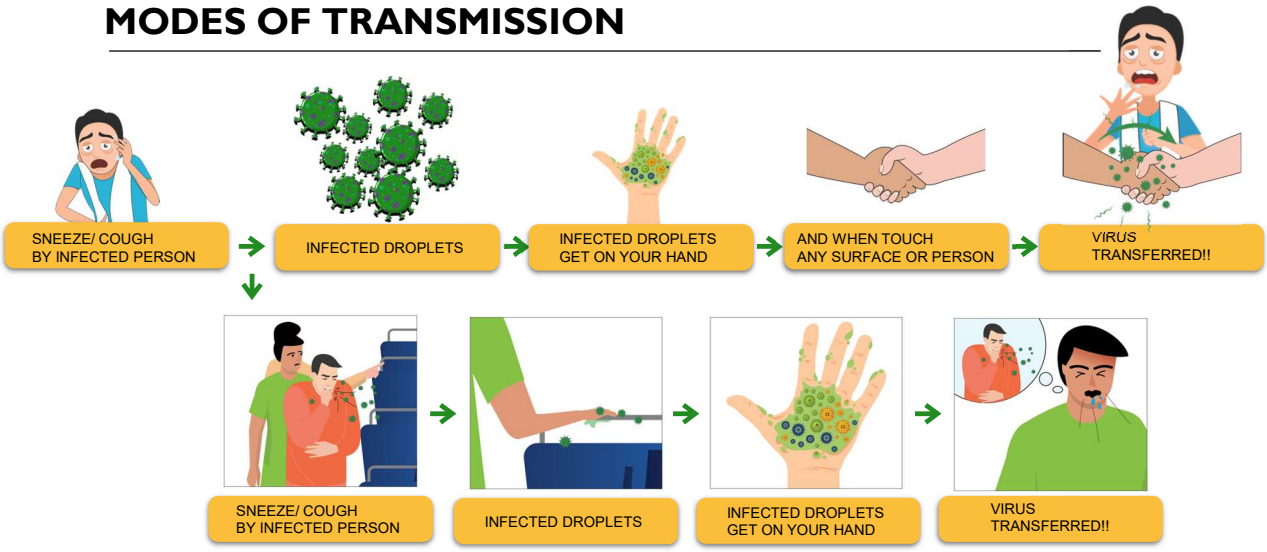
Notable Epidemiological Events Reported

- In France, for the first time outside China, a healthcare worker was diagnosed as being ill with 2019-nCoV acute respiratory disease. The health worker treated two patients who were later identified as probable cases.
- The first instance of third-generation human-to-human transmission outside China has been identified, in an individual who was exposed to a confirmed case from the cluster in Bavaria, Germany.
- For the first time, a case was exported from a country other than China: a patient was identified in South Korea following their exposure in Japan to a confirmed case.

Covid-19 events as they happen

Sl No	Date	Event
1	30th January 2020	WHO Director-General declared the 2019-nCoV outbreak a Public Health Emergency of International Concern
2	30th January 2020	India reported its first case of COVID-19 in Kerala from a student who had returned from Wuhan, China
3	11th February 2020	C-O-V-I-D hyphen one nine – COVID-19
4	11th March 2020	Declared as a pandemic
5	11th March 2020	1st death confirmed in Karnataka of a COVID-19 positive case with travel history to Saudi Arabia
6	13th March 2020	Europe becomes epicenter of the pandemic

MODES OF TRANSMISSION



DEFINITIONS – SUSPECT/PROBABLE INFECTED PERSON

A person with acute respiratory illness (fever and at least one sign/symptom of respiratory disease (eg. Cough, shortness of breath) AND

A history of travel to or residence in a country/area or territory reporting local transmission of COVID-19 disease during the 14 days prior to symptom onset OR

A person with any acute respiratory illness AND having being in contact with a confirmed COVID-19 case in the last 14 days prior to onset of symptoms OR

A person with severe acute respiratory infection {fever and at least one sign/symptom of respiratory disease (eg., Cough, shortness of breath)} AND requiring hospitalization AND with no other etiology that fully explains the clinical presentation OR

A case for whom testing for COVID-19 is inconclusive.

DEFINITIONS - WHO IS A CONTACT

A CONTACT IS A PERSON WHO IS INVOLVED IN ANY OF THE FOLLOWING:

- PROVIDING DIRECT CARE WITHOUT PROPER PERSONAL PROTECTIVE EQUIPMENT (PPE) FOR COVID-19 PATIENTS
- STAYING IN THE SAME CLOSE ENVIRONMENT OF A COVID-19 PATIENT (INCLUDING WORKPLACE, CLASSROOM, HOUSEHOLD, GATHERINGS).
- TRAVELING TOGETHER IN CLOSE PROXIMITY (LESS THAN 1 M) WITH A SYMPTOMATIC PERSON WHO LATER TESTED POSITIVE FOR COVID-19.

TYPES OF CONTACTS

HIGH RISK

- TOUCHED BODY FLUIDS OF THE PATIENT (RESPIRATORY TRACT SECRETIONS, BLOOD, VOMIT, SALIVA, URINE, FEACES)
- HAD DIRECT PHYSICAL CONTACT WITH THE BODY OF THE PATIENT, SHOOK HANDS, HUGGED OR TOOK CARE OF.
- TOUCHED OR CLEANED THE LINEN, CLOTHES, OR DISHES OF THE PATIENT.
- LIVED IN THE SAME HOUSEHOLD AS THE PATIENT.
- ANYONE IN CLOSE PROXIMITY (LESS THAN ONE METER) OF THE CONFIRMED CASE WITHOUT PRECAUTIONS.
- PASSENGER TRAVELING IN CLOSE PROXIMITY (LESS THAN ONE METER) FOR MORE THAN 6 HOURS WITH A SYMPTOMATIC PERSON WHO LATER TESTED POSITIVE FOR COVID-19.

LOW RISK

- SHARED THE SAME SPACE (SAME CLASS FOR SCHOOL/WORKED IN SAME ROOM/SIMILAR AND NOT HAVING A HIGH RISK EXPOSURE TO CONFIRMED OR SUSPECT CASE OF COVID-19).
- TRAVELLED IN SAME ENVIRONMENT (BUS/TRAIN/FLIGHT/ANY MODE OF TRANSIT) BUT NOT HAVING A HIGH-RISK EXPOSURE.

Samples to be Collected

- Essential samples:
  - Throat swab (oropharyngeal swab).
  - Nasal swab (Nasopharyngeal swab)

  } For diagnosis
- Other preferred samples:
  - Bronchoalveolar lavage
  - Tracheal aspirate
  - Sputum

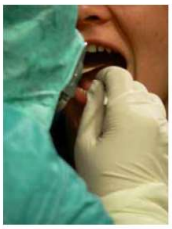
  } Wide mouth sterile plastic containers
- In lab confirmed patients:
  - Blood
  - Stool and urine - Wide mouth sterile plastic containers

Collection of OF and NP swabs

Optimal Timing:

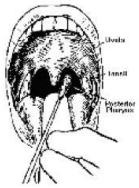
- Within 3 days of symptom onset and no later than 7 days.
- Preferably prior to initiation of antimicrobial chemoprophylaxis or therapy

Collection of Oropharyngeal Swab



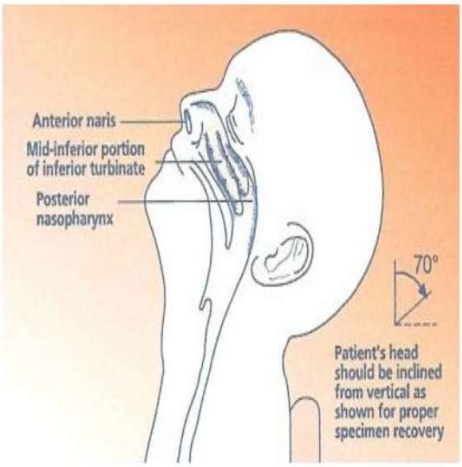
Materials:
- Sterile Dacron/Nylon flocked swab
- Viral Transport Medium (3 ml sterile VTM)

Procedure:
- Hold the tongue out of the way with a tongue depressor.
- Use a sweeping motion to swab posterior pharyngeal wall and tonsillar pillars
- Have the subject say "aahh" to elevate the uvula.
- Avoid swabbing soft palate and do not touch the tongue with swab tip.
- Put the swab in VTM



Collection of Nasopharyngeal swabs

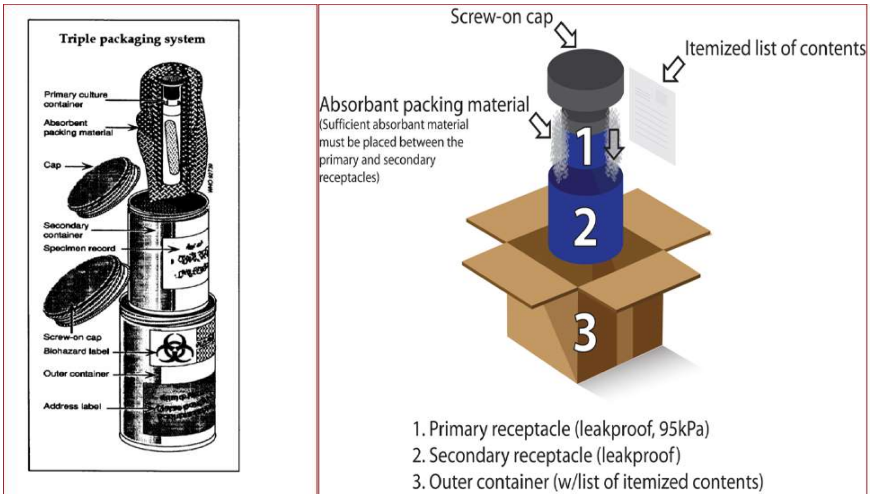
- Materials
  - Sterile Dacron/Nylon flocked swab
  - Viral Transport Medium (3 ml sterile VTM)
- Procedure
  - Tilt patient's head back 70 degrees
  - Insert swab into nostril (Swab should reach depth to distance from nostrils to outer opening of the ear
  - Leave swab in place in place for several seconds to absorb secretions
  - Slowly remove swab while rotating it
  - Place tip of swab into VTM and snap/cut off the applicator stick



Triple Packaging System

Primary Container	Secondary Container	Outer Container/ Packaging Box
•Watertight and leak proof • Cap correctly and securely closed. • Keep in upright position during transport VTM Tubes	•Watertight •Several clinical specimens may be placed into one secondary container • Containers have to be cleansed and disinfected if they are to be re-used E.g.: Disposable, zip-lock plastic bags; Large centrifuge tubes (50 ml) with screw caps	•Made of strong material that can be cleansed and disinfected •Should have the Biohazard warning label •A content list in a sealed plastic bag inside the transport box may also be included Thermocol boxes/vaccine carrier

Triple Packaging System



Highthroughput platform

Roche Cobas 6800

H & FW Dept Odisha
@HFWOdisha

Modern equipment COBAS has been installed in RMRC and with this the testing capacity has increased to 1000 per day. 3/3
#OdishaFightsCorona

10:50 PM · 10/04/20 · Twitter for Android

HIGH THROUGHPUT TESTING LABORATORY

Diagnostic facility with Roche COBAS 6800 Machine in BSL – II+ was established at OPD RMRC in record time of 3 days for ramping up the COVID-19 testing in the state of Odisha.

Inaugurated a New COBAS Laboratory on 9th April 2020

Testing algorithm

Sample receipt
Data entry
Aliquoting
RNA extraction
RT-PCR pre-analytics
RT-PCR post analytics
Data entry
Reporting

Roche COBAS-6800 machine

Daily Testing Capacity ~ 1400-2500 samples/day

The Cobas 6800 System

- It is a fully automated solution with high-throughput, that can drive laboratory efficiency in a number of ways.
- Run up to 864 results from an eight-hour shift, and up to 1,440 results in 24-hours.*
- Gain up to 8 hours of walk-away time* with just 3 user interactions per run.
- Streamline resource-intensive applications such as viral load IVD monitoring, blood screening, microbiology testing and women's health.

Roche COBAS-6800 machine

Principles of the procedure Cobas® SARS-CoV-2

The cobas® SARS-CoV-2 Test is a single-well dual target assay, which includes both specific detection of SARS-CoV-2 (ORF1) and pan-sarbecovirus detection (E gene) for the sarbecovirus subgenus family that includes SARS-CoV-2.

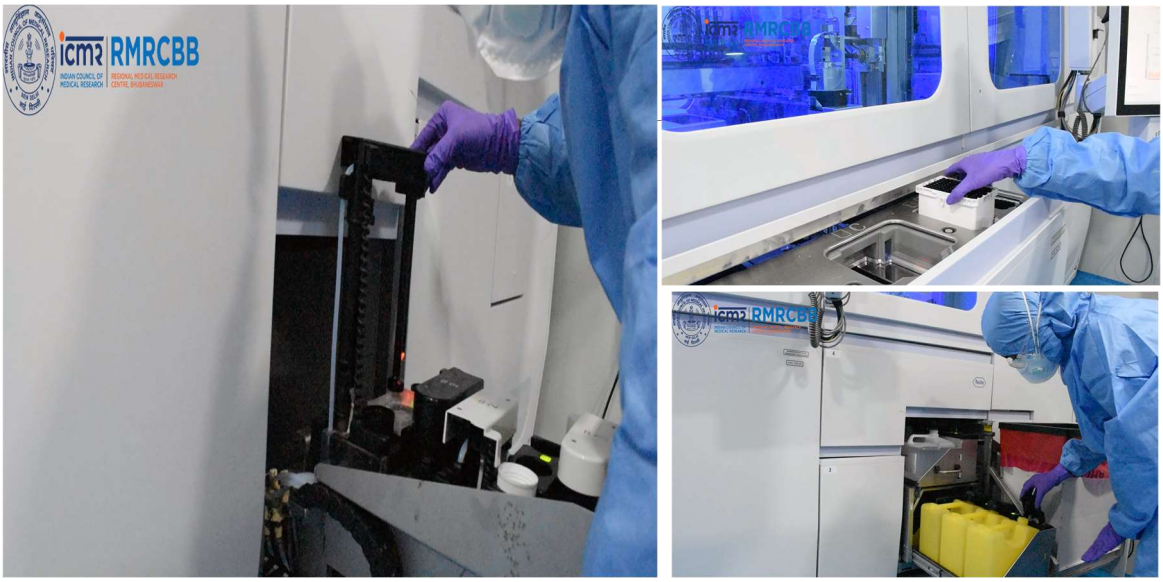
The assay has a full-process negative control, positive control and internal control.

It is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection.

The cobas® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module.

Automated data management is performed by the cobas® 6800/8800 software, which assigns test results for all tests.

Results can be reviewed directly on the system screen, and printed as a report.



Result Interpretation

Test	Sample ID	Valid	Flags	Sample Type	Overall Result	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	Yes		Swab	NA	Negative	Negative
SARS-CoV-2 400 µL	Swab_02	No	C40T	Swab	NA	Invalid	Invalid
SARS-CoV-2 400 µL	Swab_03	Yes		Swab	NA	Negative	Positive
SARS-CoV-2 400 µL	Swab_04	Yes		Swab	NA	**Positive**	**Positive**
SARS-CoV-2 400 µL	Swab_05	Yes		Swab	NA	Negative	Negative
SARS-CoV-2 400 µL	Swab_06	Yes		Swab	NA	Positive	Negative
SARS-CoV-2 400 µL	Swab_07	No	C01H2	Swab	NA	Positive	Invalid
SARS-CoV-2 400 µL	Swab_08	No	C01H1	Swab	NA	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	Valid	Valid	Valid
SARS-CoV-2	C161420284093009580264	Yes		SARS-CoV-2 (+) C	Valid	Valid	Valid

Result Interpretation

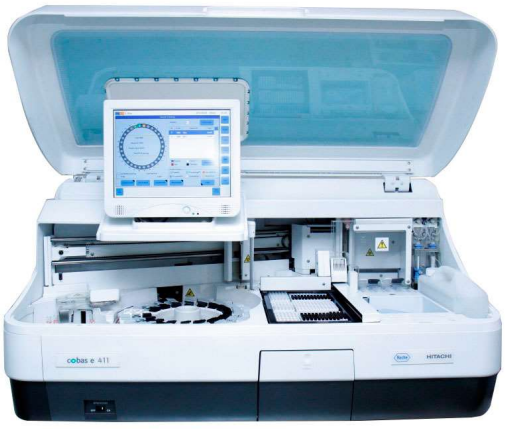
Target 1 (ORF1 gene)	Target 2 (E gene)	Interpretation
Positive	Positive	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	Negative	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected
Negative	Positive	Result for SARS-CoV-2 RNA is Presumptive Positive.
Negative	Negative	Result for SARS-CoV-2 RNA is Not Detected
Positive	Invalid	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	Positive	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive
Negative	Invalid	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	Negative	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	Invalid	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained

SEROLOGICAL TESTING LABORATORY

Immunoassays for COVID-19 detection and its' Principle

- Among the coronaviruses structural proteins (spike [S], envelope [E], membrane [M], and nucleocapsid [N], the S and N proteins are the main immunogens.
- Immunoassay for the *in vitro* determination of antibodies to the SARS-CoV-2 are developed to bind either against S protein receptor binding domain (RBD) or N protein in human serum and plasma.
- Different types of immunoassays are there in the market: enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA) and lateral flow immunoassay (LFIA).
- CLIA technologies are generally performed in automated or semi-automated platforms which has always a upper hand over conventional ELISA as it always gives high-throughput results.
- Recombinant viral antigen binds with COVID-19 patient antibodies and uses chemical probes that yield light emission through a chemiluminescence reaction to generate a positive signal.

Antibody Test at Cobas e411 analyser/Abott architech i1000SR



The centre has two semi-automated CLIA-based platforms: Roche Cobas e411 and Abbott Architect i1000SR

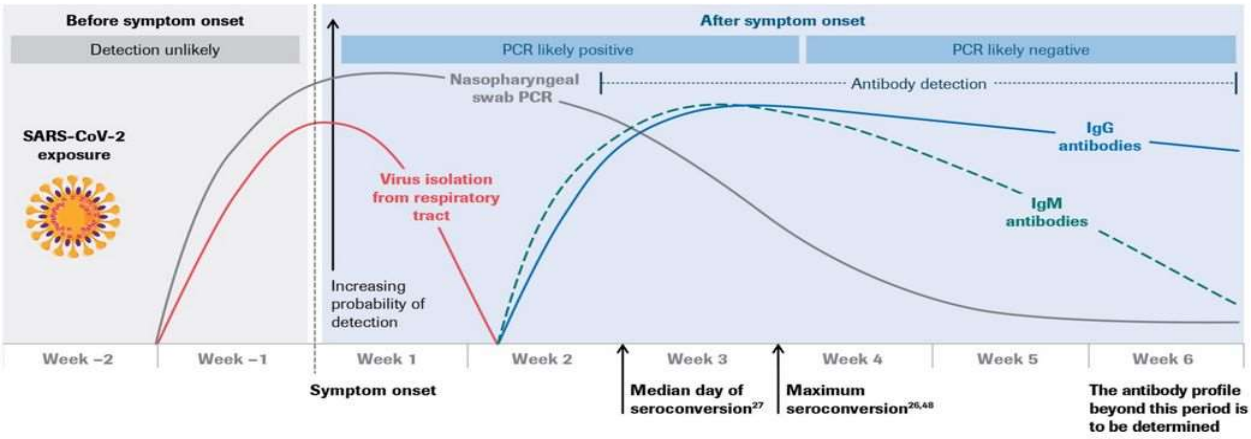
The detailed of the diagnostics features are as follows:

Machine	Technology	Kit Insert	Type	Target Epitope	Specificity	Sensitivity	Cut-off	Turn around time
Roche Cobas e411	Electro-chemiluminescence Immunoassay	Elecsys Anti-SARS-CoV-2	Qualitative	N protein	99.81 %	100% (≥14 Days Post-Positive PCR)	≥0.4 COI	~18 minutes
		Elecsys Anti-SARS-CoV-2S	Quantitative	S protein	99.98%	98.8% (≥14 Days Post-Positive PCR)	≥0.8 U/mL	
Abbott Architect i1000SR	Chemiluminescent Microparticle Immunoassay	SARS-CoV-2 IgG II	Qualitative	N protein	99.63%	100.00% (≥14 Days Post-Positive PCR)	≥1.4 index	~30 minutes
		SARS-CoV-2 IgG II Quant	Quantitative	S protein	99.55%	98.81% (≥15 Days Post-Positive PCR)	≥50 AU/mL	

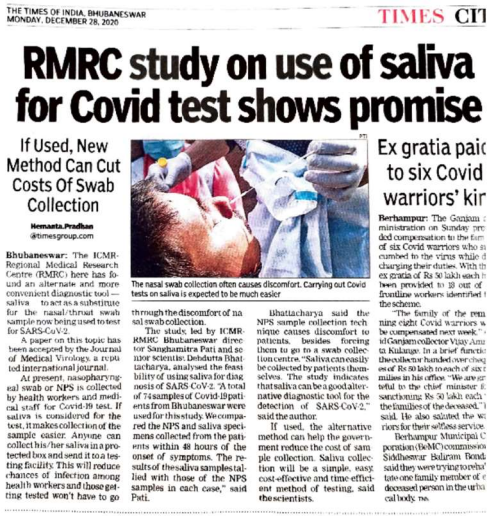
Understanding the relation of Ab & RT-PCR testing

Test Result			General Interpretation
RNA/Antigen	IgM	IgG	
+	-	-	Patient may be in the initial period of infection when antibodies are not yet produced or are under the limit of detection
+	+	-	Patient in the active stage phase of infection and has started to develop an immune response with antibody production
-	+	-	Patient may be in the early stage of infection, RNA/Antigen results may be false negative or IgM false positive
+	+	+	Patient is still in the active phase of infection; immune response has progressed
+	-	+	Patient may be in the late stage of infection or has developed a recurrent infection
-	+	+	Patient may be in the late or recovery stages of infection or RNA/Antigen false negative
-	-	+	Patient may have recovered or has been infected in the past

Seroprevalance of COVID-19 in India



Saliva for diagnosis of SARS-CoV-2: first report from India



THE TIMES OF INDIA, BHUBANESWAR
MONDAY, DECEMBER 28, 2020

TIMES CITI

RMRC study on use of saliva for Covid test shows promise

If Used, New Method Can Cut Costs Of Swab Collection

Hemanta.Pradhan
@timesgroup.com

Bhubaneswar: The ICMR-Regional Medical Research Centre (RMRC) here has found an alternate and more convenient diagnostic tool — saliva — to act as a substitute for the nasal/throat swab sample now being used to test for SARS-CoV-2.

A paper on this topic has been accepted by the Journal of Medical Virology, a reputed international journal.

At present, nasopharyngeal swab or NPS is collected by health workers and medical staff for Covid-19 test. If saliva is considered for the test, it makes collection of the sample easier. Anyone can collect his/her saliva in a protected box and send it to a testing facility. This will reduce chances of infection among health workers and those getting tested won't have to go through the discomfort of nasal swab collection.

The study, led by ICMR-RMRC Bhubaneswar director Sanghamitra Pati and senior scientist Debdutta Bhattacharya, analysed the feasibility of using saliva for diagnosis of SARS-CoV-2. "A total of 74 samples of Covid-19 patients from Bhubaneswar were used for this study. We compared the NPS and saliva specimens collected from the patients within 48 hours of the onset of symptoms. The results of the saliva samples tallied with those of the NPS samples in each case," said Pati.

The nasal swab collection often causes discomfort. Carrying out Covid tests on saliva is expected to be much easier

Bhattacharya said the NPS sample collection technique causes discomfort to patients, besides forcing them to go to a swab collection centre. "Saliva can easily be collected by patients themselves. The study indicates that saliva can be a good alternative diagnostic tool for the detection of SARS-CoV-2," said the author.

If used, the alternative method can help the government reduce the cost of sample collection. Saliva collection will be a simple, easy, cost-effective and time-efficient method of testing, said the scientists.

Ex gratia paid to six Covid warriors' kin

Berhampur: The Ganjam administration on Sunday provided compensation to the family of six Covid warriors who succumbed to the virus while discharging their duties. With the ex gratia of Rs 50 lakh each, been provided to 18 out of frontline workers identified the scheme.

"The family of the remaining eight Covid warriors will be compensated next week," said Ganjam collector Vijay Amrit Kulange. In a brief function, the collector handed over cheques of Rs 50 lakh to each of six families in his office. "We are grateful to the chief minister for sanctioning Rs 50 lakh each to the families of the deceased," said. He also saluted the warriors for their selfless service.

Berhampur Municipal Corporation (BeMC) commissioner Siddheswar Balram Bond said they were trying to celebrate one family member of deceased person in the official body. ne.

Received: 20 October 2020 | Revised: 16 November 2020 | Accepted: 7 December 2020
DOI: 10.1002/jmv.26719

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

Saliva for diagnosis of SARS-CoV-2: First report from India

Debdutta Bhattacharya[1] | Debaprasad Parai[1] | Usha K. Rout[1] | Pujarini Dash[1] | Rashmi R. Nanda[1] | Girish C. Dash[1] | Srikanta Kanungo[1] | Subrata K. Palo[1] | Sidhartha Giri[1] | Hari R. Choudhary[1] | Jaya S. Kshatri[1] | Jyotirmayee Turuk[1] | Bijay K. Mishra[1] | Rajesh K. Lenka[2] | Saroj Dash[1] | Sanghamitra Pati[1]

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[2]Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, India

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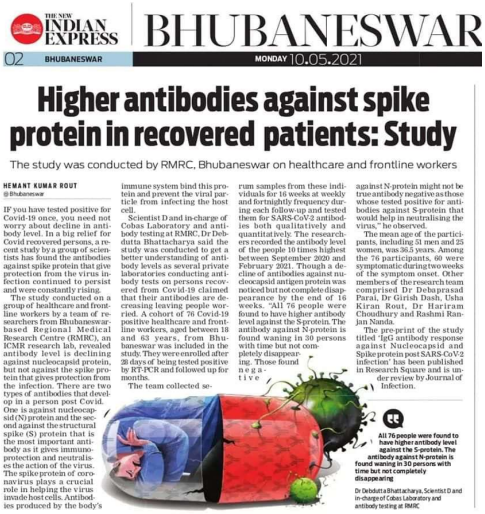
Abstract

There are very few studies in search of an alternate and convenient diagnostic tool which can substitute nasopharyngeal swab (NPS) specimen for detection of SARS-CoV-2. In the study we analyzed, the comparison and agreement between the feasibility of using the saliva in comparison to NPS for diagnosis of SARS-CoV-2. A total number of 74 patients were enrolled for this study. We analyzed and compared the NPS and saliva specimen collected within 48 h after the symptom onset. We carried out real-time quantitative polymerase chain reaction, gene sequencing for the detection and determination SARS-CoV-2 specific genes. Phylogenetic tree was constructed to establish the isolation of viral RNA from saliva. We used the Bland–Altman model to identify the agreement between two specimens. This study showed a lower cycle threshold (C_t) mean value for the detection of SARS-CoV-2 ORF1 gene (mean, 27.07; 95% confidence interval [CI], 25.62 to 28.52) in saliva methods than that of NPS (mean 28.24; 95% CI, 26.62 to 29.85) specimen although the difference is statistically nonsignificant ($p > 0.05$). Bland–Altman analysis produced relatively smaller bias and high agreement between these two clinical specimens. Phylogenetic analysis with the *RdRp* and *S* gene confirmed the presence of SARS-CoV-2 in the saliva samples. Saliva represented a promising tool in COVID-19 diagnosis and the collection method would reduce the exposure risk of frontline health workers which is one of the major concerns in primary healthcare settings.

KEYWORDS

Bland–Altman analysis, COVID-19, nasopharyngeal swab, saliva, SARS-CoV-2

Scientific Publications



THE NEW INDIAN EXPRESS | BHUBANESWAR

02 BHUBANESWAR | MONDAY 10.05.2021

Higher antibodies against spike protein in recovered patients: Study

The study was conducted by RMRC, Bhubaneswar on healthcare and frontline workers

HEMANT KUMAR ROUT
@Bhubaneswar

IF you have tested positive for Covid-19 once, you need not worry about decline in antibody level. In a big relief for Covid recovered persons, a recent study by a group of scientists has found the antibodies against spike protein that give protection from the virus infection continued to persist and were constantly rising.

The study conducted on a group of healthcare and frontline workers by a team of researchers from Bhubaneswar-based Regional Medical Research Centre (RMRC), an ICMR research lab, revealed antibody level is declining against nucleocapsid protein, but not against the spike protein that gives protection from the infection. There are two types of antibodies that develop in a person post Covid. One is against nucleocapsid (N) protein and the second against the structural spike (S) protein that is the most important antibody as it gives immunoprotection and neutralises the action of the virus. The spike protein of coronavirus plays a crucial role in helping the virus invade host cells. Antibodies produced by the body's immune system bind this protein and prevent the viral particle from infecting the host cell.

Scientist D and in-charge of Cobas Laboratory and antibody testing at RMRC, Dr Debdutta Bhattacharya said the study was conducted to get a better understanding of antibody levels as several private laboratories conducting antibody tests on persons recovered from Covid-19 claimed that their antibodies are decreasing leaving people worried. A cohort of 76 Covid-19 positive healthcare and frontline workers, aged between 18 and 63 years, from Bhubaneswar was included in the study. They were enrolled after 28 days of being tested positive by RT-PCR and followed up for months.

The team collected serum samples from these individuals for 16 weeks at weekly and fortnightly frequency during each follow-up and tested them for SARS-CoV-2 antibodies both qualitatively and quantitatively. The researchers recorded the antibody level of the people 10 times highest between September 2020 and February 2021. Though a decline of antibodies against nucleocapsid antigen protein was noticed but not complete disappearance by the end of 16 weeks. "All 76 people were found to have higher antibody level against the S-protein. The antibody against N-protein is found waning in 30 persons with time but not completely disappearing. Those found negative against N-protein might not be true antibody negative as those whose tested positive for antibodies against S-protein that would help in neutralising the virus," he observed.

The mean age of the participants, including 31 men and 25 women, was 36.5 years. Among the 76 participants, 60 were symptomatic during two weeks of the symptom onset. Other members of the research team comprised Dr Debaprasad Parai, Dr Girish Dash, Usha Kiran Rout, Dr Hariram Choudhury and Rashmi Ranjan Nanda.

The pre-print of the study titled "IgG antibody response against Nucleocapsid and Spike protein post SARS-CoV-2 infection' has been published in Research Square and is under review by Journal of Infection.

All 76 people were found to have higher antibody level against the S-protein. The antibody against N-protein is found waning in 30 persons with time but not completely disappearing

Dr Debdutta Bhattacharya, Scientist D and in-charge of Cobas Laboratory and antibody testing at RMRC

Infection
https://doi.org/10.1007/s15010-021-01651-4

BRIEF REPORT

Check for updates

IgG antibody response against nucleocapsid and spike protein post-SARS-CoV-2 infection

Hari Ram Choudhary[1] · Debaprasad Parai[1] · Girish Chandra Dash[1] · Annalisha Peter[1] · Subrat Kumar Sahoo[1] · Matrujyoti Pattnaik[1] · Usha Kiran Rout[1] · Rashmi Ranjan Nanda[1] · Sanghamitra Pati[1] · Debdutta Bhattacharya[1]

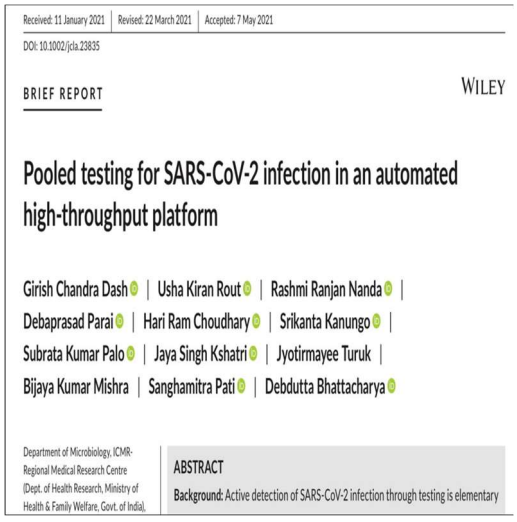
Received: 6 May 2021 / Accepted: 22 June 2021

Abstract

Objectives Coronavirus disease-19 (COVID-19) pandemic became the greatest public health challenge globally. Study of dynamicity and durability of naturally developed antibodies against SARS-CoV-2 are of great importance from an epidemiological viewpoint.

Methods In this observational cohort study, we have followed up the 76 individuals who tested positive for SARS-CoV-2 infection by real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) for 16 weeks (post-enrolment) to record the periodic changes in titre, concentration, clinical growth and persistence of naturally developed SARS-CoV-2 antibod-

Scientific Publications



Received: 11 January 2021 | Revised: 22 March 2021 | Accepted: 7 May 2021

DOI: 10.1002/jcla.23835

BRIEF REPORT

WILEY

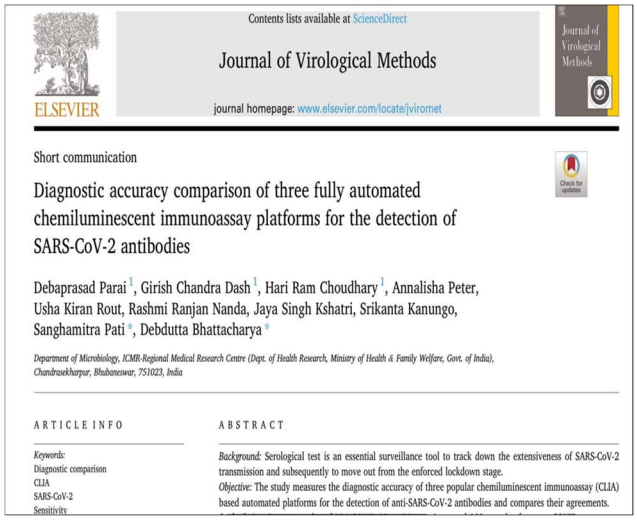
Pooled testing for SARS-CoV-2 infection in an automated high-throughput platform

Girish Chandra Dash | Usha Kiran Rout | Rashmi Ranjan Nanda | Debaprasad Parai | Hari Ram Choudhary | Srikanta Kanungo | Subrata Kumar Palo | Jaya Singh Kshatri | Jyotirmayee Turuk | Bijaya Kumar Mishra | Sanghamitra Pati | Debdutta Bhattacharya

Department of Microbiology, ICMR-Regional Medical Research Centre (Dept. of Health Research, Ministry of Health & Family Welfare, Govt. of India),

ABSTRACT

Background: Active detection of SARS-CoV-2 infection through testing is elementary



Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

ELSEVIER

Short communication

Diagnostic accuracy comparison of three fully automated chemiluminescent immunoassay platforms for the detection of SARS-CoV-2 antibodies

Debaprasad Parai[1], Girish Chandra Dash[1], Hari Ram Choudhary[1], Annalisha Peter, Usha Kiran Rout, Rashmi Ranjan Nanda, Jaya Singh Kshatri, Srikanta Kanungo, Sanghamitra Pati*, Debdutta Bhattacharya*

Department of Microbiology, ICMR-Regional Medical Research Centre (Dept. of Health Research, Ministry of Health & Family Welfare, Govt. of India), Chandrasekharpur, Bhubaneswar, 751023, India

ARTICLE INFO

Keywords:
Diagnostic comparison
CLIA
SARS-CoV-2
Sensitivity

ABSTRACT

Background: Serological test is an essential surveillance tool to track down the extensiveness of SARS-CoV-2 transmission and subsequently to move out from the enforced lockdown stage.
Objective: The study measures the diagnostic accuracy of three popular chemiluminescent immunoassay (CLIA) based automated platforms for the detection of anti-SARS-CoV-2 antibodies and compares their agreements.

Scientific Publications

Epidemiology and Infection

cambridge.org/hyg

Original Paper

Cite this article: Kshatri JS *et al* (2021). Seroprevalence of SARS-CoV-2 in Bhubaneswar, India: findings from three rounds of community surveys. *Epidemiology and Infection* **149**, e139, 1–9. https://doi.org/10.1017/S0950268821000972

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Accepted: 9 April 2021

Key words:
COVID-19; infectious disease epidemiology; SARS-CoV-2; serosurvey

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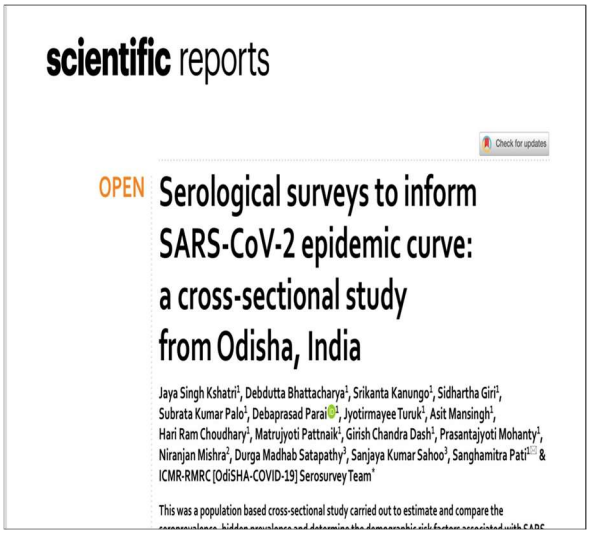
Seroprevalence of SARS-CoV-2 in Bhubaneswar, India: findings from three rounds of community surveys

Jaya Singh Kshatri, Debdutta Bhattacharya, Ira Praharaj, Asit Mansingh, Debaprasad Parai, Srikanta Kanungo, Subrata Kumar Palo, Sidhartha Giri, Matrujyoti Pattnaik, Shakti Ranjan Barik, Girish Chandra Dash, Hari Ram Choudhary, Jyotirmayee Turuk, Nitya Nanda Mandal and Sanghamitra Pati

Indian Council of Medical Research-Regional Medical Research Centre, Nalco Square, Bhubaneswar, Odisha 751023, India

Abstract

The study aims to estimate and compare the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence, the fraction of asymptomatic or subclinical infections in the population, determine the demographic risk factors and analyse the antibody development at different time points among adults in Bhubaneswar city, India. This was a serial three-round cross-sectional, community-based study where participants were selected from the residents of Bhubaneswar city using multi-stage random sampling. Blood samples were collected during household visits along with demographic and clinical data from every participant. Total anti-



scientific reports

OPEN

Serological surveys to inform SARS-CoV-2 epidemic curve: a cross-sectional study from Odisha, India

Jaya Singh Kshatri[1], Debdutta Bhattacharya[1], Srikanta Kanungo[1], Sidhartha Giri[1], Subrata Kumar Palo[1], Debaprasad Parai[1], Jyotirmayee Turuk[1], Asit Mansingh[1], Hari Ram Choudhary[1], Matrujyoti Pattnaik[1], Girish Chandra Dash[1], Prasantajyoti Mohanty[1], Niranjan Mishra[2], Durga Madhab Satapathy[3], Sanjaya Kumar Sahoo[3], Sanghamitra Pati[1]✉ & ICMR-RMRC [OdiSHA-COVID-19] Serosurvey Team*

This was a population based cross-sectional study carried out to estimate and compare the

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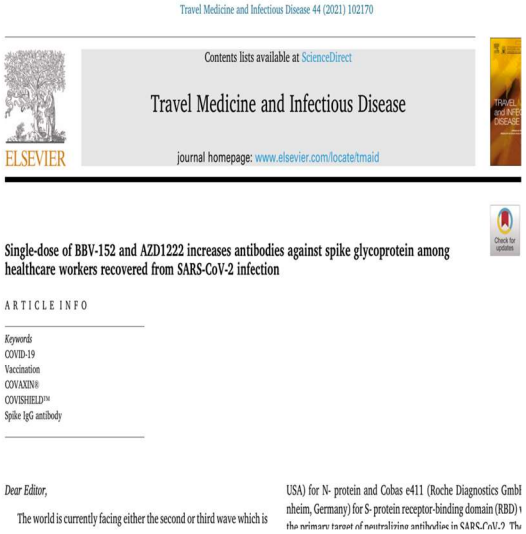


Travel Medicine and Infectious Disease 44 (2021) 102170

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Single-dose of BBV-152 and AZD1222 increases antibodies against spike glycoprotein among healthcare workers recovered from SARS-CoV-2 infection

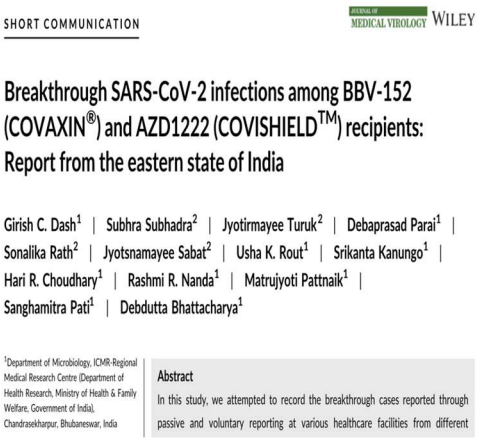
ARTICLE INFO

Keywords
COVID-19
Vaccination
COVAXIN®
COVISHIELD™
Spike IgG antibody

Dear Editor,

The world is currently facing either the second or third wave which is

USA) for N- protein and Cobas e411 (Roche Diagnostics GmbH nheim, Germany) for S- protein receptor-binding domain (RBD) the primary target of neutralizing antibodies in SARS-CoV-2. Th



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DOI: 10.1002/jmv.27382

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

Breakthrough SARS-CoV-2 infections among BBV-152 (COVAXIN®) and AZD1222 (COVISHIELD™) recipients: Report from the eastern state of India

Girish C. Dash[1] | Subhra Subhadra[2] | Jyotirmayee Turuk[2] | Debaprasad Parai[1] | Sonalika Rath[2] | Jyotsnamayee Sabat[2] | Usha K. Rout[1] | Srikanta Kanungo[1] | Hari R. Choudhary[1] | Rashmi R. Nanda[1] | Matrujyoti Pattnaik[1] | Sanghamitra Pati[1] | Debdutta Bhattacharya[1]

[1]Department of Microbiology, ICMR-Regional Medical Research Centre (Department of Health Research, Ministry of Health & Family Welfare, Government of India), Chandrasekharpur, Bhubaneswar, India

Abstract

In this study, we attempted to record the breakthrough cases reported through passive and voluntary reporting at various healthcare facilities from different

Disinfection and Decontamination

Definitions

- **Disinfection**

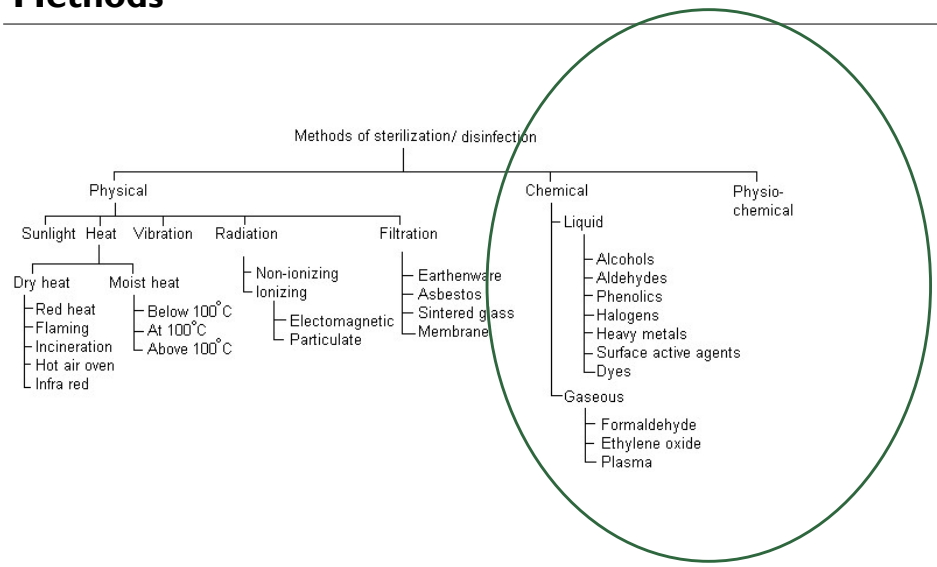
process that eliminates many or all pathogenic microorganisms, except bacterial spores on inanimate object

- **Decontamination**

removal of pathogenic microorganisms from objects so they are safe to handle, use, or discard.

WHAT ARE THE DIFFERENT METHODS OF STERILIZATION?

Methods



Classification of disinfectants

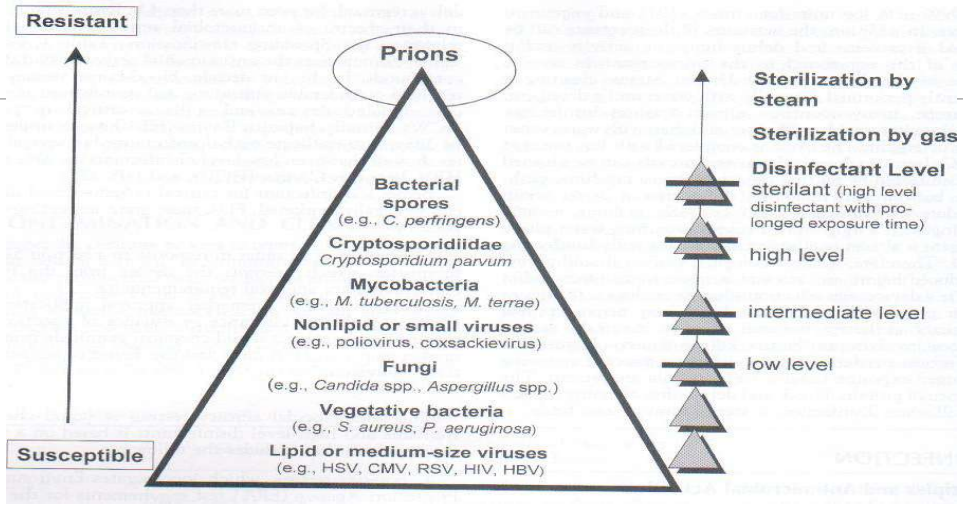
1. Based on consistency
   a. Liquid (E.g. Alcohols, Phenols)
   b. Gaseous (E.g. Formaldehyde vapour, Ethylene oxide)

2. Based on spectrum of activity
   a. High level disinfection
   b. Intermediate level disinfection
   c. Low level disinfection

Classification of disinfectants

3. Based on mechanism of action
   a. Action on membrane
      (E.g.- Alcohol, detergent)
   b. Denaturation of cellular proteins
      (Ex - Alcohol, Phenol)
   c. Oxidation of essential sulfhydryl groups of enzymes
      (Ex - H_2O_2, Halogens)
   d. Alkylation of amino-, carboxyl- and hydroxyl group
      (Ex - Ethylene Oxide, Formaldehyde)
   e. Damage to nucleic acids
      (Ex- Ethylene Oxide, Formaldehyde)

WHICH MICROORGANISMS ARE EASY AND WHICH ARE MOST TOUGH TO DISINFECT?



Levels of disinfection

High - level disinfection

- Kills most organisms including mycobacteria , except high levels of bacterial spores

Intermediate - level disinfection

- Kills non enveloped and small viruses, bacteria, fungi

Low - level disinfection

- Kills enveloped viruses, bacteria and fungi.

Spaulding classification for disinfection of medical devices

CRITICAL - objects which enter normally sterile body tissue or the vascular system
(sterilization)

SEMICRITICAL - objects that touch mucous membranes or skin that is not intact
(high-level disinfection)

NONCRITICAL -objects that touch only intact skin
(low-level disinfection)

Rutala, Weber, HICPAC. 2008. www.cdc.gov

IS THERE AN IDEAL DISINFECTANT AVAILABLE?

An ideal disinfectant

- wide spectrum of activity
- able to destroy microbes within practical period of time
- active in the presence of organic matter
- make effective contact
- active in any pH
- stable, non-toxic, non-allergic, non-irritative
- long shelf life
- high penetrating power
- Efficacy should not be lost on reasonable dilution
- Should not be expensive and must be available easily
-

Such an ideal disinfectant is not yet available

Disinfectants

Sodium hypochlorite solution stock solution (4-6%)

Use: General purpose disinfectant and for soaking contaminated metal free materials

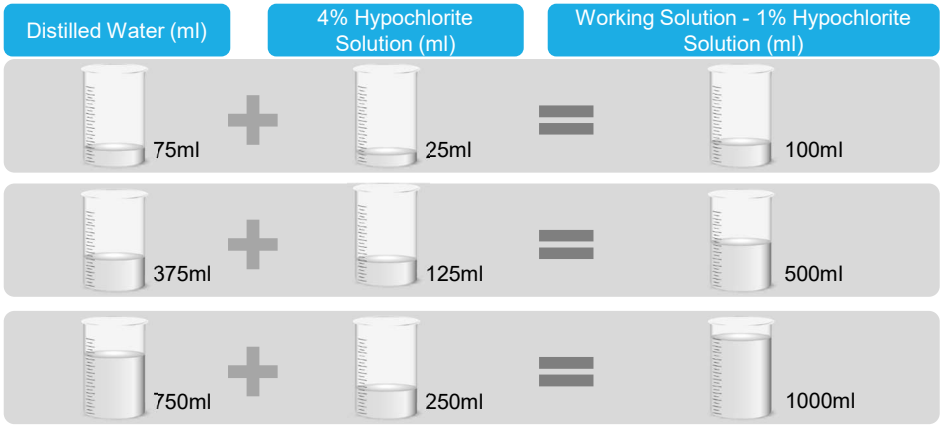
Mechanism of action: The microbicidal activity is due to undissociated hypochlorous acid (HOCl) molecule, which can react with peptide bonds and thiol groups, chemically oxidizing proteins and other biomolecules and abolish function of microbes by disruptive affect on microbial physiology including cell membrane, proteins and nucleic acids.

Working concentration (freshly prepared):
1% for sample spills
0.1% for general surface disinfection

Contact time 10 -15 min

Preparation of Disinfectants - 1% Hypochlorite Solution

- Use commercially available 4% Hypochlorite Solution
- Prepare required amount of 1% Hypochlorite Solution using appropriate amount of distilled water



Caution: It is highly hazardous and corrosive, and one should be careful while handling it

Alcohol

Alcohols, ethanol (denatured ethanol, methylated spirits)

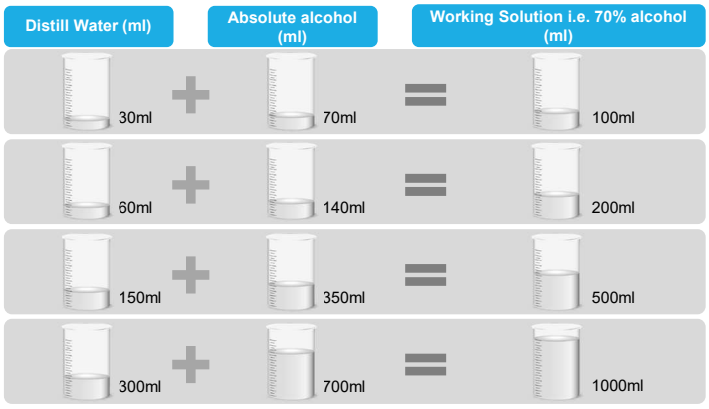
Mechanism of action: Denaturation of bacterial & viral proteins

Use: Used on laboratory benches and BSCs for routine decontamination . Should be used after disinfection with sodium hypochlorite has been carried out.

Hand cleaning: a rinse with 70% ethanol or isopropyl alcohol followed by thorough washing with soap and water is effective

Preparation of Disinfectants – 70% Alcohol

1. **Use commercially available absolute alcohol**
2. **Prepare required amount of 70% alcohol using appropriate amount of distill water and absolute alcohol**

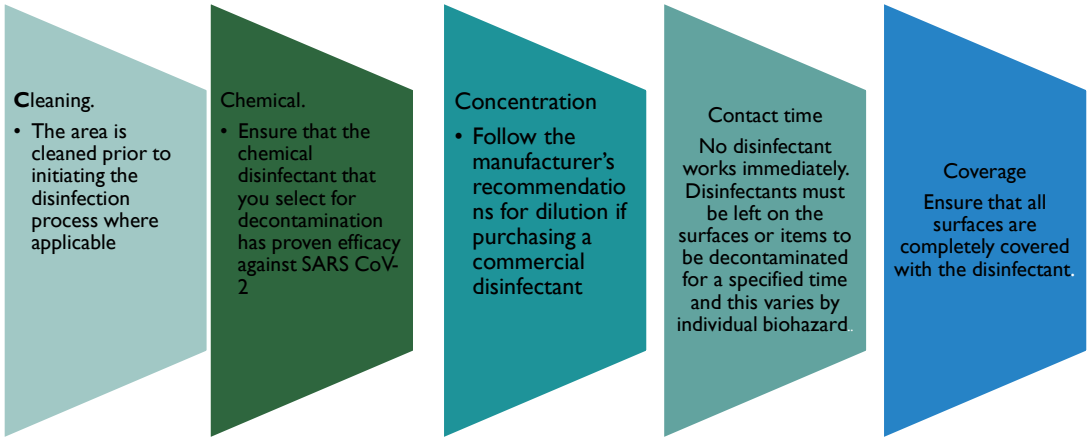


Efficacy of Disinfection/Sterilization

- Cleaning of the object
- Organic and inorganic load present
- Type and level of microbial contamination
- Concentration of and exposure time to disinfectant
- Nature of the object
- Temperature and relative humidity

"The 5 Cs"

The "5 Cs" of decontamination and disinfection should be practiced at all times in a COVID-19 testing laboratory.



SARS-CoV-2 is susceptible to disinfectants with proven activity against enveloped viruses

Povidone-iodine (7.5%), chloroxylenol (0.05%), chlorhexidine (0.05%), benzalkonium chloride (0.1%), if used according to the manufacturer's recommendations

Type	Concentration	Contact time	Comments
Freshly prepared sodium hypochlorite (bleach)	1%	10–15 minutes	A general-purpose disinfectant that can be used to soak items and disinfect spills; corrosive to metals and plastics
Freshly prepared sodium hypochlorite (bleach)	0.1%	10–15 minutes	Can be used to clean instruments
Ethanol	70%	10–15 minutes	Leaves no residue; can be used with other disinfectants to decontaminate surfaces (including metals)

WHAT ARE THE 5 C IN DISINFECTION PROCESS?

Laboratory cleaning

- The laboratories should be cleaned by the laboratory staff.
- Cleaning staff should only enter the laboratories under supervision of the laboratory staff and only be involved in cleaning of the office space and corridor
- All surface and equipment within the laboratory should be regarded as potentially infectious and should be cleaned on a daily basis
- Exterior surfaces of equipment should be wiped with appropriate disinfectant

Unidirectional Cleaning of the laboratory

- There should be effective separation of various sections and activities of lab to prevent cross contamination
- There should be dedicated cleaning items for each area.
- The cleaning items such as mops , buckets and brushes used in dirty areas of the laboratory should never be used in the cleaner areas.

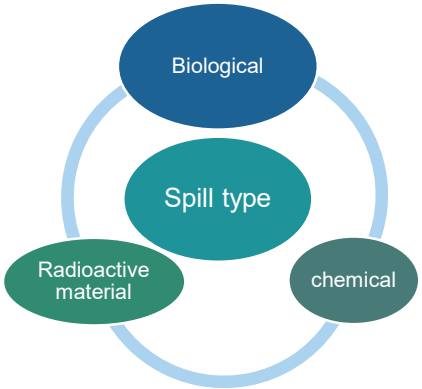
Spill Management

Reaching Impact, Saturation, and Epidemic Control (RISE)

Introduction

Cause or allow (substance) to flow over the edge of its container, especially unintentionally

Types of spill in a hospital:



Biological spill

Spillage of the blood/body fluids/human specimens

Types of biological spill:

Small spill (up to 10cm)

Large spill(>10 cm)

Spill kit

- Sodium hypochlorite – stock solution
- Premarked container for 1% sodium hypochlorite
- Falcon tube for measuring the stock solution
- 70% alcohol
- Absorbent towels
- Biohazard bags (yellow and red)
- PPE
- Spill incident logbook

PPE for Spill management

The following PPE must be worn when cleaning up a spill:

- Laboratory gown
- N95 mask
- Goggles and/or face shield
- Gloves
- Shoe covers
- Head cover

Spill cleanup procedure

- Put on gloves, a protective laboratory gown, respirator and goggles.
- Re-enter the affected area.
- Cover the spill with a cloth or paper towels to contain it.
- Pour freshly prepared 1% sodium hypochlorite over the paper towels and immediate surrounding area.
- Apply disinfectant concentrically, beginning at the outer margin of the spill and working towards the centre.
- Allow sufficient time for the disinfectant to act before clearing away any material for disposal.
- Clean up the contaminated area and place any contaminated material in a biohazard bag for disposal.
- Disinfect any contaminated equipment using 1% sodium hypochlorite. After a contact time of at least 15 minutes, remove the residual sodium hypochlorite salts by wiping the equipment with water and then 70% ethanol.
- Remove contaminated PPE before resuming your work.

Leakage in a sample transport box

- Immediately notify laboratory staff and any other individuals who are nearby.
- Cordon off the area and restrict access.
- Place the leaky transport box in a biohazard bag and close the bag.
- Wipe contaminated surfaces with paper towels soaked in freshly prepared 1% sodium hypochlorite.
- Dispose of the paper towels and other contaminated items in a biohazard bag.
- Transport the bags to an autoclave facility.
- Document the spill incident.

A spill of infectious material inside a biosafety cabinet

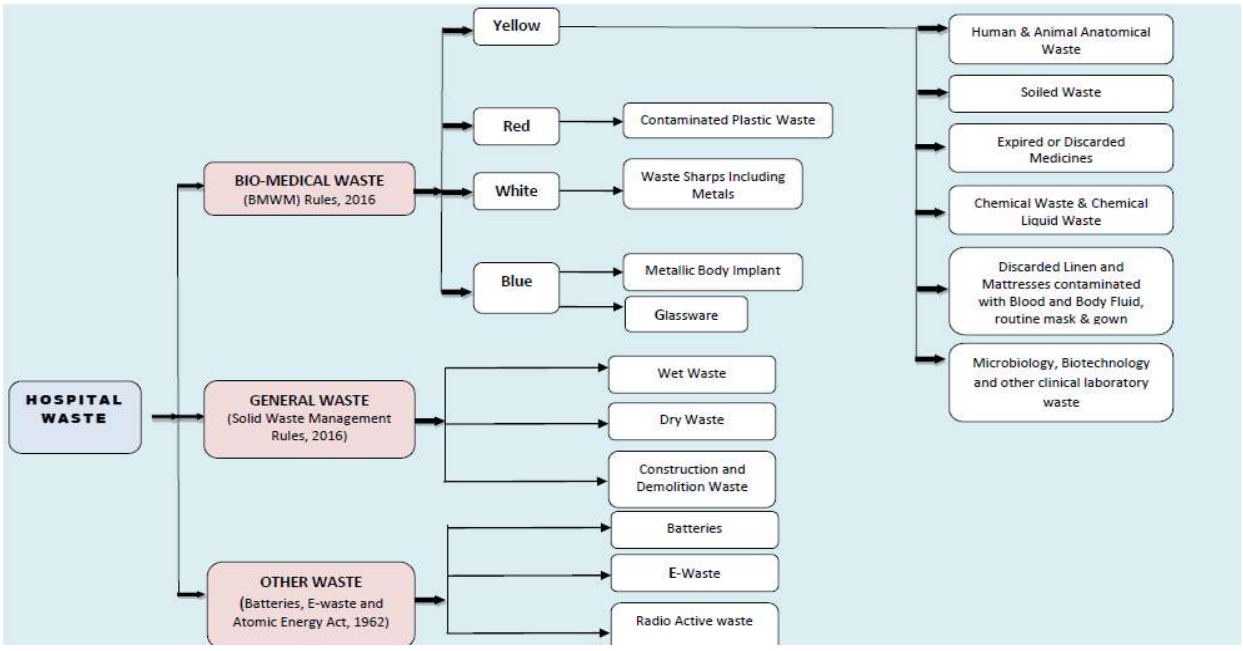
- Place absorbent tissue over the spill and pour an appropriate amount of 1% sodium hypochlorite onto the tissue.
- Leave affected areas covered with disinfectant for at least 30 minutes to allow aerosols to settle down and to act disinfectant on microbes
- Inside the BSC, carefully collect all contaminated material and place in a biohazard container for disposal.
- Wipe the spill, work surfaces, walls, and any equipment in the BSC using paper towels dampened with a disinfectant
- Any equipment or reusable material that has been splashed should be cleaned with the same disinfectant.
- Document the spill incident.

A spill of infectious material outside the biosafety cabinet, in the specimen processing area

- Ask everyone to immediately vacate the affected laboratory area.
- If the spill has contaminated your gown and shoe covers, spray them with disinfectant before stepping out of the room.
- Remove contaminated PPE and place in a biohazard bag.
- Change contaminated items of clothing and place them in an autoclave bag for decontamination later on.
- Disinfect your hands and remove your N95 mask. Discard your gloves and wash your hands thoroughly with soap and water
- Don a fresh set of PPE. SOP of spill management to be followed.
- Signs should be posted indicating that entry is forbidden during the clean-up procedure. The laboratory manager should immediately be informed of the incident. Staff must be prevented from re-entering the laboratory for at least 30 minutes to allow aerosols to be removed via the laboratory ventilation system and allow time for heavier particles to settle. Standard operating procedures for spill clean-up MUST be followed. Document the spill incident.

BIOMEDICAL WASTE MANAGEMENT

Reaching Impact, Saturation, and Epidemic Control (RISE)



Reaching Impact, Saturation, and Epidemic Control (RISE)

Bio Medical Waste

Bio-medical waste means any waste, which is generated during **the diagnosis, treatment or immunization of human beings or animals** or in **research activities** pertaining thereto or in the production or **testing of biological samples or in health camps.**

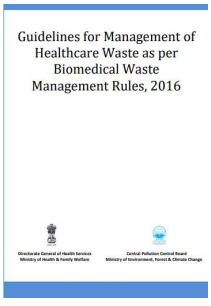
Bio-Medical waste includes all the waste generated from the Health Care Facility which can have any adverse effect to the health of a person or to the environment in general if not disposed properly.

The major salient features of BMW Management Rules, 2016 include the following:-

(a) The ambit of the rules has been expanded to include vaccination camps, blood donation camps, surgical camps or any other healthcare activity; Provide training to all its health care workers and immunize all health workers regularly;
(b) Bio-medical waste has been classified in to 4 categories instead of 10 to improve the segregation of waste at source;
(c) Pre-treatment of the laboratory waste, microbiological waste, blood samples and blood bags through disinfection or sterilization on-site in the manner as prescribed by WHO or NACO;
(d) Establish a Bar-Code System for bags or containers containing bio-medical waste for disposal;
(d) Phase-out the use of chlorinated plastic bags, gloves and blood bags within two years;

(e) Report major accidents;

(f) No occupier shall establish on-site treatment and disposal facility, if a service of `common bio-medical waste treatment facility is available at distance of seventy-five kilometers.



Reaching Impact, Saturation, and Epidemic Control (RISE)

Guidelines for BMW 2018

New Requirements	Timeline for implementation	Remarks
Pre-treatment of laboratory waste, microbiology waste, blood samples before giving to disposal	*Immediate effect.	
Phase out chlorinated bags, gloves, blood bags	**Within 2 years from date of issue for Principle BMW 2016 now extended to 27th March 2019**	
Training Health care workers about handling of Biomedical waste	*Immediate effect	Details of training to be submitted along with Annual report
Immunization of Health care workers for Hepatitis B and Tetanus	*Immediate effect	Records of vaccination to be maintained.

Guidelines for BMW 2018

New Requirements	Timeline for implementation	Remarks
Barcode system for bags or containers containing Biomedical waste	**Within 1 year from date of issue of Principle rules BMW 2016 now extended to 27th March 2019**	
Health check up of Health care workers during induction and annually there after.	*Immediate effect	Records to be maintained
Maintain and update biomedical waste management register and monthly report on website	**Within 2 years from date of issue of Principle rule BMW-2016 rules i.e. within 28th March 2018**	On Complinace Portal in ESS
Major accidents to be reported along with annual report	Should be documented and reported	Nil
Maintain records of autoclaving, Microwaving etc for a period of five years	*Immediate effect	Nil

Guidelines for BMW 2018

New Requirements	Timeline for implementation	Remarks
Annual report on website	Within 2 years from date of issue of Principle Rules 2016 now extended to 16th March 2020.	
Setup BMW management committee, and meetings be done bi-annually. Minutes submitted in annual report.	*Immediate effect	

CPCB BMWM guidelines for COVID, 2020

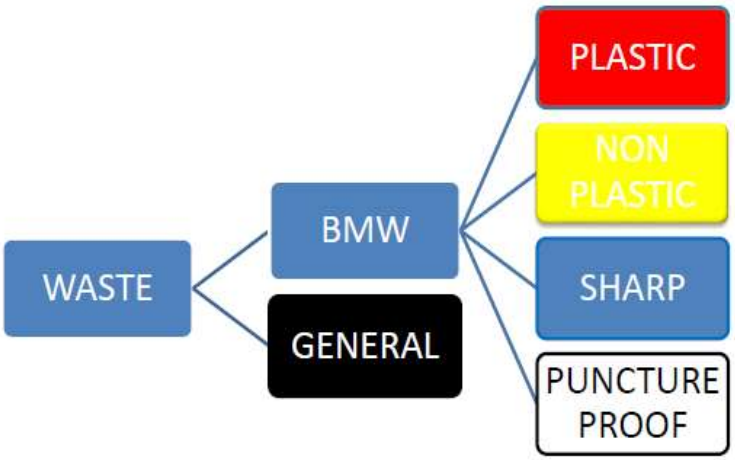
Guidelines for management of waste generated during diagnostics and treatment of COVID-19 suspected/confirmed patients, are required to be followed by stakeholders in addition to existing practices under BMW Management Rules, 2016

PICTORIAL GUIDE ON BIOMEDICAL WASTE MANAGEMENT RULES 2016

(AMENDED IN 2018 & 2019)

Including the CPCB guidelines for handling, treatment and disposal of waste generated during treatment/ diagnosis/ quarantine of COVID-19 patients

Q.1 Four types of BMW?


WASTE
BMW
GENERAL
PLASTIC
NON PLASTIC
SHARP
PUNCTURE PROOF


KEEP HOSPITALS CLEAN AND SAFE BY IDENTIFYING HAZARDS AND RISKS OF BIOMEDICAL WASTE
अपने अस्पताल को स्वच्छ एवं सुरक्षित बनाए रखने के लिए
बायोमेडिकल कचरे से सम्बंधित खतरों एवं संकट को समझें
Anatomical waste, chemical waste, soiled waste, chemotherapy waste, discarded linen and medicines and laboratory waste
शारीरिक, रासायनिक, गंदा कपड़ा, दवाइयों सम्बंधित एवं प्रयोगशाला कचरा
Contaminated plastic waste
दूषित प्लास्टिक कचरा
Glass waste and metallic implants
कांच की वस्तुएं एवं धातु प्रत्यारोपण
Metal sharps
धारदार धातु कचरा
Place the waste in designated colour coded bins
कचरे को उचित कूड़ेदान में ही डालें
Hazardous and Other waste*
परिसंकटमय और अन्य कचरा
Recyclable General waste
पुनर्चक्रण योग्य कचरा
Biodegradable General waste
स्वाभाविक तरीके से सड़ने वाला सामान्य कचरा

Q.2 Bags for VTM/nitrile gloves?

Color Coding

Yellow Category

- Anatomical Waste
- Soiled Waste
- Microbiology Waste
- Discarded Medicines
- Soiled Linen & Beddings
- Blood Bags
- Chemical Waste
- Masks, Head cover, Shoe cover, Disposable linen gown

Red Category

All contaminated Recyclable (Plastic) e.g.

- Goggles, Face shield, Splash proof apron, Plastic coverall, Hazmat suit, Nitrile gloves
- VTM
- Plastic vials, vacutainers
- Eppendorf tubes
- Plastic cryovials
- Pipette tips

White Category

- Sharp Waste (Metals)

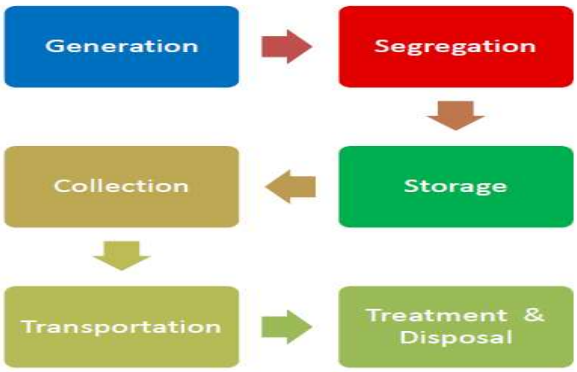
Blue Category

- Broken and unbroken glass
- Metallic body implants

Aas per 2018 guidelines it these has to be discarded in puncture proof containers with blue marking instead of cardboard boxes

Reaching Impact, Saturation, and Epidemic Control (RISE)

Process Flow for BMW



Packaging of samples

Bio-medical waste bags and sharps containers should be filled to **no more than three quarters** full. Once this level is reached, they should be sealed ready for collection.

Plastic bags should never be stapled but may be tied or sealed with a plastic tag or tie.

Replacement bags or containers should be available at each waste-collection location so that full ones can immediately be replaced.

Colour coded waste bags and containers should be printed with the bio-hazard symbol, labelled with details such as date, type of waste, waste quantity, senders name and receivers details as well as bar coded label to allow them to be tracked till final disposal.

Disposal of Liquid Waste

Liquid waste liquid from laboratories and floor washings, cleaning, house-keeping and disinfecting activities containing waste chemicals is collected and **pre-treated with 1-2% sodium hypochlorite prior to disposal through Effluent Treatment Plant.**

Labelling

All the bags/ containers/ bins used for collection and storage of bio-medical waste, must be labelled with the Symbol of Bio Hazard or Cytotoxic Hazard in accordance with "Guidelines for barcode System for Effective Management of Biomedical Waste".

Bio-Hazard Label

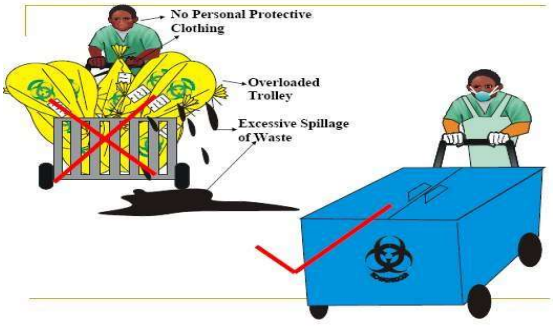
Cyto-Toxic Label

Collection of BMW

- Collect BMW: Foot operated bin with lid labelled as "COVID-19"
- Separate colour coded bins/ bags/ container/ trolleys maintain proper segregation of waste as per rules
- Inner and outer surface of containers/ bins/ trolleys – disinfect 1% sodium hypochlorite
- General waste not having contamination should be discarded as solid waste as per SWM Rules, 2016

Storage and Transportation

In house transportation of Bio Medical Waste from site of waste generation/ interim storage to central waste collection centre, within the premises of the hospital /laboratory must be done in closed trolleys / containers preferably fitted with wheels for easy manoeuvrability.



Q.3 Maximum time allowed to store BMW?

Waste disposal

- Biomedical Waste & General Waste shall not be mixed. Storage time of waste should be as less as possible so that waste storage, transportation and disposal is done **within 48 hours.**
- No secondary handling or pilferage of waste shall be done at healthcare facility. If CBWTF facility is available at a **distance of 75 km** from the HCF, bio-medical waste should be treated and disposed only through such CBWTF operator.
- **Laboratory and Highly infectious waste shall be pre-treated onsite** before sending for final treatment or disposal through a CBWTF Operator.

Autoclaving Biomedical Waste

STANDARDS FOR AUTOCLAVING OF BIO-MEDICAL WASTE:

The autoclave should be dedicated for the purposes of disinfecting and treating bio-medical waste.

When operating gravity flow autoclave:

- **Use a temperature of not less than 121° C and pressure of 15 pounds per square inch (psi) for an autoclave residence time of not less than 60 minutes**

Autoclaving Biomedical Waste

STANDARDS FOR AUTOCLAVING OF BIO-MEDICAL WASTE:

When operating a vacuum autoclave, the waste shall be subjected to the following:

- **A temperature of not less than 121°C and pressure of 15 psi per an autoclave residence time of not less than 45 minutes**
- **A temperature of not less than 135°C and a pressure of 31 psi for an autoclave residence time of not less than 30 minutes**

Autoclaving Biomedical Waste

Quality control or autoclave validation

Routine Test:

- A chemical indicator strip or tape that changes color when a certain temperature is reached
- Use more than one strip over the waste package at different locations to ensure that the inner content of the package has been adequately autoclaved
- Records for this test needs to be maintained

Autoclaving Biomedical Waste

Quality control or autoclave validation

Spore testing:

- Biological indicators in the form of vials or spore Strips; with at least 1X106 spores should be used at least once in every week to validate the autoclaving efficiency of decontamination process
- Records should be maintained

Points to consider for Lab Waste Management

Minimise waste and do not accumulate large amounts in the laboratory.

Segregate waste - Have a separate residue container . Ensure the waste container is compatible with the waste you are collecting.

Label the waste residue container with the appropriate waste label.

Store waste in a suitable area prior to collection. For example, chemicals and solvents should

Handle waste only if you are aware of the hazards associated with the waste and appropriate

Dispose waste as per relevant guidelines.

https://www.youtube.com/watch?v=avAG0BRtKO8

आज़ादी का अमृत महोत्सव

ICMR Advisory For COVID-19 Testing

USAID FROM THE AMERICAN PEOPLE

RISE Reaching Impact, Saturation, and Epidemic Control

Spectrum of Tests available for COVID-19:

- **Point-of-Care Tests:** Home or Self-Test / Rapid Antigen Test (RAT)
- **Molecular Tests:** rRT-PCR, TrueNat, CBNAAT, CRISPR, RT-LAMP, Rapid Molecular Testing Systems, Newer SARS-CoV-2 Omicron or variant detection rRTPCR assays
- The newer SARS-CoV-2 Omicron or variant detection rRTPCR assays are recommended to be used in areas with limited genomic surveillance capacity for quick detection of emergence of newer variants in such areas.

Advisory on Purposive Testing Strategy for COVID-19 in India
(Version VII, dated 10thJanuary 2022)

Scope:

This advisory on COVID-19 testing strategy is for:

- Early detection of symptomatic cases for quick isolation and care.
- Early detection of infections in elderly (>60yr) and individuals with co-morbidities (diabetes, hypertension, chronic lung or kidney disease, malignancy, obesity etc.) for quick care

Testing Categories:

- A. Routine surveillance in containment zones and screening at points of entry
- B. Routine surveillance in non-containment areas
- C. In Hospital Settings
- D. Testing on demand

People who need not be tested:

1. Asymptomatic individuals in community settings

2. Contacts of confirmed cases of COVID-19 unless identified as high risk based on age or comorbidities

3. Patients who stand discharged as per home isolation guidelines

4. Patients being discharged from a COVID-19 facility as per revised discharge policy

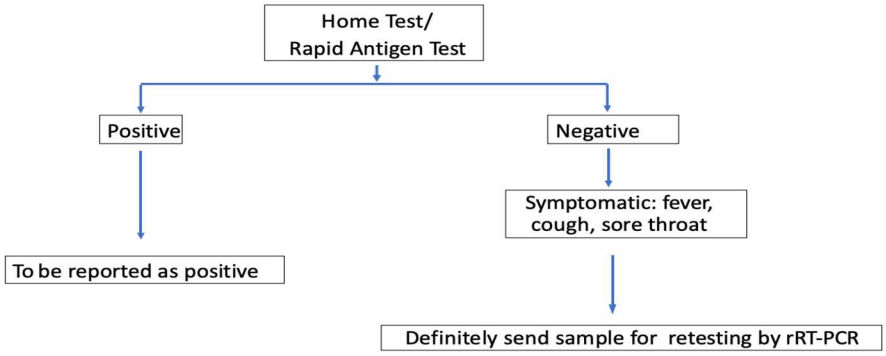
5. Individuals undertaking inter-state domestic travel

Important points to consider:

- Testing can be undertaken either through RT-PCR, TrueNat, CBNAAT, CRISPR, RT-LAMP, Rapid Molecular Testing Systems or through Rapid Antigen Test (RAT).
- A positive point-of-care test [Home or Self-test / RAT] and Molecular Test is to be considered confirmatory, without any repeat testing.
- Point-of-care test [Home or Self-test / RAT] should be interpreted as per algorithm at Annexure. Symptomatic individuals, testing negative on Home/Self-test or RAT should undertake RTPCR test as detailed in the algorithm

Annexure

Algorithm for COVID-19 Test Interpretation Using Home Test/ Rapid Antigen Point-of-Care Test



- *All positive and negative results should be entered into the ICMR portal on a real time basis after performing the test.*
- *Results of samples subjected to rRT-PCR should be entered after the rRT-PCR results are available.*

Link for Information on Kits

List of approved home tests, RAT and rRTPCR kits are available at the following links:

https://www.icmr.gov.in/pdf/covid/kits/COVID_Home_Test_Kit_23112021.pdf

https://www.icmr.gov.in/pdf/covid/kits/List_of_rapid_antigen_kits_30122021.pdf

https://www.icmr.gov.in/pdf/covid/kits/RT_PCR_Tests_Kits_Evaluation_Summ_23122021.pdf

Home Testing advisory of ICMR is available at:

https://www.icmr.gov.in/pdf/covid/kits/Advisory_Home_Test_kit_19052021_v1.pdf

Guidelines for storage of respiratory specimens collected for COVID-19 diagnosis by RT PCR platforms in Government laboratories - Dated: 25/06/2020

a) Laboratories that are serving as validation centres for COVID-19 diagnostic kits are advised to preserve adequate numbers of positive and negative samples to prepare appropriate panels for validation etc.

b) At a minimum, all samples testing positive for SARS CoV2 must be retained for at least 30 days from the date of testing before being destroyed. Depending on the freezer space availability in a particular laboratory, one or more aliquots of the positive specimen may be retained for the period.

c) A government laboratory may decide on the number of positive / negative samples to retain in the long term based on the availability of freezer space as well as perceived research agenda of the laboratory for COVID-19 in the future.

Guidelines for storage of respiratory specimens collected for COVID-19 diagnosis by RT PCR platforms in Government laboratories - Dated: 25/06/2020

d) If the number of samples tested positive at a laboratory is considerably large and the laboratory is unable to retain all positive samples beyond 30 days, a minimum of 10% of all positives detected at the laboratory in a month or 40-50 positives preferably with equal numbers of high, moderate and low viral load should be stored for a period of 1 year at the least. A single aliquot of a positive sample may be retained taking into account freezer space availability at the laboratory.

e) Considering that the number of samples tested negative at each laboratory will vary depending on the sample load and testing capacity of the laboratory, a minimum of 50 samples or 1-2% of all negative tested samples over a month, whichever is smaller should be retained at the testing laboratory for a period of 1 year. A single aliquot of a negative sample may be retained taking into account freezer space availability at the laboratory

Reaching Impact, Saturation, and Epidemic Control (RISE)

ILQC – ICMR Designated QC Lab

- All labs will have to send 5 random positive and 5 random negative samples per month to QC labs.
- ICMR has mapped COVID-19 testing labs to different QC labs (Annexure I).
- All testing labs should liaise with the recommended QC labs and will ensure regular participation in QC activity.
- All testing labs will ensure storage of samples at -80℃ and will ensure regular monthly transfer to QC labs.
- Don't forget to include your lab name and sample ID.
- While shipping, place samples in screw capped vials and proper Biosafety and Biosecurity precautions should be followed as per IATA guidelines.

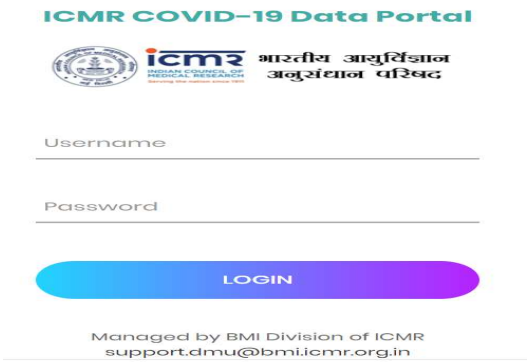
ILQC – ICMR Designated QC Lab

- In case of any discordance, additional 5 positive and 3 negative samples will need to be sent for QC check.
- If QC results are concordant, all the QC samples may be destroyed, and labs will keep record of destruction.
- Appropriate procedures to disinfect all samples prior to disposal must be followed.
- ICMR Designated QC lab
- Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura
    - ICMR-Regional Medical Research Center, Dibrugarh

Data Upload – To Do

All the QC results has to be entered in the QC portal and should not be shared with the QC lab

All rRTPCR and RAT test results should be uploaded on ICMR portal at: https://cvstatus.icmr.gov.in



Data Upload – To Do

- The vaccination status of all individuals tested for COVID-19 must be entered into the Sample Referral Form (SRF) in the RTPCR app both for individuals tested by rRTPCR and RAT. This information is of critical importance.

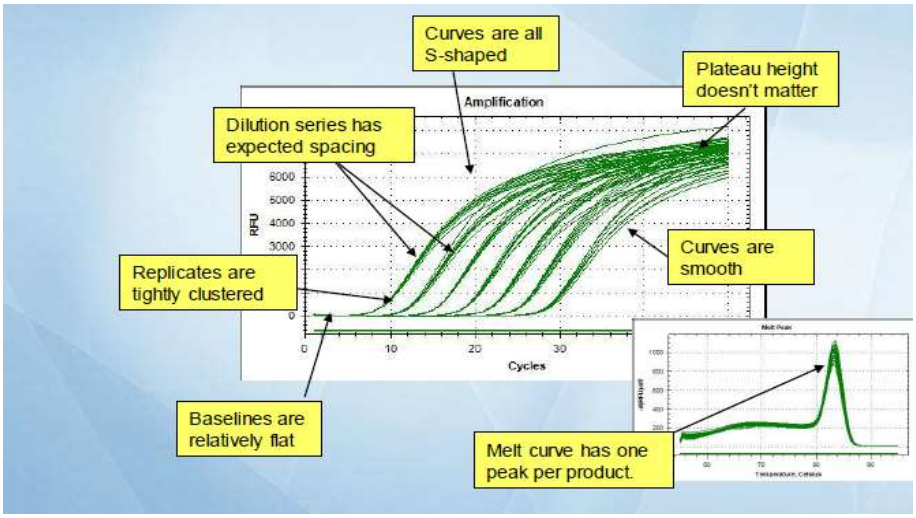
- Genome Sequencing is done for surveillance purposes and is not required to be undertaken for treatment purposes. Therefore, genome sequencing is to be performed only in a subset of positive samples as per recommendations of INSACOG (Indian SARS-CoV-2 Genomic Surveillance Consortium).

CAPA, Validation and Verification & Troubleshooting

Learning objectives

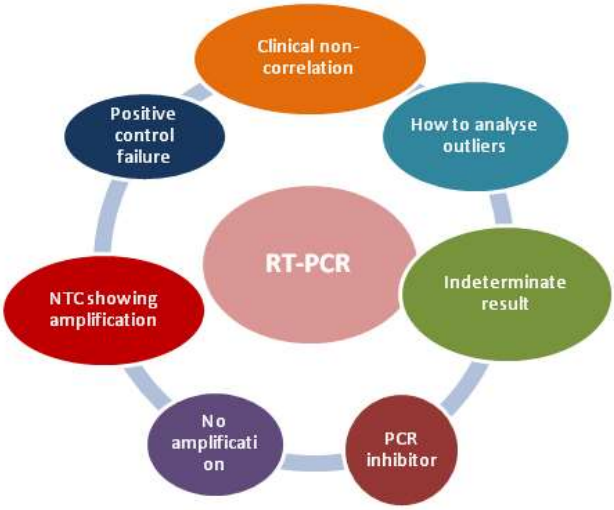
- Ideal RT-PCR
- CAPA
- Validation & Verification
- Trouble shooting Exercises

An Ideal Real Time PCR Graph



Reaching Impact, Saturation, and Epidemic Control (RISE)

Various Problems Encountered in an RT-PCR Testing



Reaching Impact, Saturation, and Epidemic Control (RISE)

What can go wrong for the clinician?

False negative results

False positive results

Unless the referring clinician or patient complains, this will not be known or investigated.

False Negative Results

- 41% of the errors
- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents
- Presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
- Failure to follow instructions /SOP

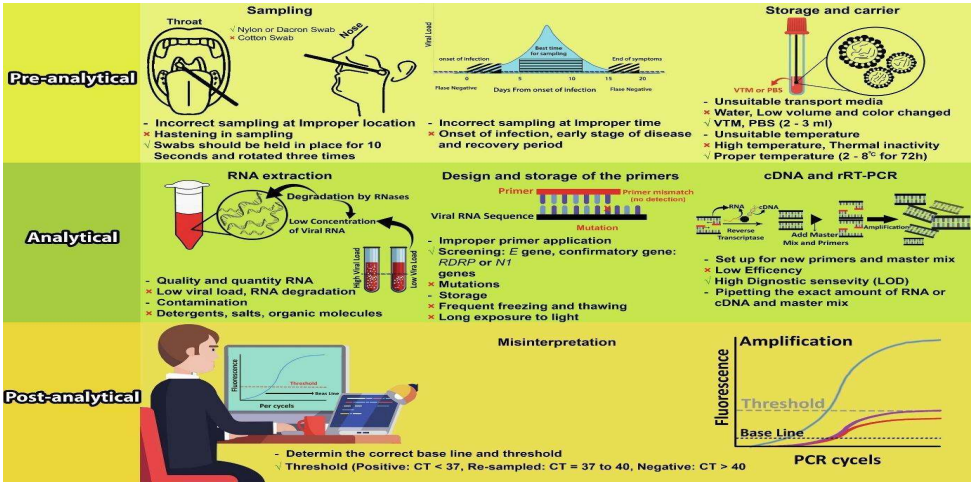
False Positives Results

- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling
- Clerical error while reporting

Almost impossible to identify and troubleshoot if controls are not affected

Strict adherence to SOP, safety and disinfection practices only can prevent

Analytical considerations when conducting RT-PCR for SARS-CoV-2



CAPA

Corrective and Preventive Action - CAPA

Corrective and preventive action (CAPA) is a process used to investigate and solve problems, identify causes, take corrective action and prevent recurrence of the root causes.

Corrective action

Corrective action is responsible for **resolving an already existing non-conformity** or an undesirable circumstance. It involves a systematic check of events that could have led to the error, including:

Clerical/transcription/labelling errors

Reagent deterioration/Reagent contamination

Pipetting errors – aerosols

Inadequate disinfection

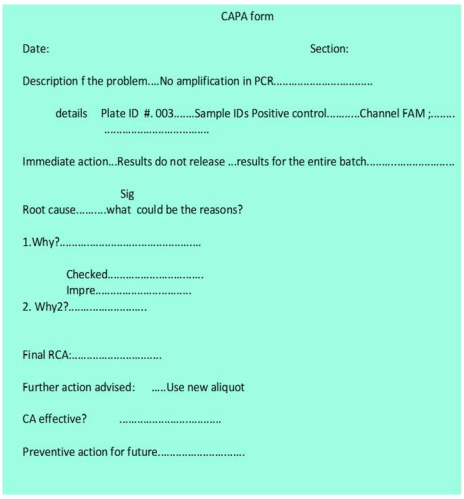
Equipment – poor maintenance, non-calibration

Preventive Action

Preventive action, on the other hand, **is taken beforehand, based on the cause of a potential non-conformity** to try to avoid it altogether. Preventive actions can include:

- Actions taken based on a risk assessment, even before an event occurs.
- Actions taken after an error, for monitoring and to prevent a recurrence. Active monitoring is carried out for a defined period of time to ensure that the corrective action taken was appropriate and effective.

CAPA Form



CAPA form

Date: Section:

Description f the problem....No amplification in PCR.....

details Plate ID #. 003.....Sample IDs Positive control.....Channel FAM ;.....
.....

Immediate action...Results do not release ...results for the entire batch.....

Sig
Root cause.....what could be the reasons?

1. Why?.....

Checked.....
Impre.....
2. Why2?.....

Final RCA:.....

Further action advised: .....Use new aliquot

CA effective? .....

Preventive action for future.....

Key Performance Indicators (KPIs)

Key performance indicators (KPIs) refer to the collection and analysis of data at each step of the testing cascade, which can serve as indicators for the correct performance of the entire testing process.

KPIs should be analysed and **reported on a regular basis (at least monthly)** and include the following:

- Number of specimens tested, by specimen type
- Number (%) of positive, negative and invalid test results
- Specimen rejection rate
- Number (%) of failed IQC results
- EQA/PT performance (pass/fail or % score)
- Turnaround time (TAT)

Validation & Verification

Validation and Verification

Validation:
Commercially available tests undergo rigorous performance **evaluations by the manufacturer**

Verification:
A **laboratory then performs their own examinations**

-compares the data they obtain in their setting with the data provided by the manufacturer

-to confirm if the performance claims made by the manufacturer (acceptance criteria) have been met.

Type of Kit	Acceptance Criteria
RT-PCR Kit	Sensitivity: 95% and above Specificity: 99% and above
RNA Extraction Kit	At least 95% concordance among positive At least 90% concordance among negative samples > 95 % samples showing amplification in internal control
VTM	100% concordance among spiked samples 100% samples showing amplification in internal control
Antibody Rapid Kit	Sensitivity: 90% and above Specificity: 99% and above
ELISA / CLIA Kit	**IgM**: Sensitivity- 90% and above Specificity- 99% and above **IgG**: Sensitivity- 90% and above Specificity- 95% and above

Note: The cut-offs have been decided after deliberation in ICMR Expert Group and with the Drug Controller General of India (DCGI).

Reaching Impact, Saturation, and Epidemic Control (RISE)

Validation and Verification Process

	Validation	Verification
Performed by	The manufacturer/test developer for commercial kits when used according to the manufacturer's instructions for use Or the laboratory when: • using non-standard methods • using in-house tests (where the central/reference laboratory develop their own COVID-19 PCR test) • using standard methods outside their scope • when modifying a previously validated method	The laboratory, on a test that has already undergone validation by the manufacture or reference laboratory
Testing criteria	• Accuracy • Precision • Limit of detection • Analyte stability during typical transport and storage of samples • Cut-off value • Interfering substances • Bias • Reference ranges • Reporting ranges	Accuracy: expresses the overall agreement between the percent positive agreement and the percent negative agreement (both of which should ideally be 100%) of the candidate method and the comparative method Precision: expresses the reproducibility of results obtained between different laboratory staff and/or at different laboratories using the same reference materials

Lot to Lot verification

Each change in reagent lots can adversely **affect the consistency and quality of patient results** and hence as part of good laboratory practice each new reagent lot must be evaluated.

- at least one known positive sample and one known negative sample must be included
- A new lot of a kit is received / A new operator performs the test / Problems (storage, operator, instrument, or other) are suspected or identified.
- Lot to lot verification should also be performed if otherwise required by the laboratory's standard QC procedures.

RT-PCR problems

What can go wrong with RT-PCR test?

We will know only after the assay is over

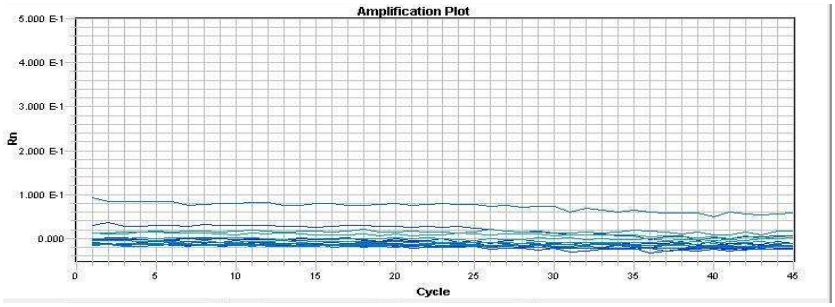
1. No amplification- failed run
2. Contamination
3. Inhibition
4. Failure of controls to give expected results
5. Indeterminate results

Questions ?

1. Was the kit a new one? New lot or shipment?
2. Was lot verification done? Was it accepted?
3. Was the storage temperatures monitored?
4. Was the PCR cycler working fine?

Trouble Shooting Exercises

Exercise 1: Flat graph …!



1. No amplification- Flat line

a) No signal is detected in all the fluorescence channels

Possible causes:

- Reagent deterioration, inactivation
- One component not added
- Steps missed/protocol not followed
- Wrong assay /program in the computer
- PCR cycler failure

Corrective action:

- No result can be concluded, and the assay must be repeated from extraction/ fresh sample has to be obtained.

1. No amplification - Flat line

b) No signal with positive controls in fluorescence channel FAM/VIC:

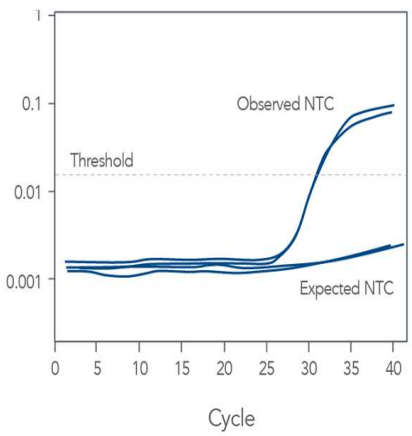
Possible Causes:

- Degradation of the positive controls
- Improper aliquoting and storage of the controls
- Improper dispensing of the required amount of the controls
- Incorrect selection of fluorescence channel
- In correct Temperature Profile
- Incorrect Configuration of PCR
- Missed to add control, instead a wrong sample or water added

Corrective action:

- Use a new aliquot of PC and repeat testing.

Exercise 2: Contamination in the NTC



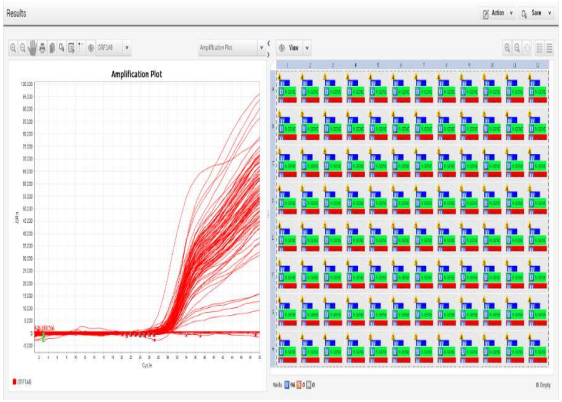
Possible Causes:

- The DNAse RNAse free water may be contaminated
- Improper decontamination of the Pipettes and the Biosafety cabinet
- Improper testing protocol carried out by the Lab technicians and requires proper training protocols and unidirectional workflow.
- Fumigation of the laboratory is lacking in proper intervals.

Corrective Action:

- Results should not be released for the entire batch of samples
- Decontamination of the laboratory thoroughly, change the tip boxes and repeat the run.

Exercise 3: Amplification see in all Wells



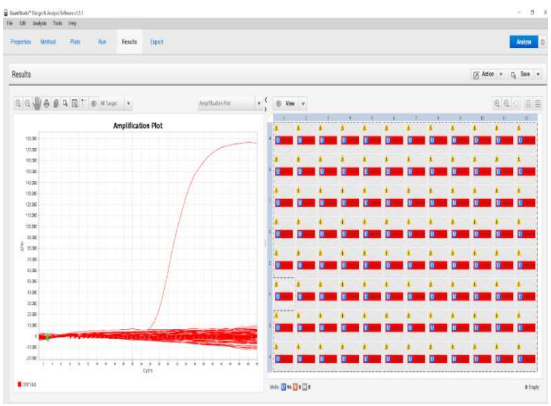
Possible Causes:

- Because of unsterile NFW while preparing reagents, There could be a chance of contamination around PCR reagents resulting in amplification of entire wells.

Corrective Action:

- Always Use good PCR grade Nuclease water (NFW) throughout the experiment.

Exercise 4: Amplification Seen in NTC well alone



<u>Possible Causes:</u>

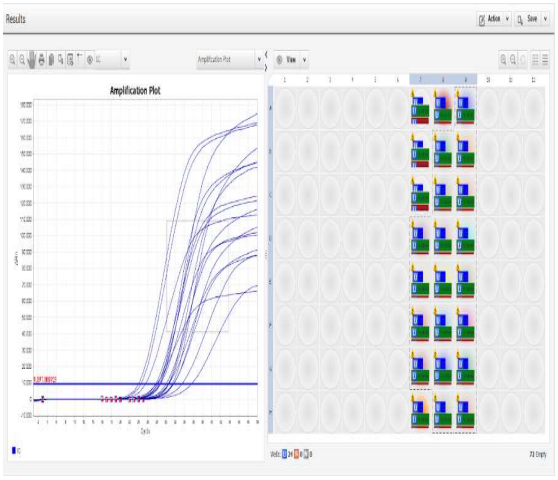
-Reagent Contamination

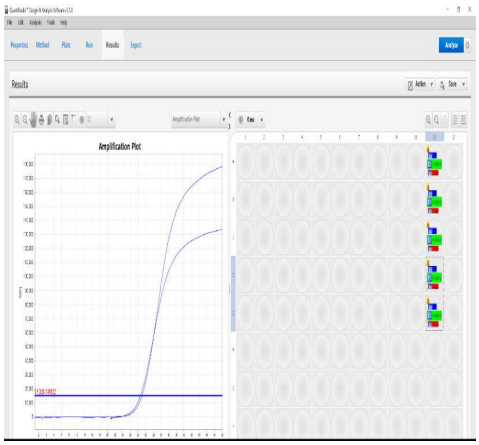
-Positive Control has been degraded

<u>Corrective Action:</u>

- Introducing New LOT for respective repeat PCR assay.
- Before you start clean bench surfaces and pipettes with a DNA decontamination reagent and 30 Mins of UV Exposure of Laminar-air flow before starting an experiment
- Use a clean separate set of pipettes and use barrier/filter tips for all work including stock reagent preparation.
- Aliquot the Positive control as it is opened and avoid multiple freeze thaws.

Exercise 5: Internal Control has Failed to Amplify





Exercise 5: Internal Control has Failed to Amplify – Contd…

Possible Causes:

- Failed to add the internal control during the time of extraction
- RNase P may be present in undetectable amounts

Corrective Action:

- In case RNase P is the internal control, then these are considered as inconclusive and must be repeated with increasing the volume of the clinical specimen or from a newly collected specimen.
- In case it is the Exogenous Internal Controls, then the RNA extraction has to be repeated with the clinical specimen

Laboratory Hazard Risk Assessment for SARS-CoV-2

Risk Assessment

All laboratories should perform a site-specific and activity-site-specific risk assessment to identify and mitigate risks.

Risk assessments and mitigation measures are dependent on:

- The procedures performed
- Identification of the hazards involved in the process and procedures
- The competency level of the personnel who perform the procedures
- The laboratory equipment and facility
- The resources available

Risk Assessment - Contd

Why is it Needed???

To minimize risks and provide a safe work environment, a risk assessment should be performed to evaluate what could go wrong by determining the **likelihood** that an undesirable incident (e.g., injury, exposure) may occur and the **consequences** (e.g., infection or disease) if that undesirable incident were to occur

Risk Assessment - Contd

When is it performed???

Formal risk assessments should be performed before work begins, and repeated when any change is introduced into the activity (e.g., changes in practices, personnel, instrumentation, or facilities). Informal risk assessments, which include short discussions among staff about current risks and mitigations, should occur much more frequently, ideally daily.

Who is involved???

A team should perform risk assessments to ensure various perspectives are considered and to reduce bias. This team could be comprised of senior leadership, clinical laboratory scientists, safety professionals, facility engineers, and others familiar with the site-specific and activity-specific laboratory and testing activities.

RISK ASSIGNMENT OF INFECTIOUS PATHOGENS

Table 1. ***Classification of infective microorganisms by risk group***

Risk Group 1 *(no or low individual and community risk)*
A microorganism that is unlikely to cause human or animal disease.

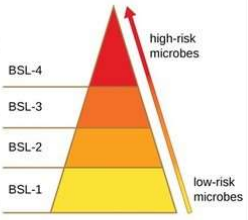
Risk Group 2 *(moderate individual risk, low community risk)*
A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 *(high individual risk, low community risk)*
A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

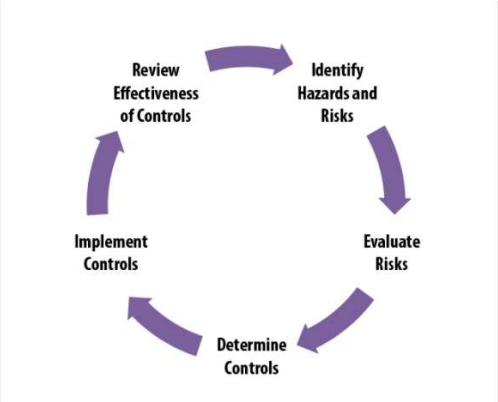
Risk Group 4 *(high individual and community risk)*
A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

SARS COV-2 is a Group 4 Risk

Table below depicts the different biosafety level laboratories [WHO, 12.02.2020]

Biosafety Levels			
Biological Safety Levels	**Description**	**Examples**	**CDC Classification**
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	*Mycobacterium tuberculosis*	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	*Staphylococcus aureus*	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of *Escherichia coli*	

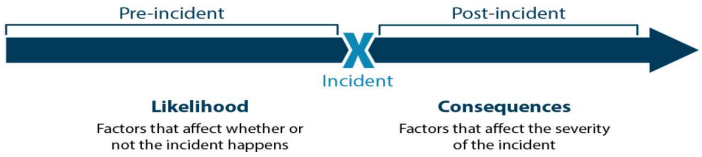
Risk Management Process



How is it conducted: Step 1 – Identify the Hazard

Instructions: *Provide a brief overview of the laboratory work and summarize the laboratory activities to be conducted that are included in the scope of this risk assessment.*	
Describe the biological agents and other potential hazards (for example, transmission, infectious dose, treatment/preventive measures, pathogenicity).	
Describe the laboratory procedures to be used (for example, culturing, centrifugation, work with sharps, waste handling, frequency of performing the laboratory activity).	
Describe the types of equipment to be used (PPE, centrifuges, autoclaves, biological safety cabinets [BSCs]).	
Describe the type and condition of the facility where work is conducted.	
Describe relevant human factors (for example, competency, training, experience and attitude of personnel).	
Describe any other factors that may affect laboratory operations (for example, legal, cultural, socioeconomic).	

Step 2 – Evaluate the Risks



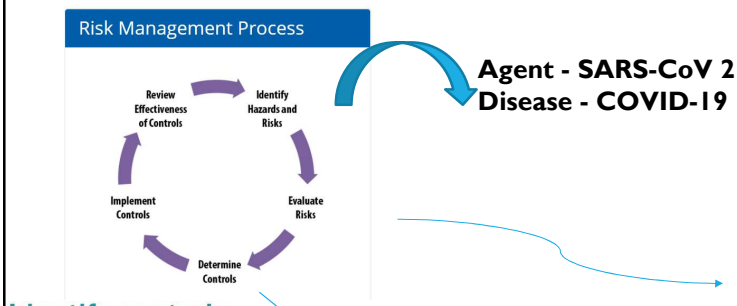
		Likelihood of exposure/release		
		Unlikely	Possible	Likely
Consequence of exposure/release	Severe	Medium	High	Very high
	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium
Laboratory activity/procedure		**Initial risk (very low, low, medium, high, very high)**	**Is the initial risk acceptable? (yes/no)**	**Priority (high/medium/low)**
Select the overall **initial** risk.		☐ Very low	☐ Low	☐ Medium	☐ High	☐ Very high
Should work proceed without additional risk control measures?		☐Yes ☐No				

Step 3 – Develop a Risk Control Strategy

For risks that are determined unacceptable by the institution, a mitigation control plan should be implemented.

Instructions: *List any requirements that have been prescribed by international and national regulations, legislation, guidelines, policies, and strategies on biosafety and biosecurity.*	
Describe the measures required by national legislation or regulations (if any).	
Describe the measures advised by guidelines, policies and strategies (if any).	

Instructions: *Describe the resources available for risk control and consider their applicability, availability, and sustainability in the local context, including management support.*	
Are resources sufficient to secure and maintain potential risk control measures?	
What factors exist that may limit or restrict any of the risk control measures?	
Will work be able to proceed without any of the risk control measures; are there alternatives?	



Agent - SARS-CoV 2
Disease - COVID-19

Risk = likelihood X consequence

Risk Assessment Matrix		Hazard Consequence				
		Insignificant	Minor	Moderate	Major	Critical
Hazard Likelihood	Highly likely	Medium	Medium	High	Extreme	Extreme
	Likely	Low	Medium	High	High	Extreme
	Possible	Low	Medium	High	High	High
	Unlikely	Low	Low	Medium	Medium	High
	Rare	Low	Low	Low	Medium	Medium

Identify controls

Task	Risk	Controls
Prepping respiratory specimen for BioFire FilmArray	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Work in a certified BSC* (if possible) in a BSL2 lab • Use Standard Precautions • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to washing hands
Preparing respiratory specimen for RT-PCR 2019-nCoV assay	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Work in a certified BSC* (if possible) in a BSL2 lab • Use Standard Precautions • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to washing hands
Touching contaminated surfaces and instruments	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Decontaminate work surfaces and equipment with EPA-registered hospital disinfectants found on the EPA's List N • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to washing hands

Task	Likelihood	Consequence	Risk
Prepping respiratory specimen for BioFire FilmArray	Likely	Moderate	High
Preparing respiratory specimen for RT-PCR Diagnostic Test	Likely		High
Touching contaminated surfaces and instruments	Possible		High

SARS-CoV-2 Sample Type Risk Assessment

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/ processes
Point of Care sample collection	-Droplet -Fomites -Aerosol	-Negative air flow, if available -Dispersal if outside collection	-Standard precautions -Biohazardous waste -Agent-specific training -Disinfection SOP -Doffing SOP	-Solid front gown, -Single gloves, -Eye protection plus fa shield (if available), -Respirator (or mucosal protection if a respirator is not available)	-Hand hygiene
Swabs (collection)	-Droplet -Fomites -Aerosol	Negative air flow, if available	-Standard precautions -Biohazardous waste -Agent-specific training -Disinfection SOP -Doffing SOP	-Solid front gown, -Single gloves, -Eye protection plus face shield (if available), -Respirator or mucosal protection (if a respirator is not available)	-Placed in a transport tube containing either viral transport medium or sterile saline[3] -Hand hygiene
Processing blood, plasma/serum (convalescent, confirmed negative)	-Droplet -Fomites -Aerosol	-Negative air flow (BSC preferred) -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -SARS-CoV-2 -specific training -Disinfection SOP -Doffing SOP -BSL-2 proficiency documented -Bloodborne Pathogens training	-Solid front gown, -Single gloves, -Eye protection, -Respirator or mucosal protection (if a respirator is not available)	-Hand hygiene

SARS-CoV-2 Sample Type Risk Assessment

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/ processes
Processing Blood (suspected or confirmed SARS-CoV-2)	-Droplet -Fomites -Aerosol	-Negative air flow -BSC -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 specific training -BSL-2 proficiency documented -Bloodborne Pathogen training	-Solid front gown (fluid resistant) -Eye protection -Mucosal protection -Face shield (if risk for splash) -Double gloves	-Cytometry that has Aerosol control features and/or specimens are inactivated -If BSC not available, work behind a splash shield -Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological
Processing swab (suspected or confirmed SARS-CoV-2-)	-Droplet -Fomites Aerosol	-Negative air flow -BSC -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	-Solid front gown (fluid resistant) -Eye protection -Mucosal protection -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological Substance, Category B
Urine (suspected or confirmed SARS-CoV-2)	-Droplet -Fomites -Aerosol	-Negative air flow -BSC -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	-Solid front gown (fluid resistant) -Eye protection -Mucosal protection -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological Substance, Category B

SARS-CoV-2 Sample Type Risk Assessment

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/ processes
Stool (suspected or confirmed SARS-CoV-2-)	-Droplet -Fomites Aerosol	-BSC -Negative air flow -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	--Solid front gown (fluid resistant) -Eye protection -Respirator or mucosal protection (if a respirator is not available) -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological Substance, Category B
Lower Respiratory samples	-Droplet -Fomites -Aerosol	-BSC -Negative air flow -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	--Solid front gown (fluid resistant) -Eye protection -Respirator or mucosal protection (if a respirator is not available) -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological Substance, Category B
Higher Respiratory samples	-Droplet -Fomites -Aerosol	-BSC -Negative air flow -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	--Solid front gown (fluid resistant) -Eye protection -Respirator or mucosal protection (if a respirator is not available) -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Specimens, cultures, or isolates should be packaged and shipped as UN 3373 Biological Substance, Category B

Reaching Impact, Saturation, and Epidemic Control (RISE)

SARS-CoV-2 Sample Type Risk Assessment

Sample types	Potential hazards	Engineering controls	Administrative controls	PPE	Additional controls/ processes
Environmental samples (wastewater/ sewage)	-Higher concentration of virus -Droplet -Fomites -Aerosol	-BSC -Negative air flow -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	--Solid front gown (fluid resistant) -Eye protection -Respirator or mucosal protection (if a respirator is not available) -Face shield (if risk for splash) -Double gloves	-Hand hygiene -Precipitation; membrane filtration[1]
Environmental samples- (Surface sampling)	Droplet -Fomites -Aerosol	-Negative air flow -Centrifuge cup & lid or sealed rotor	-Universal precautions -Biohazardous waste -Disinfection SOP -Doffing SOP -SARS-CoV-2 -specific training -BSL-2 proficiency documented	--Solid front gown (fluid resistant) -Eye protection -Mucosal protection -Face shield (if risk for splash) -Double gloves	-Hand hygiene

1. https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html#testing
2. https://www.cdc.gov/coronavirus/2019-ncov/php/water.html
3. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

Step 4 – Evaluate Effectiveness of Controls

The effectiveness of implementing additional controls (e.g., engineering controls, administrative and work practice controls, and use of PPE) should be reviewed and evaluated.

Instructions: *Describe where and when risk control measures are needed, the level of* ***residual*** *(remaining) risk when these risk control measures are in place, and an assessment of the availability, effectiveness, and sustainability of the risk control measures.*

Laboratory activity/procedure	Selected risk control measure(s)	Residual risk (very low, low, medium, high, very high)	Is the residual risk acceptable? (yes/no)	Are risk control measures available, effective, and sustainable? (yes/no)

Instructions: *Evaluate the* ***residual*** *risk that remains after risk control measures have been selected, to determine whether that level of risk is now acceptable and whether work should proceed.*
Circle the ***residual*** *risk of the laboratory activities after risk control measures are in place.*

Residual Risk

Task	Risk	Controls	Residual Risk
Prepping respiratory specimen for BioFire FilmArray	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Work in a certified BSC* (if possible) in a BSL2 lab • Use Standard Precautions • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to washing hands	Medium
Preparing respiratory specimen for RT-PCR 2019-nCoV assay	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Work in a certified BSC* (if possible) in a BSL2 lab • Use Standard Precautions • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to washing hands	Medium
Touching contaminated surfaces and instruments	High	• Follow CDC guidance for Laboratory Biosafety for COVID-19 • Decontaminate work surfaces and equipment with EPA-registered hospital disinfectants found on the EPA's List N • PPE: gloves, lab coat/gown, and eye protection • Must wash hands after glove removal and prior to exiting the laboratory • Refrain from touching face or mucous membrane prior to	Low

Risk Assessment Development

Key Considerations for Risk Assessment Development

- Address the "unusual" situations.
  1) Leaking specimens.
  2) Broken specimens.
  3) Transport between lab areas.
  4) Small spills.
  5) Serum.

Risk Assessment Best Practices

- Identify and mitigate the risks of working in your laboratory environment with the current facts about COVID-19 infection
- Workforce
  - Identify personnel that will be affected
  - Assess competency for necessary procedures
- Risk Characterization
  - Identify the hazards
  - Modes of transmission and potential exposure
  - Identify activities with increased risk
- Risk Mitigations
  - Identify required appropriate safety practices
  - Communicate risks and required safety practices to staff
  - Review safety practices to make sure they were effective

आज़ादी का अमृत महोत्सव

Good Clinical Laboratory Practices

USAID FROM THE AMERICAN PEOPLE

RISE Reaching Impact, Saturation, and Epidemic Control

GCP, GLP & GCLP

- GCP - Good Clinical Practice
- GLP - Good Laboratory Practice
- GCLP - Good Clinical Laboratory Practice

GCP, GLP & GCLP

GCP- international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects

GLP- intended to promote the quality and validity of test data

GCLP- applies those principles established under GLP for data generation used in regulatory submissions relevant to the analysis of samples from a clinical trial or clinical laboratory

At the same time it ensures that the objectives of the GCP principles are carried out.

What is GCLP ?

GCLP

- GCLP is intended to ensure that the requirements of GCP applicable to the analysis of clinical samples are met.

GCLP guidelines

- WHO guidelines, 2009
- ICMR guidelines, 2021
- DAIDS (Division of AIDS) guidelines

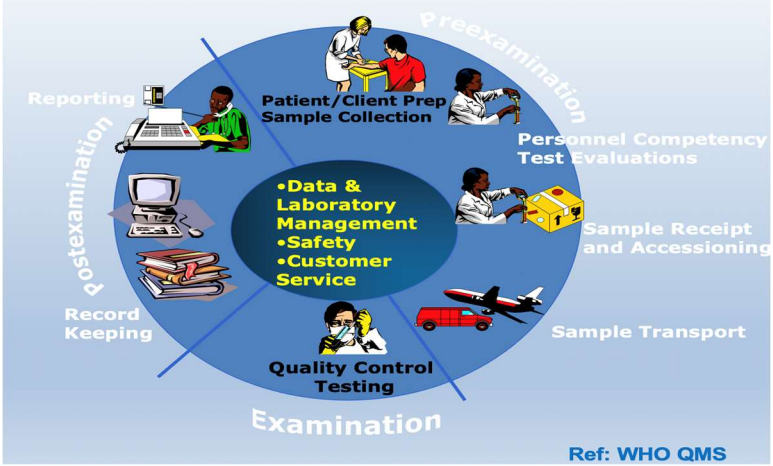
https://main.icmr.nic.in/sites/default/files/upload_documents/GCLP_Guidelines_2020_Final.pdf

Why should we follow GCLP in our laboratories???

GCLP

- Good Clinical Laboratory Practices should be followed by all laboratories where tests are done on biological specimens for diagnosis, patient care, disease control and research
- GCLP- an international quality system for laboratories which undertake the analysis of samples from clinical trials

Elements of a Laboratory System



GCLP Key principles

- Organisation & Personnel
- Facilities
- Equipment, materials & reagents
- Standard Operating Procedures
- Planning of the work
- Conduct of the work
- Reporting results
- Quality Control
- Quality Audit
- Storage & Retention of Records
- Confidentiality

Laboratory tests are influenced by

- Laboratory environment
- Knowledgeable staff
- Competent staff
- Reagents & equipment
- Quality control
- Communications
- Process management
- Occurrence management
- Record keeping

ORGANISATION and PERSONNEL

- Trial Facility Management Responsibilities
  - Ensure qualified personnel, appropriate facilities, equipment and materials are available
    - Organogram
  - Maintain record of qualifications, training , experience and job description for each personnel in trial
  - Health and safety precautions within trial facility
  - Appropriate SOPs
  - Quality audit programme with designated personnel
  - Quality control programme
  - Maintain copies of all trial protocols and analytical plans
  - Proper archiving

IMPORTANT

- Ensure that personnel clearly understand the functions they are to perform and where necessary, provide training for these functions.
- Ensure that Health and Safety precautions within the laboratory are applied according to national regulations

Clinical/Public Health Laboratory Staff Responsibilities

Be aware of guidelines applicable to work

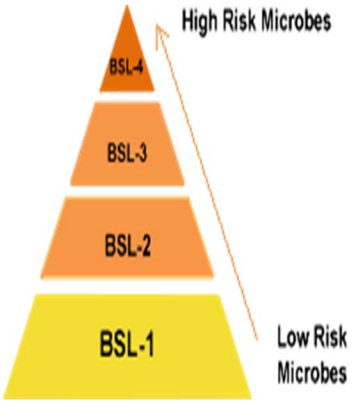
Record raw data promptly and accurately and in compliance with guidelines

RESPONSIBLE FOR THE QUALITY OF THEIR DATA

Follow instructions in SOPs, trial protocols and analytical plans if applicable

Facilities

- Laboratories- appropriate and according to regulations
  - Q-What are the different levels of BSL laboratories?
- Archiving facilities
- Waste Disposal- Biomedical waste disposal



Safety in Laboratories

- Protection of the staff and the environment
  - General safety measures
  - Biosafety precautions
    - WHO risk groups
    - Levels of Biosafety
  - Bio-medical waste management

Equipment

- Appropriate equipment
- Suitable location
- SOPs
- Periodic inspection, cleaning and maintenance
- Calibration before routine use
- Internal QC results
- Calibration of analytical and non-analytical equipment
  - In-house calibration?

Equipment

Equipment used in the analysis of trial material and operation of the clinical laboratory should be suitably located and of appropriate design and adequate capacity.

Equipment used should be periodically inspected, cleaned, maintained, and calibrated, as appropriate. Records of such maintenance and any unscheduled maintenance or calibration should be retained.

An equipment service schedule listing all relevant equipment and the schedule of planned service and calibration activities should be maintained.

Equipment

Any equipment that is out of service for any reason should be clearly identified as such.

DO NOT USE
OUT OF ORDER

Equipment

Equipment users should be suitably qualified and trained in the operation of the equipment.

In all cases equipment used should be demonstrably fit for purpose.

Materials and Reagents

Materials used in the analysis of trial materials should be demonstrably fit for purpose.

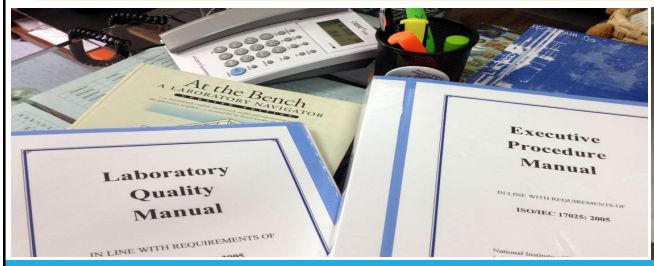
Reagents should be suitably labeled and indicate the identity, concentration, specific storage instructions and stability. Stability information may include the preparation date and expiration date.

SOPs

- Standard Operating Procedures
  - For trials- Manual of Procedures
- Current, Accurate and Available
- Many types
  - Assay/ Test SOPs
  - Equipment
  - Record keeping
  - Collection and receipt of samples
  - Chain of custody
  - Quality control procedures
  - Quality audit procedures

Types of SOPs

Documents	Records
Creation	Collection
Revisions & review	review
Control & distribution	storage
	retention



Documentation Structure

Data Management

- Absence of records implies that the work was never done
  - **"If it ain't written, it ain't there"**
- Format of recording and reporting results should be described in the SOPs
- "Raw data" or "source data"
- **Raw Data: all original records and documentation, or verified copies thereof,** which are the result of the original observations and activities during the conduct of the work and are necessary for the reconstruction and evaluation of the reported results. For the purposes of GCLP, 'source data' (ICH GCP) and 'raw data' are regarded as equivalent.

Computer Systems

- In all cases computer systems should be appropriately validated and maintained and be demonstrably fit for purpose.
- Procedures that address the security and operation of the computer systems should exist. These should include the maintenance of a data audit trail, the date/time and individual responsible for the collection of the data, system change control procedures, maintenance and system security procedures that ensure the integrity of trial data.
- Access to computer systems should be restricted to authorized personnel.
- If data is retained electronically means should exist to ensure the data held can always be retrieved.
- LIMS- Laboratory Information Management System

Quality Control

- The trial facility should maintain appropriate quality control procedures to ensure the quality and accuracy of all aspects of the work performed and reported.

Quality Control

QC procedures may apply, but are not limited, to the following aspects of the work:

a) Within analytical batch acceptance criteria
b) External proficiency scheme results
c) Production of analytical plans and consistency with clinical protocol
d) Acceptability of materials and reagent supplied
e) Secondary tube labelling or aliquoting
g) Sample receipt, handling and storage
h) Results/reports reflect raw data accurately

Internal Quality Control

- **Quality Control (QC):** in process, systematic checking to ensure the quality and accuracy of the work performed and reported, and to eliminate errors.

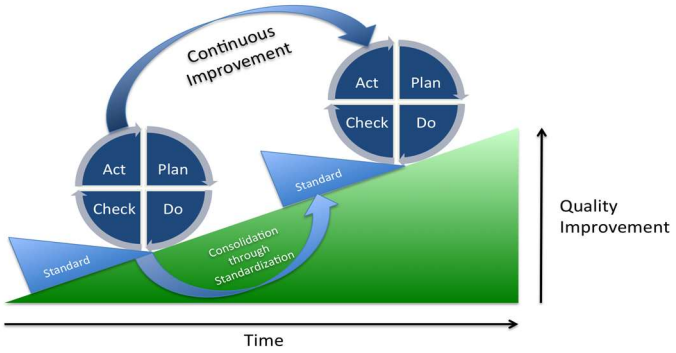
Quality Control

- Where appropriate, test facilities should subscribe to membership of external accreditation/performance/proficiency schemes to demonstrate the competency of the work performed.

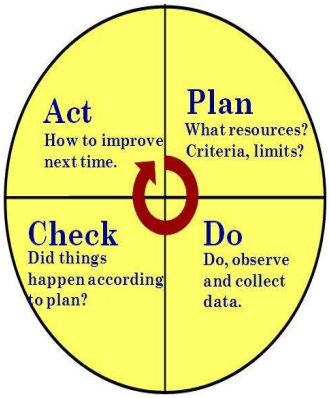
Quality Assurance Programme

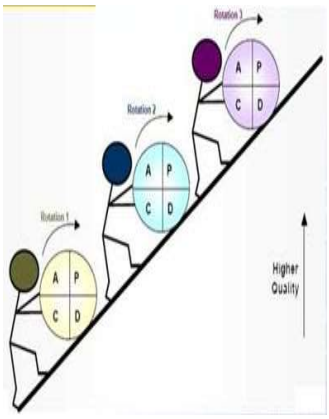
- PDCA cycle
- CAPA

Deming's PDCA/Quality Cycle



Continual Improvement by PDCA cycle





Storage and Retention of Records

The following should be retained for the period specified by the appropriate authorities or as defined by any accreditation bodies:

- The analytical plan, data, samples/specimens (where appropriate), analytical results and if issued the final analytical report
- Records of all audits performed by the quality audit function
- Personnel records
- Document histories of SOPs
- Records and reports of the maintenance and calibration of equipment
- The records and results of all the quality control tests performed to confirm
- the accuracy of the work.

Setting Up RTPCR Lab for COVID-19 and Biosafety

Learning objectives

Steps and sequence in planning

- Pre-planning
- Facility design
- Selection, installation of equipment
- Selection of kits and reagents
- HR Staffing

Safety in laboratory

GMPP

Chemical safety

Physical/fire and electrical

Immunization

Points to consider before action -1

- Anticipated workload for COVID19 RT-PCR
- Expected TAT –How soon the results should be released ?
- Level of training and skill of manpower- Are they trained enough in molecular lab ?
- Cost – cannot be at the expense of quality and safety
- What is the available menu for RT-PCR testing?

a. Fully automated closed system – high throughput, accuracy and precision, expensive, can be run with other assays

b. Open RT-PCR kits- not expensive, need very good training, skills, safety, troubleshooting capability.

c. CBNAAT/Truenat- POCT, less skill and training, quicker, low throughput, cost effective

Points to consider before action-2

Combinations	May be considered
Low , infrequent workload(20-30 samples per day) + space small	Closed system with small throughputs- CBNAAT and Truenat
Low volume + limited staff availability with other testing responsibility	As above
Moderate volume (about 80-100 per day) + small space + shared staff	As above with more channels
High volume + less space + less manpower High Volume + adequate space + sufficient manpower (at least 2 /3 per shift)	Fully automated closed system Open system with dedicated rooms, processes and equipment

Steps to plan and implement open RT-PCR testing

- Following order is practical and convenient with minimal loss of time
- Select the RT-PCR kit based on the regulatory approval (ICMR/USFDA EUA, CE IVD), get the validation document, and select the extraction kit that is compatible and validated by the manufacturer

(Advantage : The extraction kit, PCR equipment can be selected form the manufacturer validation document, reduces verification efforts by the lab.)

- Select kits from 2/3 manufacturers with similar performance
- characteristics as back-up.
- Order equipment and supplies as required by the manufacturer including the infrastructure and electrical supplies
- While the orders arrive, prepare the BSL-2 facility

Steps to plan and implement open RT-PCR testing-contd…

- Install equipment and training of staff
- Draft SOPs, worksheets and TRFs – Perform test validation/verification using known samples procured from reference laboratories to confirm performance characteristics
- Finalise SOP, train all staff , perform competence assessment-trial testing with patient samples
- Interlab comparison –confirm reproducibility
- Start patient testing – All set to go!

2. Facility and Accommodation

Designing a molecular testing laboratory is a meticulous task, because the laboratory will require each of the following:

- Mechanical barriers to prevent contamination (contamination is the most common problem in a molecular testing laboratory)
- Each area should be fascinated fitted with adequate requirements.
- Unidirectional work flow
- Maintenance of air pressure
- Temperature and humidity control
- Exhaust ventilation
- Reliable water supply
- Electricity
- Back-up power system
- ***Surfaces and finishes -*** All walls and floors must be smooth, non-absorbent, and skid-proof, continuous surfaces. Materials used for walls and floors must be easy to clean and impermeable and resistant to chemicals and disinfectants used in the laboratory.

Is it important to have designated rooms?

- PCR is an extremely sensitive technique liable for cross-contaminations and hence false positive result.
- SARS CoV2 is highly infectious and spread easily.
- RNA is labile, subject to easy degradation by nuclease that it ubiquitous. Hence can lead to false negative results, which is not easy to recognize .
- Amplified products are too tiny, an aerosol from a micropipette handling is likely to have as much a 10^6 amplicons .These can stay suspended in the air/contaminate surfaces, ventilation systems etc.
- If lab design is not robust, it is very easy to get wrong or inconsistent results.

Lab design – Open RT-PCR Lab

A laboratory performing open RT-PCR system testing on diagnostic samples should be divided into four, physically separate rooms:

- Room 1: Sample Processing
- Room 2: Reagent Preparation
- Room 3 Template Addition Room
- Room 4: Amplification and Detection

Each room must have its own equipment, protective clothing and consumables, and there should be no transport of materials or equipment between the rooms . In addition, separate rooms are required for each of the following:

- Sample receiving area
- Donning room
- Doffing room
- Staff room/reporting room
- Designated consumables storeroom
- Autoclave area
- Space for handling biomedical waste

Lab design – POCT Lab

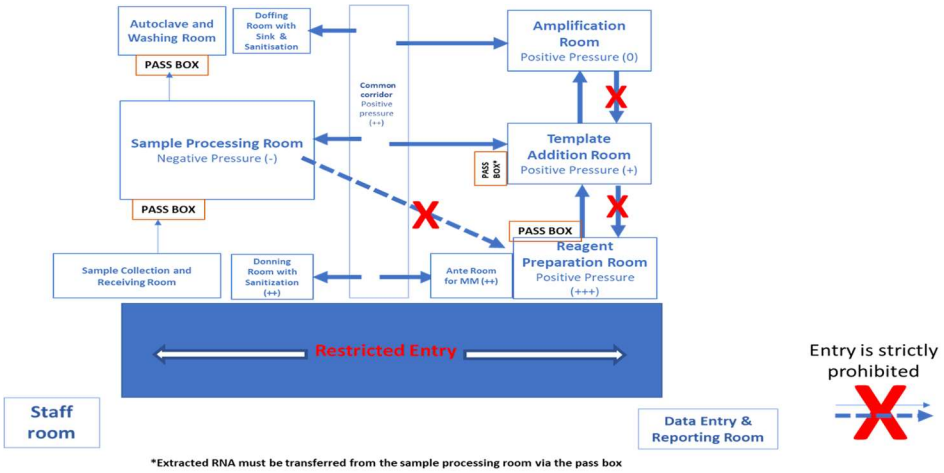
The essential requirements for a CBNAAT/ Truenat laboratory are as follows:

- Designated sample reception area
- Designated area for a biosafety cabinet for sample processing* (loading of samples to the cartridge or extracting using the Truenat extractors)
- Designated area for sample run in the GeneXpert instrument or the Truenat PCR analyser
- Designated storeroom for consumables
- Designated reporting/documentation room
- Designated autoclave area
- Designated space for handling biomedical waste

With the CBNAAT/Truenat sample processing and sample run in GeneXpert can be performed in a single room with a partition or in two physically separate rooms.

* Biosafety cabinet and BSL2 facility is not mandatory and safety measures are minimal

A schematic diagram of the unidirectional workflow in an open RT-PCR system laboratory



3. Equipment - requirement

Depending on the type of test/level of automation planned

- Biosafety cabinet Class II A2/, automated NA extraction equipment
- Laminar Flow cabinet, PCR hood
- Centrifuges , if manual extraction is planned
- Vortex, minifuges in each room
- Micropipettes with barrier tips
- RT- PCR instrument
- Refrigerators, Deep freezers -20^0 C and -80^{0}C
- Autoclave

All equipment to have Installation, operation and Performance qualifications done and documented.

4. Kits and Consumables

- Kits that are approved by ICMR are only to be procured. (USFDA /CE are also approved by ICMR)
- Research-only kits or in-house developed not recommended without adequate expertise and validation .
- Validation report to be obtained from the manufacturer before hand to understand the targets, LOD and performance of the kit.
- After selection of PCR kit, it is better to choose RNA extraction kit, validated already by the manufacturer for the PCR kit, as well as the RT- PCR instrument.
- Fluorescence channels has to be checked. This ensures that the laboratory achieves similar analytical sensitivity and specificity as claimed.

Kits and Consumables – Contd…

- In case of closed systems, the entire supplies, including swabs, VTM/lysis buffer should be from the same/recommended manufacturer only.
- The kit should be used only for the sample types mentioned .
- If the laboratory extends the test to other sample types(stool etc),it may not work and complete validation will be needed.
- It is **necessary** to do verification/validation using atleast 20 samples, using different staff for reproducibility before starting patient testing.

4. Verification Protocol for Kits

Once the kits and reagents are received, for verification, the laboratory needs to establish the following, in comparison with manufacturer's claim.

1. Correlation – positive and negative results approximate Ct values

2. Reproducibility- in duplicates by each designated staff – the difference in Ct values of duplicates to be within 0.5

3. Precision- the range of Ct values achievable by the lab for one sample by repeating atleast 2 or 3 times on different days by different techs

Final Checklist

- Lab facility ready all rooms are as per requirement.
- Equipment installed and checked for correct working, trainings done and instructions ready
- Kits and supplies received.
- Training of persons done for RT-PCR, biosafety, disinfection protocols and policies with the SOPs on place
- Method verification and process flow ensured
- Mock drills have been done
- Interlaboratory comparison of results done with mentoring laboratory

The lab is now ready to start the testing of patient samples

Safety in COVID-19 laboratory

Infection Control in laboratory – what is in place?

Objective	How
Protect Lab staff	1. GMPP 2. Barriers, Engineering control 3. Training 4. Immunization
Protect environment and community	1. BMW management 2. Sterilisation and disinfection 3. Engineering controls
Protect Patient samples	1. Prevention of contamination- training, SOPs, 2. Disinfection and sterilization 3. Transport, storage, temperature

Core requirements

1. Good microbiology practices and procedures
2. Primary barriers
   - Biosafety Cabinet
   - PPE
   - Immunization
3. Secondary Barriers
   - Engineering controls- facility design- Access control, airflow ,hand wash, anteroom,
   - splash free basins,
4. Disinfection and sterilization
5. Biomedical waste management
6. Packaging and Transport
7. Incident management

1. Good Microbiological Practice and procedures (GMPP)

GMPP is a term given to a set of standard operating practices and procedures, or a code of practice, that is applicable to all types of activities with biological agents.

Includes general behaviour, best practices and technical procedures.

GMPP – contd…

1. Never store food or drink, or personal items such as coats and bags in the laboratory. Activities such as eating, drinking, smoking, and applying cosmetics are only to be performed outside the laboratory.
2. Never put materials, such as pens, pencils or gum in the mouth while inside the laboratory, regardless of having gloved hands or not.
3. Wash hands thoroughly , with running water and soap, after handling biological material , before leaving the laboratory or when hands are known or believed to be contaminated.
4. Ensure open flames or heat sources are never placed near flammable supplies and are never left unattended.

GMPP – contd…

01 Ensure that cuts or broken skin are covered before entering the laboratory.

02 Before entering the laboratory, ensure that there are adequate supplies of laboratory equipment and consumables, including reagents, PPE and disinfectants

03 Ensure that supplies are stored safely and according to storage to prevent spills reduce accidents and incidents.

04 Ensure proper labelling of all biological agents and chemicals

05 Protect written documents from contamination using barriers (such as plastic coverings).

GMPP – contd…

- Refrain from using portable electronic devices (for example, mobile telephones, tablets, laptops, flash drives, memory sticks, cameras, or other portable devices, including those used for DNA/RNA sequencing) when not specifically required for the laboratory procedures being performed.
- Keep portable electronic devices in areas where they cannot easily become contaminated or act as fomites that transmit infection. Where close proximity of such devices to biological agents is unavoidable, ensure the devices are either protected by a physical barrier or decontaminated before leaving the laboratory.

GMPP - Technical procedures

- Avoid inhalation of biological agents. Use GMPP techniques to minimize the formation of aerosols and droplets when manipulating specimens.
- Avoid ingestion of biological agents and their contact with the skin and eyes.
- Always wear disposable gloves when handling specimens.
- Avoid gloved hands coming into contact with the face.
- Shield or otherwise protect the mouth, eyes and face during procedures where splashes may occur.

GMPP - Lab dress code- GOI

- Laboratory coats should be fully buttoned, and must be worn by all laboratory staff at all times in the laboratory. These coats should be left in the laboratory when going out for lunch or breaks and when leaving the laboratory.
- Laboratory coats should be decontaminated and laundered regularly (never taken home for laundering).
- Comfortable, water repellent closed shoes with non-skid soles should be worn and must enclose the entire foot.
- Long, dangling jewellery is not permitted in the laboratory.
- Long hair and beards must be tied back to avoid contamination and interference with laboratory work.
- A spare, clean laboratory coat must be available in case of a spill or an emergency.

Good housekeeping practices

- Work areas should be kept free of clutter, dirty glassware and contaminated articles such as paper towels or lint-free tissues.
- Decontaminate equipment and work benches upon entering the laboratory and before leaving the work area with a freshly made 1:10 dilution of household bleach.
- Clean up spills immediately and properly as per laboratory policy.
- Do not submit worksheets that have become contaminated; transfer results and data to new worksheets before submission.

Standard precautions

- Hand hygiene
- Respiratory hygiene (cough etiquette)
- PPE according to the risk
- Safe practices, sharps management and injury prevention
- Safe handling, cleaning and disinfection
- Environmental cleaning
- Sterilisation/ decontamination
- Waste management

2. Personnel competence and training

- Introductory training that includes laboratory layout, codes of practice, local guidelines, safety manuals, risk assessments, legislative requirements, and emergency response procedures. (A training SOP with PPT)
- Job-specific training - on COVID 19 RT-PCR testing
  All laboratory personnel, irrespective of specific job requirements must be trained on GMPP.
- Competency and proficiency assessment must be used and verified before working independently, followed by regular review and refresher training.
- Relevant information such as new procedures must be updated and communicated to applicable personnel.
- Safety and security training
  All personnel must be aware of the hazards present in the laboratory and their associated risks as well as safe working procedures, security measures, and emergency preparedness and response.

3. Facility design

- Other than the designated rooms, there must be ample space , hand-wash with restriction of access.
- Doors properly labelled, and laboratory walls, floors, and furniture smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory.
- Reliable electricity supply with back up.
- Safe handling and storage of infectious and other hazardous materials, such as chemicals and solvents (Reference to MSDS)
- Facilities for eating and drinking must be provided outside the laboratory, and first-aid-facilities must be accessible.
- Appropriate methods for decontamination of waste, for example disinfectants and autoclaves, must be available close to the laboratory.
- Safety systems must cover fire, electrical emergencies, and emergency/incident response facilities, based on risk assessment.

4. Specimen receipt and storage

- A specimen received by the laboratory must be accompanied by sufficient information to identify
  - what it is,
  - when and where it was taken or prepared, and
  - which tests and/or procedures (if any) are to be performed (Ref SRF)
- Consider unpacking the items in the BSC.
- Personnel unpacking and receiving specimens must be adequately trained on the hazards involved; how to adopt necessary precautions according to GMPP described earlier; how to handle broken or leaking containers; and how to handle spills and use disinfectants to manage any contamination.
- Specimens must be stored in sterile vials that are temperature specific, labelled properly, and are leakproof.

5. Decontamination and waste management

- Any surface or material known to be, or potentially be, contaminated by biological agents during laboratory operations must be correctly disinfected to control infectious risks.
- Proper processes for the identification and segregation of contaminated materials must be adopted before decontamination or disposal.
- Where decontamination is not possible in the laboratory area, or onsite, contaminated waste must be packaged in a leakproof fashion, for transfer to another facility with decontamination capacity.

6. Personal Protective Equipment : General Instructions

- Protective coats and aprons in all areas as appropriate.
- Laboratory coats must have long sleeves, preferably with elasticated or fitted cuffs, and must be fastened when worn in the laboratory.
- Sleeves should never be rolled up. Coats must be long enough to cover the knees, but not trail on the floor.
- Where possible, the fabric of the laboratory coat should be splash-resistant.
- Laboratory coats must only be worn in designated areas. When not in use, they should be stored properly; they should not be hung on top of other laboratory coats, or kept in lockers or on hooks with personal items.

PPE Contd….

- Appropriate sterile disposable gloves must be worn and must be changed frequently
- They must not be disinfected or reused, as exposure to disinfectants and prolonged wear reduces the integrity of the glove and decreases protection to the user.
- Gloves should always be inspected before use, to check that they are intact.
- Safety glasses or goggles, face shields (visors) or other protective devices must be worn whenever necessary to protect the eyes and face from splashes, impacting objects or artificial ultraviolet radiation. Eye protection devices can be re-used but must be cleaned each time after use. If splashed, devices must be decontaminated with an appropriate disinfectant.

Recommended biosafety level and PPE for COVID – 19 testing

Activity	Recommendation	Safety level
Specimen Collection	Full PPE (Single use, overall with sterile gloves, Eye & face protection and N95 respirator)	NA
Transport, Receipt	Lab coat, N95 Respirator and Gloves	NA
Specimen opening and RNA extraction	Single use gown, with sterile gloves, N95 respirator, eye protection	BSL- 2 (Negative Pressure)
Master mix, PCR setup	Laboratory coat/apron, face mask , sterile gloves	NA (PCR Hood, Positive Pressure)

7. Laboratory Equipment

- When used effectively together with GMPP, the safe use of laboratory equipment will help to minimize the likelihood of exposure of personnel when handling or manipulating biological agents.
- Regular maintenance and training of personnel on equipment maintenance is important.
- Operation of Biosafety cabinet, centrifuges, autoclave, RT-PCR instruments and correct pipetting practices are to be documented and persons trained.

8. Emergency/Incident Response Plan

- A contingency plan must be developed by the lab to reduce incidents.
- Personnel must be trained on these procedures and have periodic refresher training to maintain competency.
- First-aid kits, including medical supplies, such as bottled eye washes and bandages, must be available and easily accessible to personnel. These products must be checked routinely to ensure that they are within their use-by dates and are in sufficient supply.
- Reporting of lab incidents and investigation of the same must be done with proper documentation.

9. Occupational health

- Immunization for the staff- for Hepatitis B, Influenza, Tetanus and COVID-19.
- Annual medical examination to ensure that staff are healthy to handle infectious agents in the laboratory.

TOOL KIT
A comprehensive manual on SARS-CoV-2 diagnostics

USAID

RISE
Reaching Impact, Saturation, and Epidemic Control

Reaching Impact, Saturation, and Epidemic Control (RISE)

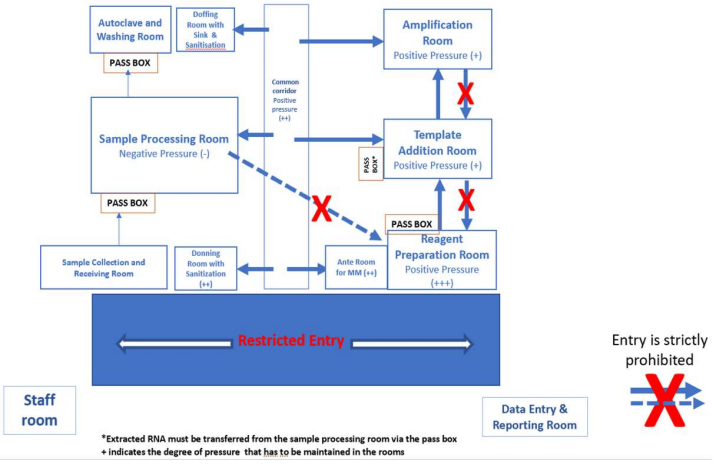
Concepts Covered

1. Introduction to SARS-CoV-2 Testing
2. Complete Laboratory workflow Process
3. Biosafety and Biomedical Waste Management Aspects
4. Samples for COVID-19 diagnosis
5. Complete Details of the Molecular Tests for COVID -19 Diagnosis
6. RT-PCR open systems
7. CBNAAT
8. Truenat
9. Quality Management
10. Trouble shooting of RT-PCR Testing
11. Equipment Management
12. Laboratory Assessment Tools - Audit and Accreditation

Ideal Requirements for the Molecular testing Labs (Page Nos: 9 to 83)

- Physical site
- Facility Design
- Unidirectional Workflow with differential Pressures
- Surfaces and Finishes
- Walls and Partitions
- Passbox, windows, doors, lab furniture's, electrical supplies and plumbing's including lan connections
- Monitoring mechanisms, emergency preparedness

A schematic diagram of the unidirectional workflow in an open RT-PCR system laboratory



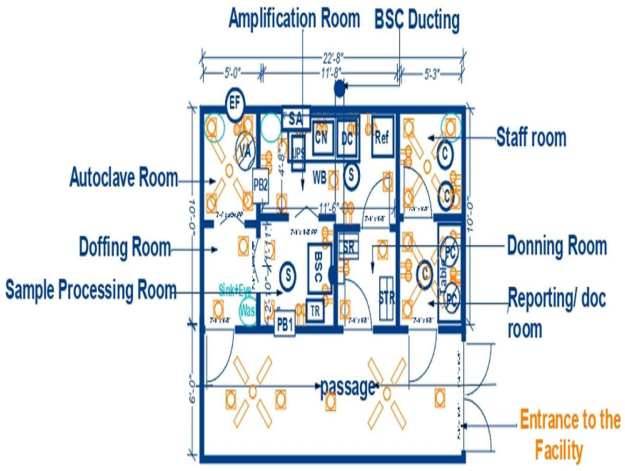
Laboratory floor plans - Types of laboratory models and their testing capacity

Type of laboratory model	Testing capacity (per 8-hour shift)
Truenat laboratory (Quattro system)	Up to 32 samples per system
CBNAAT laboratory (GeneXpert system)	Up to 32 samples per system
Open RT-PCR system ± CBNAAT/Truenat	
Type 1 a	Up to 200 samples
Type 1 b	200–500 samples
Type 2 a	500–1000 samples
Type 2 b	1000–2000 samples
Type 3	2000–5000 samples
Type 4 (referral laboratory)	5000–10 000 samples
Mobile laboratory (Quattro Truenat system)	4 samples per hour per Truenat system

Laboratory Layouts

The space and design requirements of a laboratory will vary according to the type of molecular testing platform used and testing capacity of the laboratory

Floorplan of a laboratory for COVID-19 testing using the CBNAAT (GeneXpert) system



Area Required (in SQFT)

Total area: 230-250
1.Sample Processing Room : 40
2.Amplification Room: 60
3.Donning Room: 20
4.Doffing Room:25
5.Autoclave Room:30
6.Reporting+Staff Room:50

Equipment Details and dimensions (In Feet(')& Inches(")(LengthxWidthxHeight)):

BSC: Class II A2 Biosafety Cabinet (2'x2'x4')
Ref: Refrigerator (2'x2'3"x5'6")
CN: CBNAAT (GeneXpert® Systems)(11"x11"x1')
DC: Desktop Computer (2'3"x2'9")
UPS: 2 KVA Online UPS with atleast 2 hr backup
VA: Vertical Autoclave(2'6"x2'2"x47")
PC: Personal Computer(2'3"x2'9")
SA: Split AC(1TR)(3'6"x2'x2'5")

Others:

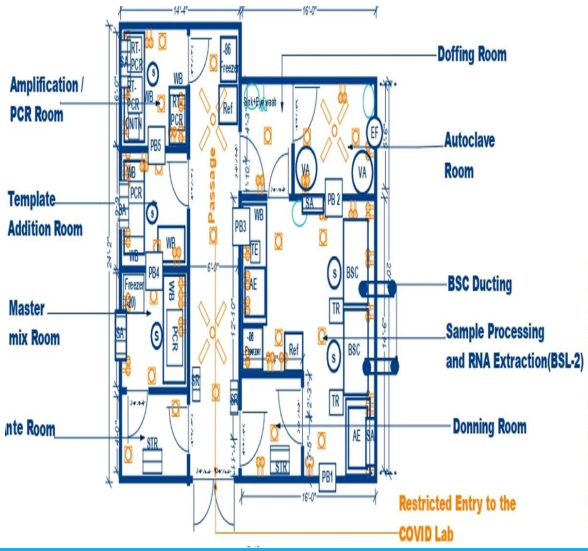
WB: Work bench
C: Laboratory chair
S: Laboratory Stool
TR: Trolley
STR: Storage Rack
SR: Shoe Rack
PB1: Pass box for passage of sample
PB2: Pass box for passage of waste discard
EF: Exhaust Fan

Legends:

- Ceiling Lights
- 15/5 Amp Single phase electrical Switch & Socket
- Ceiling Fan
- Hands Free Wash Basin
- SA: Split Air Conditioner
- Sliding Door

Laboratory Layouts – Contd…

Floorplan of an open RT-PCR system laboratory for COVID-19 testing – type 2 (500–2000 samples)



Area Required (in SQFT)

Total area: 730-750

1.For Sample Processing and Automatic RNA Extraction Room:200
2.Donning Room: 35
3.Doffing Room:35
4.Autoclave Room:50
5.Template addition Room:60
6.Amplification/PCR Room:50
7.Master Mix Room:45
8.Ante Room of MMX Room:30

Equipment Details & Dimensions
(In Feet(')& Inches(")(LengthxWidthxHeight)):

BSC: Class 2 A2 Biosafety Cabinet(4'x2'6"x7')
AE: Automatic RNA Extraction machine(2'6"x2'6"x3')
VA: Vertical Autoclave(2'6"x2'2"x4'7")
-86 Freezer(2'6"x2'6"x6')
-20 Freezer(2'x2'x5')
PCR: PCR Workstation/hood(2'x1'6"x2'3")
RT-PCR: Real Time PCR(2'x1'6"x1'6")
CN/TN:CBNAAT /True Nat(11"x1'x1')
TE: TrueNAT Extractor (11"x1'x1')
Ref: Refrigerator(2'x2'3"x5'6")
SA: Split AC(3'6"x2'x2'5")

Major items of equipment required for a COVID-19 testing laboratory

Type of laboratory	Testing capacity of the laboratory (per day)*	Biosafety cabinet#	Freezers*		Autoclaves$	RNA extraction and the number required		RT-PCR instrument modules with numbers required		Truenat	CBNAAT
			-86°C	-20°C*		Manual**	Automated				
								96 well	384 well		
Truenat laboratory (with one Truenat system)	Up to 32 samples	Small, 1	0	0	1	0	0	0	0	1	0
CBNAAT laboratory with one GeneXpert system with 4 modules	Up to 32 samples	Small, 1	1	-	1	-	-	-	-	-	1
RT-PCR*** ± CBNAAT/Truenat											
Type 1a	Up to 200 samples	Small, 1	1	1	1	1	-	1	0	+/-	+/-

Major items of equipment required for a COVID-19 testing laboratory contd…

Type 1b	200–500 samples	Small, 2	1	1	1	1	0/1	1	0	+/-	+/-
Type 2a	500–1000 samples	Large, 1	2	1	2	-	1	2	0	+/-	+/-
Type 2b	1000–2000 samples	Large, 2	2	1	2	-	2	3	0	+/-	+/-
Type 3	2000–5000 samples	Small, 1 large, 2	3	1	2	-	3	3	1	+/-	+/-
Type 4 (Referral laboratory)	5000–10 000 samples	Large, 3	4	1	2	-	4	3	3	+/-	+/-
Mobile Laboratory** **with three Truenat systems**	12 samples per hour	Small, 1	-	-	-	-	-	-	-	3	0/1

*For small facilities with Truenat or CBNAAT, a refrigerator– freezer is recommended in place of a dedicated freezer for the storage of samples. Also, for small healthcare facilities where long-term storage is not feasible, samples should be transported to a higher-level facility where a -86°C freezer is available.

#Class 2 Type A2; small 2 × 2 × 2 feet; large, 4 × 2 × 2 feet.

Biosafety and Waste Management Protocols for the COVID-19 laboratory (Page Nos: **83 -128)**

- Risk assessment and Risk group classification
- Exposure, and general safety aspects including electrical, chemical and fire safety
- Biosafety level requirement for a molecular testing laboratory with details on
  - PPE
  - BSC
- Training of Laboratory Personnel
- Laboratory workflow process – Dos and Don'ts
- Decontamination and Disinfection Protocols
- Biomedical Waste management protocols including autoclave usage
- Maintenance of equipment's and Reagent storage details

Sample collection, registration, transportation and storage, report management, including sample management for whole genome sequencing (Page Nos: 129 -166)

Sources of Various samples that can be used with

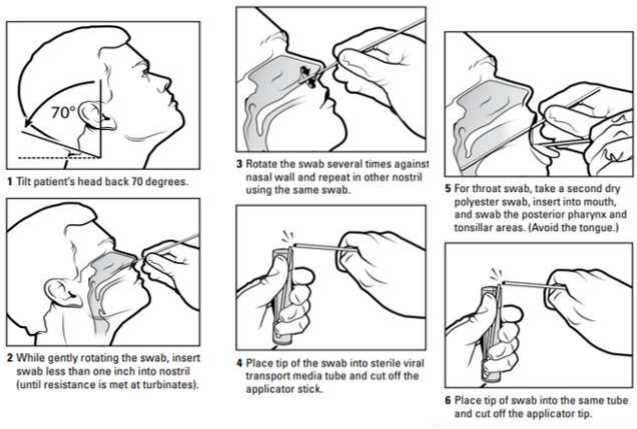


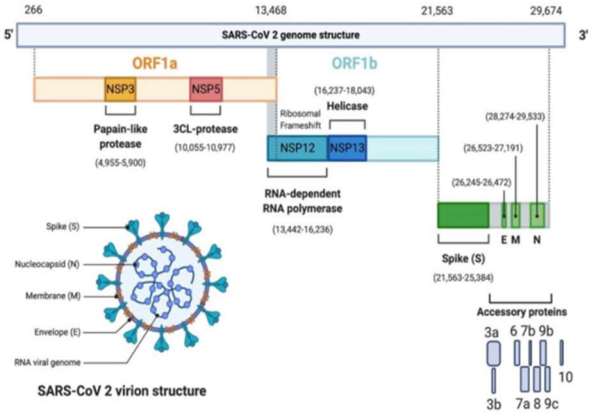
Table 2: The recommended temperatures for transportation and storage of various types of specimens for SARS-CoV-2 testing[6]

Specimen type	Collection material	Recommended temperature during transportation	Recommended temperature during storage in the laboratory	Comments
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs*	4°C	≤5 days: 4°C >5 days: –70°C	Nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load
Bronchoalveolar lavage	Sterile container*	4°C	≤48 hours: 4°C >48 hours: –70°C	There may be some dilution of pathogens, however, it can be considered as a valid specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	Sterile container*	4°C	≤48 hours: 4°C >48 hours: –70°C	-
Sputum	Sterile container*	4°C	≤48 hours: 4°C >48 hours: –70°C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	Sterile container with saline	4°C	≤24 hours: 4°C >24 hours: –70°C	Autopsy sample collection preferably to be avoided
Serum (two samples – acute and convalescent)	Serum separator tubes (adults: collect 3–5 mL whole blood)	4°C	≤5 days: 4°C >5 days: –70°C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later

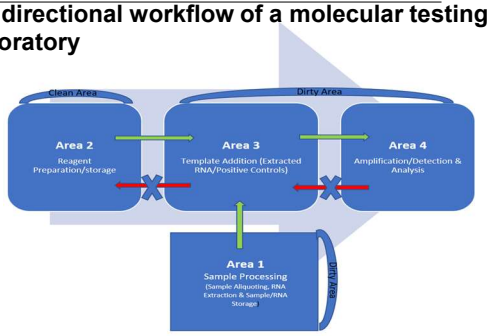
*For transport of samples for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements avoid repeated freezing and thawing of specimens.

Testing Protocols for RT-PCR Open Systems (Page Nos: 168 – 197)

The genomic organization of SARS-CoV-2[2]



Unidirectional workflow of a molecular testing laboratory



Overview of COVID-19 RT-PCR testing procedures

Testing Protocols for CBNAAT (Page Nos: 200 – 210)

Figure 6. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

Table 1: Possible results from a CBNAAT[14]

Result displayed	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+	+/-
	+	-	
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-
ERROR	NO RESULT	NO RESULT	NO RESULT
No Result	NO RESULT	NO RESULT	NO RESULT

Table 3: GeneXpert instruments and their respective capacities[3]

Instrument configuration	Number of modules	Capacity per 8-hour shift/day*
GeneXpert GX-IV	4	32
GeneXpert GX-XVI	8	64
GeneXpert GX-XVI	16	128
GeneXpert GX-INF 48s	48	384
GeneXpert GX-INF 80	80	640

*The reference document provides the capacity of GeneXpert systems for 24 hours; however for this document the capacities have been extrapolated to 8 hours per day.

Testing Protocols for Truenat(Page Nos: 212- 247)

Figure 1: A Truelab Real-Time PCR workstation field case

Trueprep AUTO Universal Cartridge Based Sample Prep Device

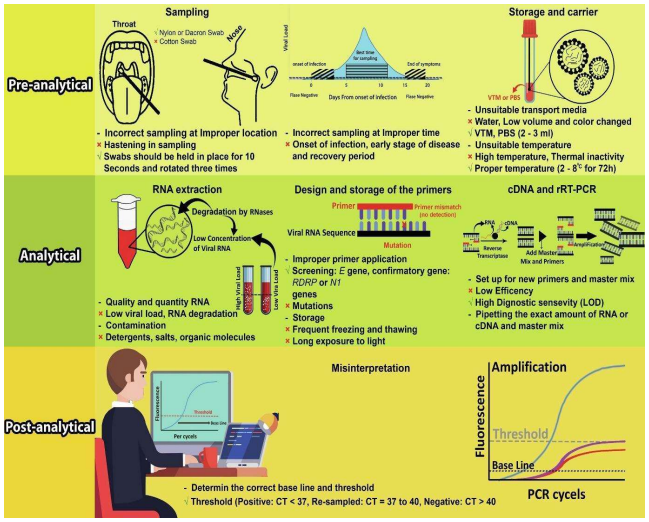
Truelab micro PCR Analyzer Available with 1, 2 or 4 chip ports

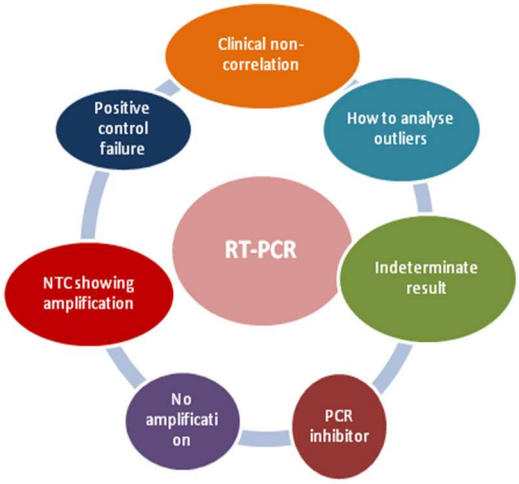
Truelab micro PCR printer

Table 1: Number of tests performed by different types of Truelab analyzer

Type of Truelab micro PCR analyzer	Tests/8 hours
Truelab Uno Dx	10–12 tests
Truelab Duo	20–24 tests
Truelab Quattro	40–48 tests

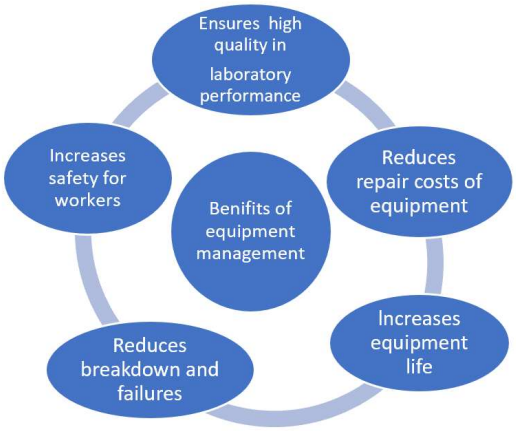
Troubleshooting COVID-19 testing (Page Nos: **249 – 259)**





Equipment Management (Page Nos: 260 -265)

The benefits of good equipment management



Elements of equipment management →

- Selection and purchasing
- Equipment inventory
- Installation
- Calibration and performance evaluation
- Maintenance
- Troubleshooting
- Service and repair
- Retiring and disposing

COVID-19 Laboratory Assessment Tools - Audit and Accreditation (Page Nos: **266 – 315)**

- Audit mechanism
- Critical points for audit preparation
- Documents required during an audit
- Laboratory accreditation
- Laboratory Assessment Checklist

COVID-19 Laboratory Assessment Tools - Audit and

Annexure 15: WHO laboratory assessment tool

Source: https://www.who.int/publications-detail-redirect/laboratory-assessment-tool-for-laboratories-implementing-covid-19-virus-testing[3]

Laboratory identification

Country	
Region/Province/District	
Name of the laboratory	
Address Telephone	
Fax	
E-mail	
Name of the laboratory director	
Qualification and contact details of the laboratory director	

Date of the assessment (DD/MM/YYYY)	
Name of the assessor/s	
Contact details of the assessor/s	
Name of the responding person/s	

5. Consumable and Reagent Management:

Procurement

5.1	Is there a responsible staff for consumable and reagent management (inventory, order, etc.)?			
5.2	Does the laboratory experience problems with reagent delivery like delays, temperature not adequate, reference error, etc. (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)?			

Inventory and storage

5.3	Is there an inventory system for consumables and reagents?	X		
5.4	Are consumables and reagents inspected upon receipt?	X		
5.5	Are consumables and reagents appropriately stored (temperature, humidity, etc.) with storage conditions monitored (thermometer, etc.)?			

Reaching Impact, Saturation, and Epidemic Control (RISE)

Tool Kit Chapters Details on…..

- The ICMR guidelines and relevant videos are provided as references and links in every chapter
- Details on the trouble shooting of RT-PCR testing with root cause and probable reasons and corrections are provided with examples
- A complete list of laboratory check lists for all aspects for audit readiness is also included as the chapter in the manual
- Apart from these the log sheets and other relevant details are provided as annexures with SOPs (Page Nos: 316 – 341)

Trainer's Script

Title: Demonstration and hands on training on the use of BSC in the COVID-19 molecular diagnostics laboratory.

Purpose: This document describes demonstrations and hands on exercises for safe work practices inside the BSC. The key steps performed inside the BSC during COVID-19 specimen aliquoting are demonstrated and practiced.

Trainee Learning Objectives:

To operate and use the BSC correctly

To carry out the procedure inside the BSC safely

To learn the correct procedure for handling the infectious specimen and proper disposal of infectious waste arising from aliquoting procedures.

Title: Demonstration and hands on exercise: Safe practices in using class II biological safety cabinets for COVID specimen processing.

Reference documents:

- SOP for specimen aliquoting
- SOP for BSC use & maintenance

Equipment & material: Refer to check list provided in SOP for specimen processing

Equipment

- Certified Biosafety Cabinet Class II Type A2
- Smoke/fume tester
- Vortex mixer
- -70°C/ -80°C (with free space for sample storage)
- 4°C Refrigerator
- Tabletop refrigerated centrifuge

Consumables

- Sterile Transfer pipettes
- Absorbent liner
- Cryovials
- Biohazard bags

- Markers
- Twist tag (to tie the bags)

Personal Protecting Equipment

- Coverall/ Gowns
- Gloves
- Goggles
- Shoe covers
- Head covers

Disinfectants

- Ethanol
- Sodium Hypochlorite stock solution

Updated Logbooks

- BSC use logbook
- Maintenance logbook
- Centrifuge use logbook

Mock Specimens

- 10 (or more) VTM tubes with appropriate labeling

Items to be provided to the trainee beforehand

- SOP for specimen processing
- SOP for BSC cabinet use

Instructions to the trainer:

- Please read the SOP and trainer script before hand
- Prepare for demonstration by collecting all the required material
- Ensure you have the space and time for the number of trainee in your demonstration
- Provide the SOP to the trainee and ensure its review by the trainee before the start of exercise
- Provide safety guidelines in case of adverse events such as chemical spills etc., ensure that is is read and understood by trainees.
- Ensure that trainees are aware of biomedical waste management procedures followed.

Trainer's Script

Demonstration and hands on training on the use of BSC for COVID-19 specimen aliquoting		
Training Steps	**Trainers Task**	**Trainer's observations**
Turning on the BSC	Trainer shows the correct order of turning on the BSC: • Correct sash level • Turn on the fan for 10 min • Magnahelic gauge reading and comparing with certification values. • Fume testing • Entering the details in the logbook	
Getting ready to work	**Task: setting up the work area** • Assigns a trainee to set up the work area. Observes, whether following practices followed: o Checklist consulted o Disinfection carried out as per the protocol o Solid and liquid biohazard waste collection o Clean to dirty work area division achieved • Comments on the right vs wrong practice	
Working inside the BSC - Centrifugation of VTM tubes	**Task: How to centrifuge VTM tubes** • Trainer demonstrates how VTM tubes are loaded into the centrifuge. • Trainer emphasizes on balance of the rotor • Trainer emphasizes on decontamination of the centrifuge bucket	

Working inside the BSC – Aliquoting specimen to cryovials	**Task: Aliquoting the specimen into cryovials** • Trainer demonstrates the right practice to aliquote atleast 4-5 samples (mock) • Trainer assigns minimum two samples per trainee todemonstrate aliquoting • Focus on pipetting techniques to avoid crosscontamination and aerosol generation • Focus on discarding the used Pasteur pipette and VTMtubes Comments on right vs wrong practice	
Working inside the BSC – Cleaning the BSC post work	**Task: Cleaning the work area after work completion** • Trainer assigns trainees to perform: • Cleaning of the work area • Removing solid and liquid waste from the BSC • Final surface cleaning • Transfer of cryovials to the freezer/refrigeratorDiscusses right vs wrong practice	
Working inside the BSC – Transfer of cryovials to refrigerator freezer	**Task: Storing the specimen and shut down of BSC.** • Demonstrates correct storage procedure • Cleaning up the laboratory before leaving • Turning off BSC • Documenting lab activity • Exiting the Lab	

Trainer's Script

Title: Micropipetting for PCR

Purpose: This document describes demonstrations and hands on exercises on the correct use of micropipettes in a PCR based diagnostics laboraotry.

Equipment & material:

Micropipettes:

- Certified micropipettes: (at least 2 sets for 10 trainees)
- 1-20 µl
- 200 µl
- 1000 µl
- Micropipettes out of use (?)
- Multichannel pipettes

Other consumables:

- Tube racks
- PCR tubes/96 well plates
- Plate covers
- Plate sealers
- 1.5 ml centrifuge tubes
- Tips for all the micropipettes
- Troughs for multichannel plates
- 96 well RNA extraction plate
- Precision balance (accuracy up to 10 mg)
- Paper boats
- Distilled water aliquots
- Notebook
- Marker
- Pens

Disinfectants:

- 1% sodium hypochlorite
- 70%Ethanol

Mock Reagents

- Nuclease free water
- 80% glycerol
- Dye solution

Reference material for the trainer:

- Micropipetting:
- https://www.youtube.com/watch?v=VEkfBStZSNc (reverse pipetting)
- https://www.youtube.com/watch?v=1Y0U9jf5ZbI (reverse pipetting multichannel)

- https://www.youtube.com/watch?v=QGX490kuKjg
- https://www.youtube.com/watch?v=uEy_NGDfo_8&t=195s
- Sealing the thermocycler plate
- https://www.youtube.com/watch?v=25OOn6W5gU0&t=10s

Instructions to the trainer:

- Please read the script before hand
- Prepare for demonstration by collecting all the required material beforehand and setting up thedemonstration area
- Practice your training to get an idea on time requirements and preparation gaps
- Ensure you have the space and time for the number of trainees in your demonstration

Trainer's Script

Demonstration and hands on exercises on the use of micropipettes in PCR based diagnosticlaboratory		
Training Steps	**Trainer's Task**	**Trainer's observations**
Introductio n& Learning objectives	• Describes the importance of correct pipetting techniques and theirmaintenance, especially calibration. • Informs about learning objectives of the training.	
Micropipette volume range – Calibration & Volume setting	• Shows types of micropipettes, describes their volume range. • Shows the certification label on the pipettes (or the calibrationrecord). • Shows how to correctly hold the pipette • Shows how to correctly set the volume and attach tips for eachmicropipette	
Aspiration and dispensing liquids correctly	• Shows how to aspirate the liquids • Angle of aspiration • How much to dip the tip in the liquid (immersion depth) duringaspiration • How to do reverse pipetting • How to dispense • How to eject the tip into the wastebin	
Measuring consistency of repeat pipetting- A	• Shows the accuracy of repeat measurement by carrying outrepeat pipetting of a dye solution on a piece of paraffin/wax paper. (Use 5-10-20 volume for this exercise). • Repeated volumes are visually inspection of the size of the dye-drops is carried out to show consistency of repeated pipetting. • Asks the trainee to test their pipetting skills in this way	
Measuring pipetting accuracy	• Shows the accuracy of measurement by carrying out repeatpipetting of distilled water on a paper boat. (Use 10-20 ul volume for this exercise). • Repeated pipette volumes are noted on the paper andcompared later for accuracy. • Asks the trainee to test their pipetting skills in this way, oncethey return to their labs	

Using Multichannel pipettes	• Shows proper way of attaching tips • Using troughs for dispensing molecular grade reagents • Reverse pipetting • Dispensing liquids to 96 well plates, avoiding bubbles • Common issues with multichannel pipettes	
Pipetting PCR reaction mixes in 96 well plates	• Shows how to load the PCR reaction in a 96 well plate • How to seal the plate • Shows how to use PCR tubes • How to cap them • Shows how to spin the 96 well plates/tubes • Look for air bubbles and how to get rid of them	

Trainer's Script

Title: Demonstration and hands on training on the steps performed in the RTPCR assay in a COVID-19 molecular diagnostics laboratory.

Purpose: This document describes the various steps that are followed in the RTPCRassay for COVID- 19 testing.

Trainee Learning Objectives:

To carry out the RT-PCR assay for the VTM samples and interpret the results

To understand the need for the Quality Control (QC), External Quality Assurance (EQA)and proficiency testing (PT)

To demonstrate the internal controls usage and the inclusion of known positive andnegative controls for every individual testing

To learn the corrective actions in case of QC failures

Duration of Training:

Presentation – 1hr and 30 minutes

Hands on Training – 3 hrs

Reference documents:

- SOP for RNA Extraction
- SOP for RT-PCR
- Equipment & material: Refer to check list provided in SOP for RNA Extraction and RT-PCR

Equipment

- Certified Biosafety Cabinet Class II Type A2
- Vortex mixer
- -70°C/ -80°C (with free space for sample storage)
- -20°C Freezers
- 4°C Refrigerator
- Tabletop refrigerated centrifuge
- Spinner
- Pipettes-1000ul, 200ul, 100ul, 10ul
- 1.5ml Centrifuge tubes
- 2ml screwcap tubes
- Auto plates and spin tips (Automated Extraction)
- Spin tips (Automated Extraction)
- Real Time PCR Instrument

'Consumables

- Sterile Transfer pipettes

- Absorbent liner
- Cryovials
- Biohazard bags
- Markers
- Twist tag (to tie the bags)
- Kits for Extraction and PCR

Personal Protecting Equipment

- Coverall/ Gowns
- Gloves
- Goggles
- Shoe covers
- Head covers

Disinfectants

- Ethanol
- Sodium Hypochlorite stock solution

Updated Logbooks

- BSC use logbook
- Maintenance logbook
- Centrifuge use logbook
- Instrument use logbook
- Worksheets for sample entries

Mock Specimens

3 (or more) VTM tubes with appropriate labeling

Items to be provided to the trainee beforehand

- SOP for Viral RNA Extraction
- SOP for RTPCR

Instructions to the trainer:

- Please read the SOPs, QA plan and trainer script before hand
- Prepare for demonstration by collecting all the required material
- Ensure you have the space and time for the number of trainees in your demonstration
- Provide the SOP to the trainee and ensure its review by the trainee before the start of exercise
- Provide safety guidelines in case of adverse events such as chemical spills etc., ensure that is read and understood by trainees.
- Ensure that trainees are aware of biomedical waste management procedures followed.

Trainer's Script

Demonstration and hands on training on the RTPCR assay for COVID-19 testing		
Training Steps	**Trainer's Task**	**Trainer's observations**
Getting ready the Lab to work	**Task: Setting up the work area** o Assigns a trainee to set up the workarea. Observes, whether following practices followed: o Checklist entry and cross checked o Check reagents date and expiry o Check and arrange the VTM samplesand cross check with the lab numbers o Proper labelling of the vials and entering the respective worksheets o Ensure the internal controls are aliquoted and stored in appropriatetemperature as per the SOP o Ensures the known samples are aliquoted and in required amount forthe assay o Ensures proper biosafety practicesincluding PPE are in place o The instruments and the pipettes arecalibrated and proper check is done before starting the assay Entering the details in the logbook Comments on the right vs wrong practice	
Working on the Viral RNA Extraction Protocol	**Task: How to carry out the Viral RNA Extraction** • Trainer demonstrates how the RNA extraction is carried out stepwise emphasizing on the protocol with at least 3 samples and kit controls(mock) • Trainer ensures that the extraction is carried out in the room designated	

	for Extraction. • Trainer emphasizes on the importance of proper usage of pipettes and how to avoid cross contaminations • Trainer emphasizes on proper labelling and storage of the extracted RNA • Trainer demonstrates the importance of cross check signatures during each and every step • Trainer ensures that the internal controls and the positive and the negative controls provided in the kit are added as per the kit instructions along with the samples • Trainer also ensures that the Comments on right Vs wrong practice	
Working on the Setting up of the RTPCR Protocol	**Task: Storage and transport of the RNA samples into cryovials** • Trainer assigns minimum 3 samples per trainee to demonstrate the aliquoting and preparation of master mixes • Focus on pipetting techniques to avoid cross contamination and aerosol generation • Trainer ensures that the internal controls and the positive and the negative controls provided in the kit are added as per the kit instructions along with the samples • Focus on the setting up of PCR and initiate the run • Focus on the return the RNA samples and master mix reagents back to the proper temperature storage • Emphasize on the cleaning of the work area and pipettes after work completion • A known positive and a known negative sample are added in the	

	assay along with the unknown samples as a part of the IQC procedures. • Demonstrates the quality control indicators • Comments on right vs wrong practice	
Working on the Interpretation	**Task: Interpretation of the RTPCR Results** • Trainer assigns trainees to perform: • Checking if the positive and the negative controls have worked as expected • Ensures the Known control samples results are as expected • Ensures all the results are cross verified • Interpret the results of the samples as per the SOP • Document and release the reports if the controls are fine • Discusses right vs wrong practice	
ICMR designated ILQC Testing	**Task: ILQC Testing (ICMR)** • Ensures the ILQC testing with the ICMR designated Lab (RMRC, Bhubaneshwar) is done every quarterly as per the ICMR guidelines • Ensures 5 positive and 5 negative samples that are already tested earlier in the week are selected for ILQC testing and stored at appropriate temperature. • Ensures the samples with a higher ct Values and the ones with a ct value of not detected are selected for the positive samples and the negative samples respectively. • Ensures the proper aliquoting, storage and the transport of the samples to the designated lab • Demonstrates the proper entry of the reports on the ICMR QC portal	

	Ensures proper documentation of the ILQC tests	
External Quality Control	**Task: How to process and report the EQA Results** • Demonstrates the ILC protocol as part ofthe EQAS and the samples are tested yearly once. • Ensures the Inter Laboratory Comparison samples are processed as per the unknown samples • Ensures the ILC samples are tested as per the protocol along with the internal controlsprovided with the kit • Ensures the reports are interpreted anduploaded as per the requirement Samples are stored appropriately once thetesting is completed	
Working on the Trouble shooting Aspects	**Task: Trouble Shooting of RTPCR Assay** • Trainer assigns 2 reports per trainee to demonstrate the same • Trainer to focus on the reports of the controls and instruct the action to be taken	

Trainer's Script

Title: Demonstration and hands on training on the steps performed in the CBNAAT assay in a COVID-19 molecular diagnostics laboratory.

Purpose: This document describes the various steps that are followed in the CBNAATplatforms (**Cepheid Xpert Xpress SARS CoV-2 system)** for COVID- 19 testing.

Trainee Learning Objectives:

To carry out the CBNAAT assay for the nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/aspirate specimens and interpret the results

To understand the need for the Quality Control (QC), External Quality Assurance (EQA)and proficiency testing (PT)

To demonstrate the internal controls usage and the inclusion of known positive and negative controls for every individual testing

To learn the corrective actions in case of QC failures

Duration of the Training:

Presentation – 45 minutes

Hands on – 1 hr

Interpretation and Results Discussion – 15mins

Reference documents:

- SOP for CBNAAT systems
- Equipment & material: Refer to check list provided in SOP
- Checklist for Protocol
- SOP for Biomedical Waste Protocol

Equipment

- Certified Biosafety Cabinet Class II Type A2
- Vortex mixer
- -70°C/ -80°C (with free space for sample storage)
- 4°C Refrigerator
- Spinner
- GeneXpert Instrument Systems

Consumables

- Xpert Xpress SARS-CoV-2 kit
- Disposable Transfer Pipettes
- Absorbent liner
- Biohazard bags

- Markers
- Twist tag (to tie the bags)

Personal Protecting Equipment

- Coverall/ Gowns
- Gloves
- Goggles
- Shoe covers
- Head covers

Disinfectants

- Ethanol
- Sodium Hypochlorite stock solution

Updated Logbooks

- BSC use logbook
- Maintenance logbook
- Worksheets

Mock Specimens

Two unknown specimen along with the controls provided in the kit with appropriate labeling

Items to be provided to the trainee before hand

- SOP for CBNAAT Assay
- QA Manual

Instructions to the trainer

- Please read the SOPs and trainer script before hand
- Prepare for demonstration by collecting all the required material
- Ensure the checklist is filled before the start of the assay
- Ensure you have the space and time for the number of trainees in your demonstration
- Ensure on the importance of PPE- donning and doffing occurs in the correct place and all the trainees are aware of the same.
- Ensure that the assay is carried in the BSC in room designated for extraction.
- Provide the SOP to the trainee and ensure its review by the trainee before the start of exercise
- Provide safety guidelines in case of adverse events such as chemical spills etc., ensure that is read and understood by trainees.
- Ensure that trainees are aware of biomedical waste management procedures followed.

Trainer's Script

Demonstration and hands on training on the CBNAAT assay for COVID-19 testing		
Training Steps	**Trainer's Task**	**Trainer's observations**
Timelinefor the Assay	Time taken for 2 or 4 samples is 45 mins to 1hour	
Getting ready theLab to work	**Task: setting up the work area** o Assigns a trainee to set up the workarea. Observes, whether following practices followed: o Ensures proper biosafety practicesincluding PPE are in place o Checklist entry and cross checked o Check reagents and the cartridgeexpiry dates o Check and arrange the specimensand cross check with the lab numbers o Proper labelling of the cartridge andentering the respective worksheets o Ensure the internal controls are aliquoted and stored in appropriatetemperature as per the SOP o Proper check of the required instruments and the pipettes are calibrated and proper check is donebefore starting the assay Comments on the right vs wrong practice	

Workingon the Test	**Task: How to carry out the CBNAAT Assay** • Trainer demonstrates and emphasizes on the correct position of the cartridges and make sure the sample is added within the timeline as instructed in SOP. • Trainer to demonstrate the addition ofthe sample • Internal controls provided in the kit areadded as per protocol along with the samples in the correct positions • Trainer emphasizes on the importanceof proper volume to be dispensed. • Trainer demonstrates the instrumenthandling and proper labelling of the sample as per the kit instructions. • Trainer demonstrates the instrumenthandling and proper labelling of the sample as per the kit instructions.	
	• Trainer to demonstrate the importanceto monitor and check the module and the software version of the kit • Trainer demonstrates the disposal protocol and make sure the modules areproperly switched off after use. Comments on right Vs wrong practice	
Working on the Interpretation Results	**Task: Interpretation of the Results** • Trainer assigns trainees to perform: • Checking if the Sample Processing Control and the Probe Check Controlhave worked as expected • Interpret the results of the samples asper the SOP • Document and release the reports if thecontrols are fine Discusses right vs wrong practice	

ILQC Testing	**Task: ILQC Testing (ICMR)** • Ensures the ILQC testing with the ICMR designated Lab (RMRC, Bhubaneshwar)is done every quarterly as per the ICMR guidelines • Ensures 5 positive and 5 negative samples that are already tested earlier inthe week are selected for ILQC testing • Ensures the proper aliquoting, storage and the transport of the samples to thedesignated lab • Demonstrates the proper entry of thereports on the ICMR QC portal Ensures proper documentation of the ILQCtests	
External Quality Control	**Task: How to process and report the EQAResults** • Demonstrates the ILC protocol as part ofthe EQAS and the samples are tested yearly once. • Ensures the Inter Laboratory Comparison samples are processed asper the unknown samples • Ensures the ILC samples are tested as per the protocol along with the internal controls provided with the kit • Ensures the reports are interpreted anduploaded as per the requirement	
	• Samples are stored appropriately oncethe testing is completed	

Workingon the Trouble shooting Aspects	**Task: Trouble Shooting** Trainer assigns 2 reports per trainee todemonstrate the same • Trainer to focus on the error and invalid reports and demonstrate the action to betaken • Trainer to demonstrate the limitation ofthe assay and the retest criteria as perthe SOP • Demonstrates the root cause analysis for the failed assay and completes theCAPA for the same	

Trainer's Script

Title: Demonstration and hands on training on the steps performed in the TrueNat assay in a COVID-19 molecular diagnostics laboratory.

Purpose: This document describes the various steps that are followed in the **TrueNat system** for COVID- 19 testing.

Trainee Learning Objectives:

To carry out the TrueNat assay for the nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/aspirate specimens and interpret the results.

To understand the need for the Quality Control (QC), External Quality Assurance (EQA)and proficiency testing (PT)

To demonstrate the internal controls usage and the inclusion of known positive and negative controls for every individual testing

To learn the corrective actions in case of QC failures

Duration of the Training

Presentation – 45
minutes

Hands on Training – 45 minutes to 1 hour

Reference documents:

- SOP for TrueNat Assay
- Equipment & material: Refer to check list provided in SOP
- Checklist for Protocol
- SOP for Biomedical Waste Protocol

Equipment

- Certified Biosafety Cabinet Class II Type A2
- Vortex mixer
- -70°C/ -80°C (with free space for sample storage)
- 4°C Refrigerator
- Spinner
- Molbio Truelab micro PCR Systems

Consumables

- TrueNat™ COVID-19 micro PCR chip
- Micro \tube with freeze dried RTPCR reagents
- Lysis Buffer
- Transport medium for swab specimens
- Disposable Transfer Pipettes
- Dessicant pouch

- Absorbent liner
- Biohazard bags
- Markers
- Twist tag (to tie the bags)

Personal Protecting Equipment

- Coverall/ Gowns
- Gloves
- Goggles
- Shoe covers
- Head covers

Disinfectants

- Ethanol
- Sodium Hypochlorite stock solution

Updated Logbooks

- BSC use logbook
- Maintenance logbook
- Worksheets

Mock Specimens

Two unknown specimens along with appropriate labeling

Items to be provided to the trainee before hand

- SOP for TrueNat Assay

Instructions to the trainer

- Please read the SOPs and trainer script before hand
- Prepare for demonstration by collecting all the required material
- Ensure the checklist is filled before the start of the assay
- Ensure you have the space and time for the number of trainees in your demonstration
- Ensure on the importance of PPE- donning and doffing occurs in the correct place and all the trainees are aware of the same.
- Ensure that the assay is carried in the BSC in room designated for the RNA Extractions.
- Provide the SOP to the trainee and ensure its review by the trainee before the start of exercise
- Provide safety guidelines in case of adverse events such as chemical spills etc., ensure that is read and understood by trainees.
- Ensure that trainees are aware of biomedical waste management procedures followed.

Trainer's Script

Demonstration and hands on training on the CBNAAT assay for COVID-19 testing		
Training Steps	**Trainer's Task**	**Trainer's observations**
Timeline for the assay	Time taken for 2 samples is 45 mins to 1 hour	
Getting ready to the Lab Work	**Task: setting up the work area** • Assigns a trainee to set up the work area.Observes, whether following practices followed: o Checklist entry and cross checked o Check reagents and the cartridgeexpiry dates o Check and arrange the specimens andcross check with the lab numbers o Proper labelling of the cartridge andentering the respective worksheets o Proper check of the requiredinstruments to be used • Comments on the right vs wrong practice	
Working on the Test	**Task: How to carry out the Trunat Assay** • Trainer demonstrates and emphasizes onthe correct position of the cartridges and make sure the sample is added within thetimeline as instructed in SOP. • Ensure that the positive and the negative controls provided in the TrueNat universal kit are added along with the samples in every run as per the SOP. • Trainer emphasizes on the importance ofproper volume to be dispensed.	

	• Trainer demonstrates the instrumenthandling and proper labelling of the sample as per the kit instructions. • Trainer to demonstrate the importance tomonitor and check the proper profile in the analyser and the chip is inserted in correct position • Trainer demonstrates the disposal protocol and make sure the system isproperly switched off after use. • Comments on right Vs wrong practice	
Working on the interpretation Results	**Task: Interpretation of the RTPCR Results** • Trainer assigns trainees to perform: • Checking if the Controls have worked asexpected • Interpret the results of the samples as perthe SOP • Document and release the reports if thecontrols are fine Discusses right vs wrong practice	
ICMR designated ILQC Testing	**Task: ILQC Testing (ICMR)** • Ensures the ILQC testing with the ICMR designated Lab (RMRC, Bhubaneshwar)is done every quarterly as per the ICMR guidelines • Ensures 5 positive and 5 negative samples that are already tested earlier inthe week are selected for ILQC testing • Ensures the proper aliquoting, storage and the transport of the samples to thedesignated lab • Demonstrates the proper entry of the reports on the ICMR QC portal Ensures proper documentation of the ILQC tests	

External Quality Control	**Task: How to process and report the EQAResults** • Demonstrates the ILC protocol as part ofthe EQAS and the samples are tested yearly once. • Ensures the Inter Laboratory Comparisonsamples are processed as per the unknown samples • Ensures the ILC samples are tested as per the protocol along with the internal controls provided with the kit • Ensures the reports are interpreted anduploaded as per the requirement • Samples are stored appropriately oncethe testing is completed	
Working on the troubleshooting aspects	**Task: Trouble Shooting** • Trainer assigns 2 reports per trainee todemonstrate the same • Trainer to focus on the error and invalid reports and demonstrate the action to betaken • Trainer to demonstrate the limitation of the assay and the retest criteria as per theSOP • Report the problem in case of controlsfailed • Demonstrates on the upload format for the QC and the EQA results Demonstrates the root cause analysis forthe failed assay and completes the CAPAfor the same	

Trainers Manual

1. **Biosafety Lab Training**
   - **i)** BSL2 Lab work Protocols
   - **ii)** Guidelines on Use of PPE
   - **iii)** Guidelines for Usage of BSC, Pipettes and All Instruments
   - **iv)** Biomedical Waste Management

2. **Laboratory Testing Protocol Training**

   i) Sample Reception and Registration

   (1) TRF Verification
   (2) Sample Acceptance and Rejection Criteria
   (3) Storage of Samples at appropriate temperature till testing is initiated

   ii) Sample Processing for RT-PCR

CBNAAT/TRUNAAT

(1) Processing of VTMs inside the BSC for CBNAAT (Room 1)
(2) Checking of Reagents and Consumables as per the Checklist
(3) Storing of VTMs after aliquoting as per the SOP
(4) Discarding of the samples as per the protocol
(5) Interpretation of the results
(6) Quality check with the internal controls tested
(7) Documentation

Real Time PCR

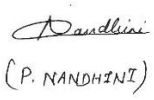
(1) Checking and aliquoting of the reagents and controls
(2) Processing the VTMs for RNA Extraction (Room 1)
(3) Storage of RNA at the appropriate temperature
(4) Processing of Extracted RNA for PCR (Room 2)
(5) Storing of Reagents and extracted RNA after testing
(6) Interpretation of the Results
(7) Quality check of the internal controls tested
(8) Trouble shooting

3. **Quality Control Procedures Training**

(1) Personnel Responsibilities
(2) Proficiency Testing
(3) Interlaboratory Comparison Testing
(4) Instrument Maintenance and Calibrations
(5) Competency Assessment
(6) Training for Laboratory technicians at regular intervals

4. **References**

FIND Because diagnosis matters	Specimen collection and transport of clinical specimens			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-001	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist		13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager		21 Jan 2022

Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
	Nil		

SOP Annual Review	Name, Title	Signature	Date
	Nil		

Distributed Copies to	Name (or location)	# of copies	Name (or location)	Date

1. Purpose

To describe the procedure of collection of clinical specimens and the transport from the sample collection centers to the diagnostic laboratory and Referral Labs for SARS-CoV-2 testing. Detection of viral RNA from clinical specimens is the key to the diagnosis of SARS-CoV-2 infection. The sample collected from the patient should contain viable microorganism with minimal contamination. Appropriate sample collection ensures quality laboratory test.

2. Responsibilities

It is the responsibility of the laboratory 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.

3. Pre-requisites

3.1 Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Hygiene requirements
- Donning and doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials
- Workflow in the laboratory
- Waste handling
- Importance of laboratory results for patient management

3.2 Equipment & materials

The process starts with sample collection, packaging and transport and these processes will require needs for performing the activities (Table 1). It is important to be prepared prior to performing the respective activity.

Table 1. Requirements for sample collection and transport

Biosafety	Appropriate PPE, waste disposal bags and disinfectants
Sample collection	Swabs, transport medium, suitable collection tools and containers
Sample labelling	Marker pens or barcodes
Triple packaging	Absorbent material, plastic bag, sturdy outer container, racks, cooler box, thermometer
Documentation	COVID-19 test request form, sample register, transport register form
Transportation	Regular schedule and method to move the samples to the laboratory, personnel trained in sample handling

4. PPE for specimen collection

- Long-sleeved isolation gown that fully cover your torso and extend from your neck to your knees and cover your arms to the end of your wrists.
- Head coverings, shoe covers or dedicated shoes
- N95 respirator that is secured properly and fits your face
- Eye protection (e.g. goggles or disposable full-face shield) that can be adjusted to fit your face
- Clean non-sterile disposable gloves that cover the wrist area of your isolation gown

5. Procedure

Recommended upper and lower respiratory specimens obtained from suspected SARS-CoV-2 infected individuals are collected in suitable transport medium and transported to the diagnostic laboratories in a triple layer packing at 2-8°C.

5.1 Patient preparation:

The first step in laboratory or diagnostic procedure is patient preparation or patient teaching before the performance of the procedure.

This pretesting explanation to the patient or caregiver follows essentially the same pattern for all sites and types of studies and includes the following:

5.1.1 Statement of the purpose of the study. The level of detail provided to patients about the test purpose depends on numerous factors and should be individualized appropriately in each particular setting.

5.1.2 Description of the procedure, including site and method. It is a good idea to explain to the patient that you will be wearing gloves throughout the procedure. The explanation should help the patient understand that the use of gloves is standard practice established for his or her protection as

well as yours. The collection procedure may require hand washing at the beginning and end of each specimen collection.

5.1.3 Description of the sensations, including discomfort and pain, that the patient may experience during the specimen collection procedure. Address concerns about pain related to the procedure and suggest breathing or visualization techniques to promote relaxation. For pediatric patients, the parents may be accompanied during the collection. Where appropriate, Sensitivity to cultural and social issues, as well as concern for modesty, is important in providing psychological support.

5.1.4 Instruction regarding pretesting preparations related to diet, liquids, medications, and activity as well as any restrictions regarding diet, liquids, medications, activity, known allergies, therapies, or other procedures that might affect test results. To increase patient compliance, the instructions should include an explanation of why strict adherence to the instructions is required.

5.1.5 6.5 Recognition of anxiety related to test results. Provide a compassionate, reassuring environment. Be prepared to educate the patient regarding access to the appropriate counselling services. Encourage the patient to ask questions and verbalize his or her concerns.

5.2 Sample Collection:

5.2.1 Nasopharyngeal swab:

- Tilt patient's head back 70 degrees
- Insert swab (sterile dacron/nylon flocked) into nostril (Swab should reach depth to distance
- from nostrils to outer opening of the ear
- Leave swab in place for several seconds to absorb secretions
- Slowly remove swab while rotating it
- Place tip of swab into VTM and snap/cut off the applicator stick

5.2.2 Oropharyngeal swab:

- Hold the tongue out of the way with a tongue depressor.
- Use a sweeping motion to swab posterior pharyngeal wall and tonsillar pillars
- Avoid swabbing soft palate and do not touch the tongue with swab tip.
- Put the swab in VTM
- The collection of combined nasopharyngeal and oropharyngeal swabs can improve test sensitivity

- If you collect both nasopharyngeal and oropharyngeal swabs, place them in the same tube

- Swabs should be transported in the viral transport medium. If transport medium is not available, use sterile saline

5.2.3 Nasopharyngeal wash/aspirate or nasal wash/aspirate (performed by a trained healthcare provider)

- Attach catheter to suction apparatus.

- Tilt patient's head back 70 degrees.

- Instill 1 mL-1.5 mL of non-bacteriostatic saline (pH 7.0) into one nostril.

- Insert the tubing into the nostril parallel to the palate (not upwards). Catheter should reach depth equal to distance from nostrils to outer opening of ear.

- Begin gentle suction/aspiration and remove catheter while rotating it gently.

- Place specimen in a sterile viral transport media tube.

5.2.4 Bronchoalveolar lavage, tracheal aspirate, pleural fluid (generally performed by a physician in the hospital setting)

- Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

- Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/or fatal cases.

5.2.5 Sputum (collected under the guidance of a trained healthcare professional)

- For patients who develop a productive cough, sputum can be collected and tested when available for SARS-CoV-2. However, the induction of sputum is not recommended.

- Educate the patient about the difference between sputum (deep cough) and oral secretions (saliva/spit).

- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap collection cup or sterile dry container.

5.3 Labelling:

Each sample should be clearly labelled with:

5.1.6 The patient's first and last name with consent

5.1.7 A unique barcode generated by the hospital for sample labeling or UHID number

5.1.8 The time and date of collection

5.1.9 The initials of the person-in-charge

5.4 Specimen storage:

The storage of the specimens are to be done as per the table.1 below

Table.1

Specimen collection details:

(Adapted from the WHO guidelines on 2019-nCoV):

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container*	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container*	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Not applicable
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	Autopsy sample collection preferably to be avoided
Serum (2 samples – acute and convalescent)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later

**For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens.*

Image: ICMR-NIV SOP for specimen, collection & transport (Reference 1)

5.5 Specimen Packaging and Transport:

Samples for COVID-19 testing must be triple packaged. Triple packaging protects the specimen from breaking or leaking in transit and prevents contamination of the courier and the environment if breakage/leakage does occur. The three layers that constitute triple packaging (i.e. primary receptacle, secondary and outer packaging) are shown in figures 1, 2 and 3.

5.5.1 Triple Packaging Procedure:

The packaging consists of three layers as follows.

- Primary receptacle: A labelled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

- Secondary receptacle: A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

- Outer shipping package: The secondary receptacle is placed in an outer shipping package which protects it and its content from outside influences such as physical damage and water while in transit.

Fig 1: Triple layer packaging

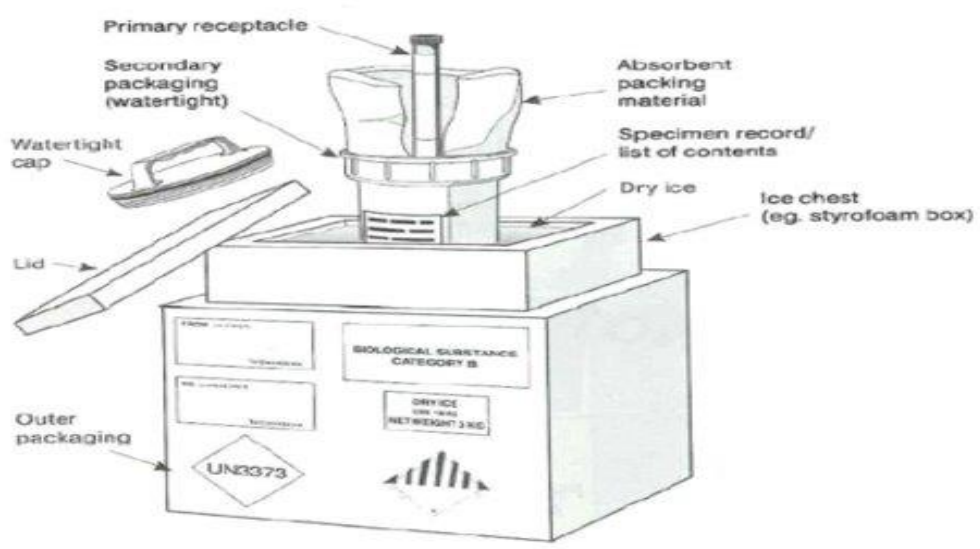


Fig 2: Procedure for Specimen Packaging and Transport – part 1

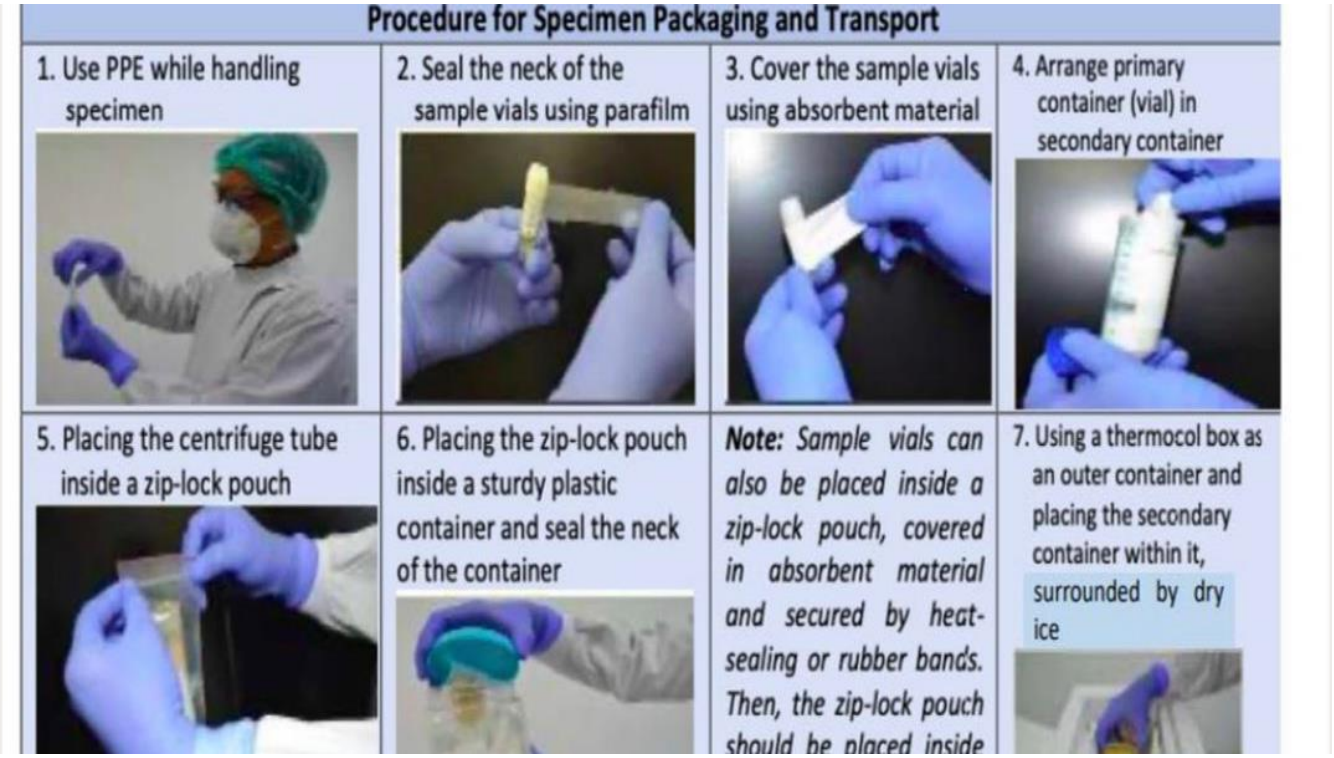
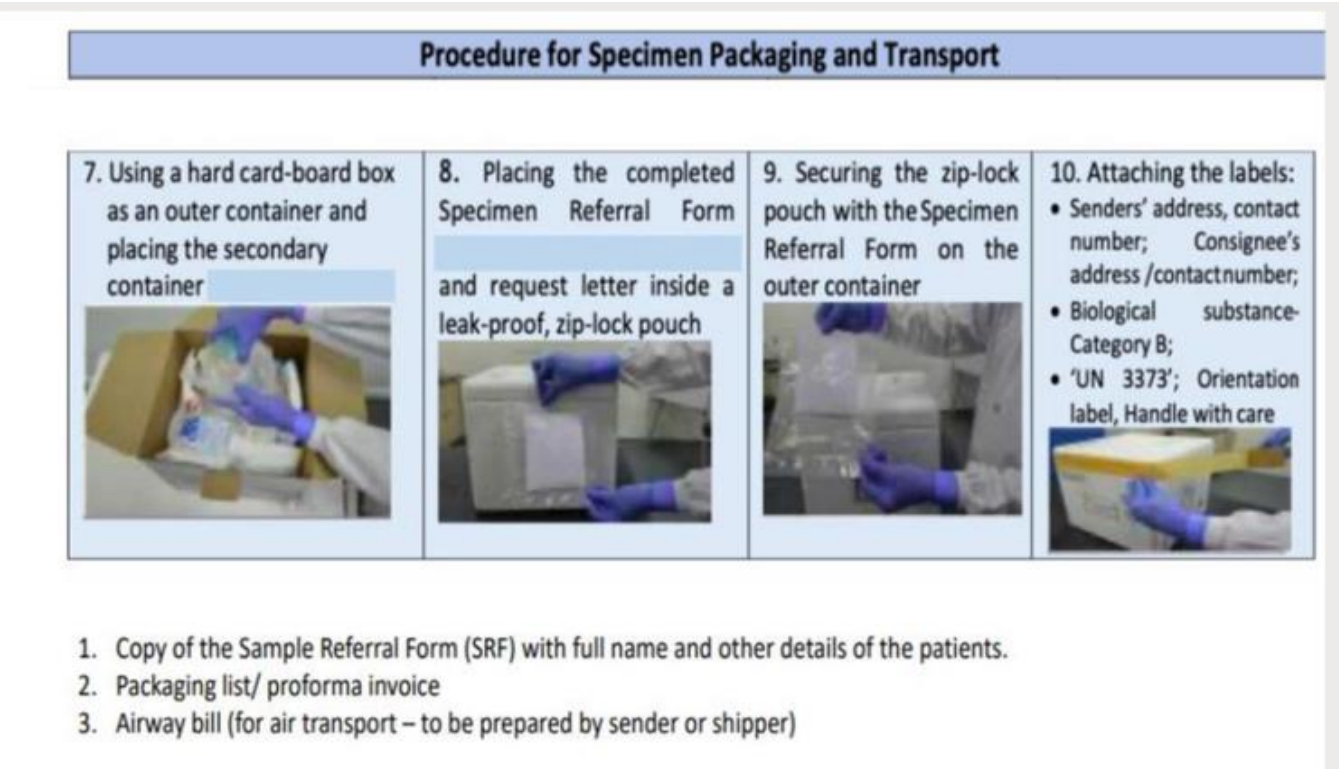


Fig 3: Procedure for Specimen Packaging and Transport – part 2



5.5.2 Packing and transport of Specimens for Whole Genome Sequencing (to Regional Genome Sequencing Laboratory (RGSL))

- Only those samples which are *positive for SARS-CoV-2 by RT PCR preferably with a Ct value of 25 or less* should be packaged & transported.
- After carrying out the RT-PCR test the remaining samples (within 72 hours of collection, stored at 2-8°), which are RT-PCR positive (Ct value <30), will be transported in VTM with cool pack (4-8 degree) or in ice.
- Alternatively, *remaining RNA samples may be stored and aliquoted in the 1.5 ml micro-centrifuge tubes followed by proper labelling and sealing with the parafilm (stored at -70degree).*
- *RNA placed together in plastic/ cardboard cryobox and packed in the thermocol box with dry ice should be shipped to the respective RGSL for sequencing*.
- Samples should be packaged and transported with all biosafety precautions and should be accompanied with line list and details of samples including the Ct values of all the target genes detected in standard triple packaging.
- Line list excel with the below details has to be accompanied along with the samples or RNA that are sent to RGSL (Fig.4)

Fig.4 Line list details to be sent for WGS laboratory

Name of the COVID-19 positive sample referral lab/health care facility:										
Date:										
Sr. No	SRF ID	Name	Age	Gender	Address	Patient Mobile	Type of Specimen	Date of collection of sample	Ct Value of all target genes detected by RTPCR Test for SARS-CoV-2	Status (Symptomatic / Asymptomatic)

6. Spill Management

Refer to the SOP on spill management

7. Waste Management

- All uninfected solid waste collected in the reception room should be discarded only in labelled discard bins.

- Removed PPE should be discarded in marked designated bins lined with biohazard bags.

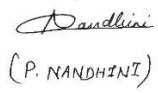
- Biohazard bags containing soiled PPEs should be handed over to the Autoclave Team

- Refer SOP on Biomedical Waste Management.

8. References:

1. ICMR-NIV SOP for specimen transport: https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20%202019-nCoV.pdf

FIND Because diagnosis matters	Specimen Registration and Report Management of Clinical Specimens			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-002	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist		13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager		21 Jan 2022

Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
	Nil		

SOP Annual Review	Name, Title	Signature	Date
	Nil		

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PURPOSE

The purpose of this document is to enable compliance with government guidelines for registration of COVID-19 specimens received and updating of the reports on the portal.

2. Introduction

At the onset of COVID-19 pandemic, the challenge was to standardize the format of sample data from various Tests conducted across the country. ICMR designed the Specimen Referral Form (SRF) for use with every COVID-19 sample; NIC developed the RT-PCR and RATI mobile apps on Android, iOS and Windows mobiles for ICMR along with the web-portal to whitelist phlebotomists (sample collectors) for using the mobile apps and web-portal. It is mandatory to fill this form for each and every sample being collected.

2.1 RTPCR APP

RT-PCR App is a handheld tool for the Medical Staff at Sample Collection Centres spread across the country. The Sample Collection Facility will be sending the sample for various type of specimen to ICMR labs conducting the RT-PCR test for confirmation of COVID-19. Advance intimation is being shared through the App with ICMR. On successful saving any sample, the Collection Centre and the User can view the collection details.

2.2 Data Reporting

The data management system covers RT-PCR, Rapid Antigen and Rapid Antibody tests.

Salient features of the COVID-19 Data Management System developed by NIC

- Robust, reliable Cloud infra, with redundancy, zero down-time and region-wise Databases with responsive portal and apps, tested for 50,000 concurrent users
- Single sign-on using Government official email IDs and Mobile number-based access for Labs (including Private), Collection Centres and Sample Collectors
- Extensive training material, videos, FAQs AVAILABLE
- SRF data accessible on real-time basis by ICMR Labs. Data Analytics, Auto Alert SMS/ Emails and GIS integrated
- Option to enter offline data after generation of SRF-ID
- Configurable- Skip Patient OTP for verification and warning on multiple use of same patient mobile
- RT-PCR, Rapid Antigen and Rapid Antibody tests covered
- Patient mobile and location details to help in tracking of patients and tracing of contacts

RESPONSIBILITY

Authorized specimen collection personnel, Data entry team and In-charge.

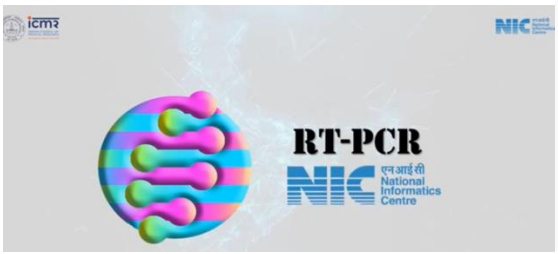
PROCEDURE

STEP WISE (WITH IMAGES) SOP FOR USING RTPCR APP

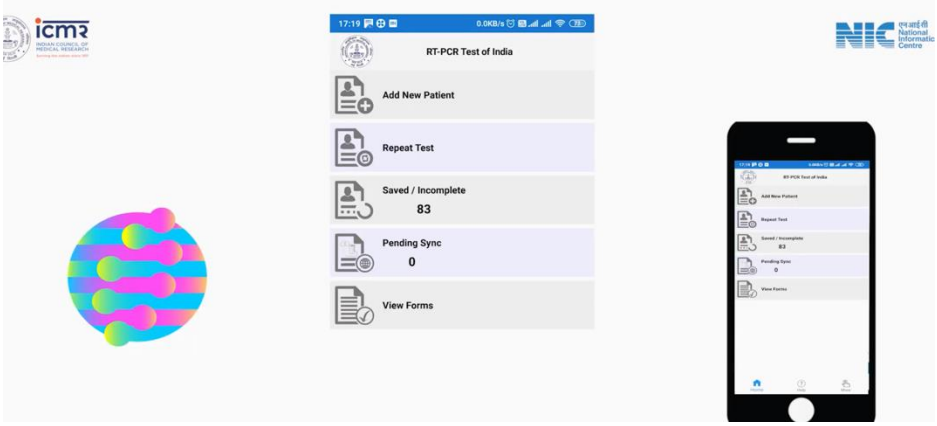
While using the app, please ensure your internet connection is active

A. REGISTERING A PATIENT FOR THE FIRST TIME

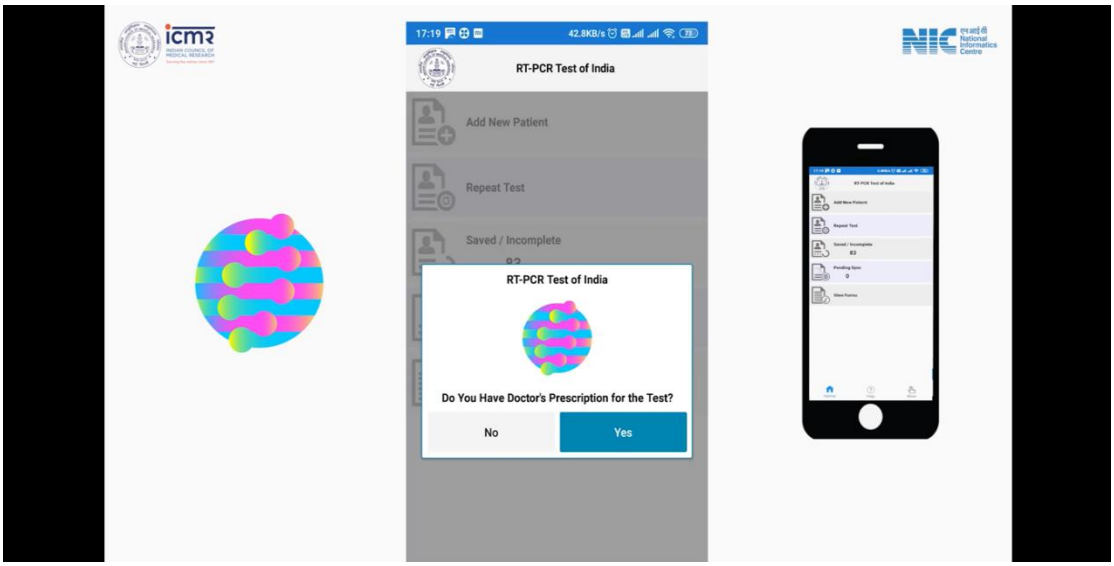
1. STEP 1: Download and install the app from the portal/playstore. Before accepting terms and conditions, check your mobile no. authorization by visiting over the portal



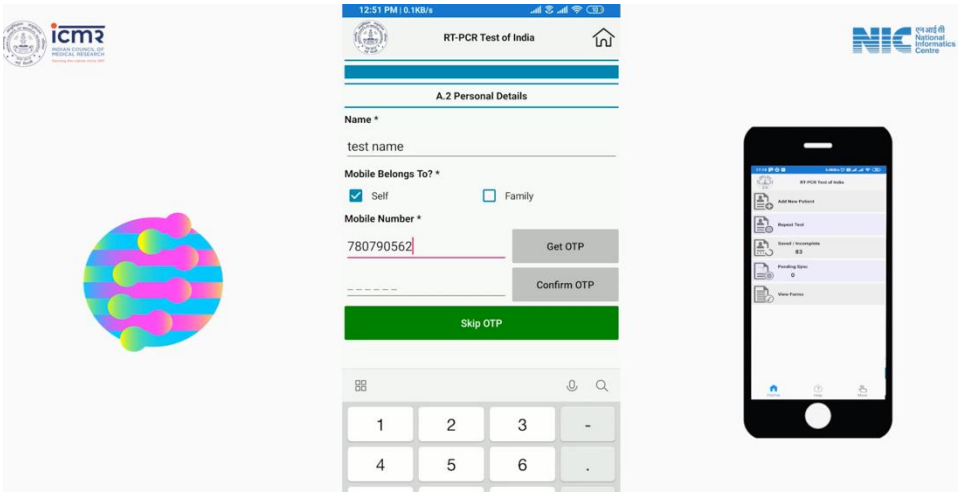
2. **STEP 2:** This app will now ask you to enter your mobile no. against which an OTP will be generated The very 1st menu at the top will ask you to add New Patient.

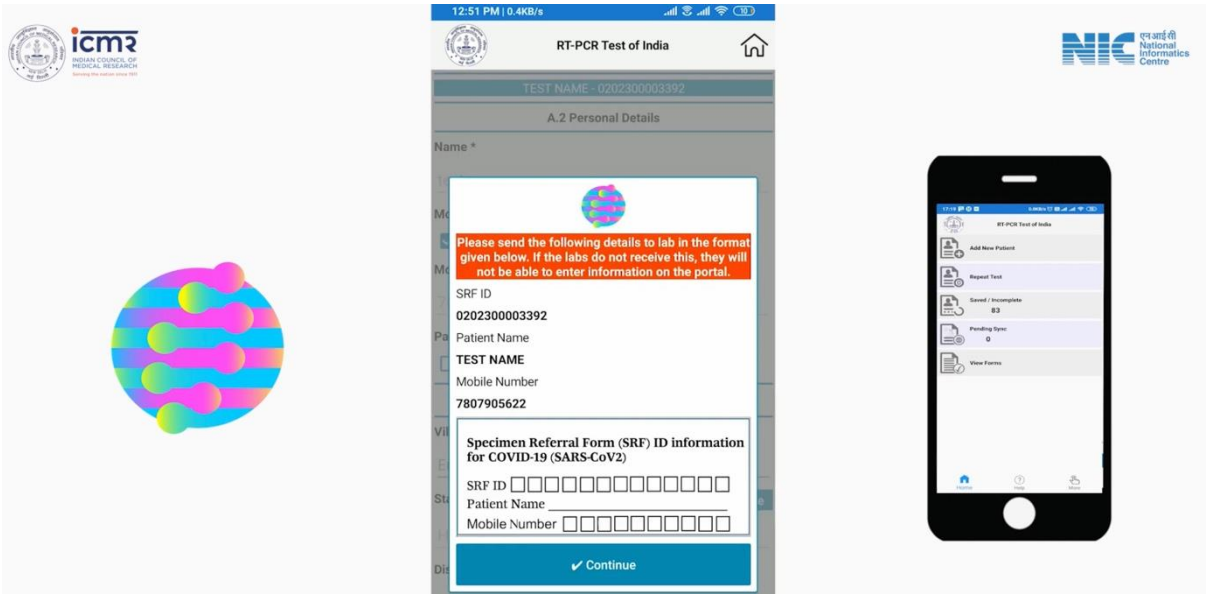


3. **STEP 3:** After adding a new patient the app will ask for Doctor's prescription (not a mandatory field anymore).



The app will then ask the name and mobile no. of the patient and OTP will be sent to patient's registered phone no. provided

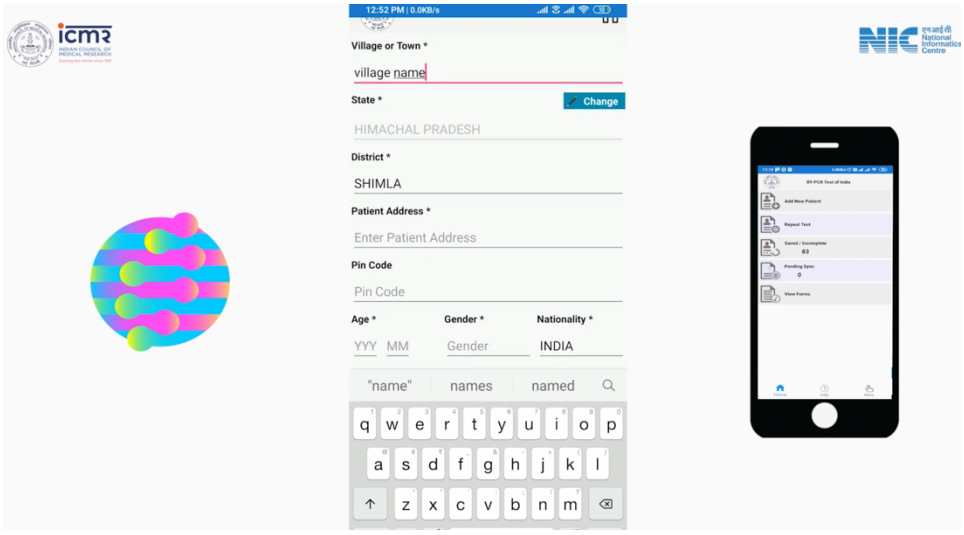


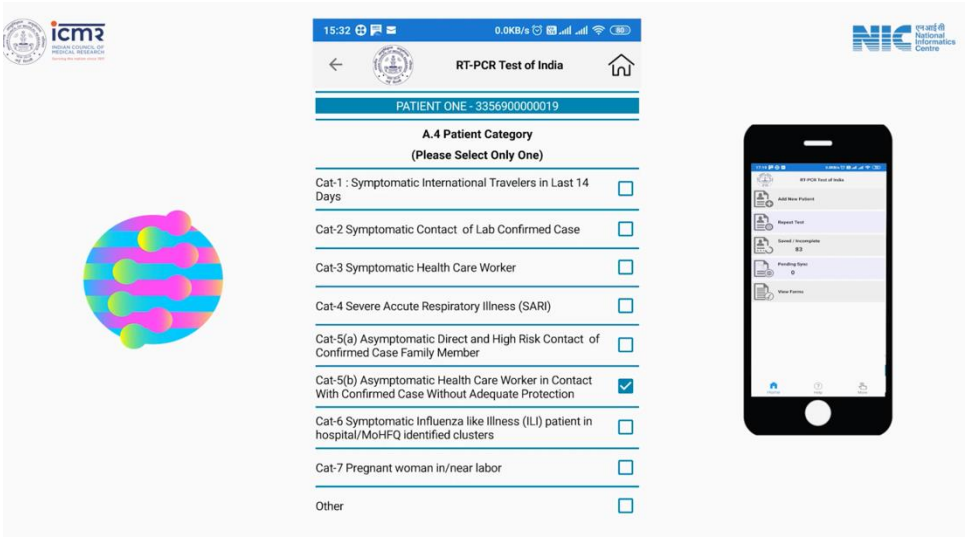


After validating the OTP partial form is saved and other sections can be filled immediately/later.

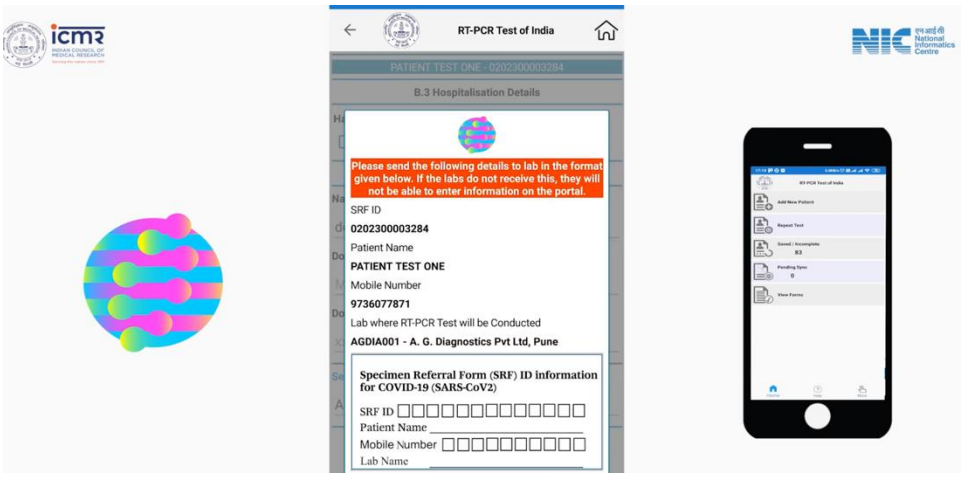
4. **STEP 4**

This app will ask you to enter personal details, patient credential and clinical presentation questions as per latest Specimen Referral form.

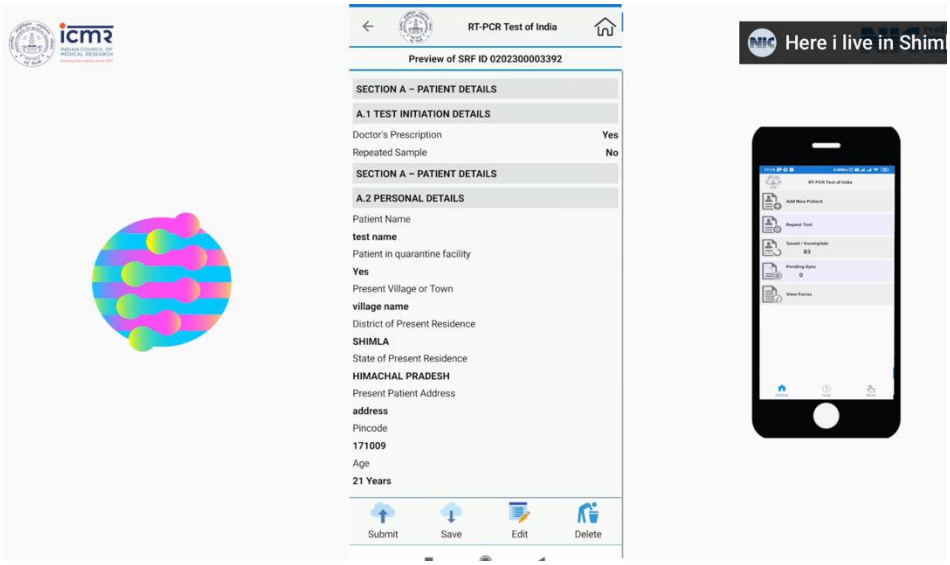




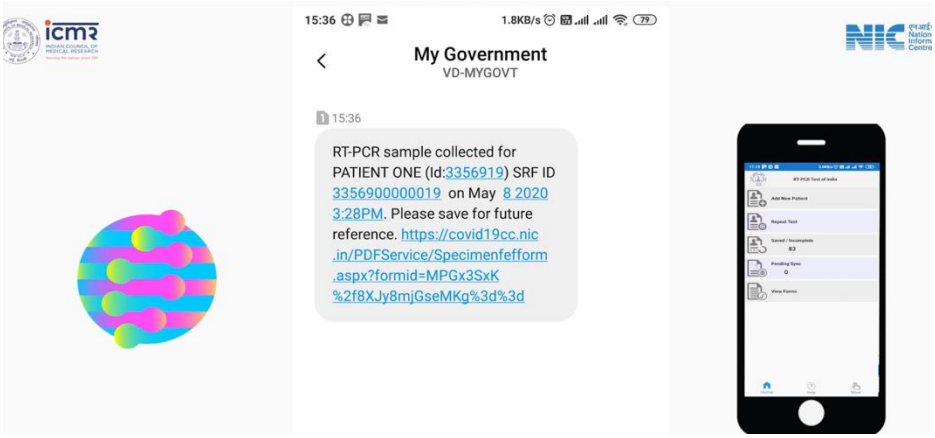
5. **STEP 5:** After saving the information, before making final submission, screen the pop up displaying your SRF ID.



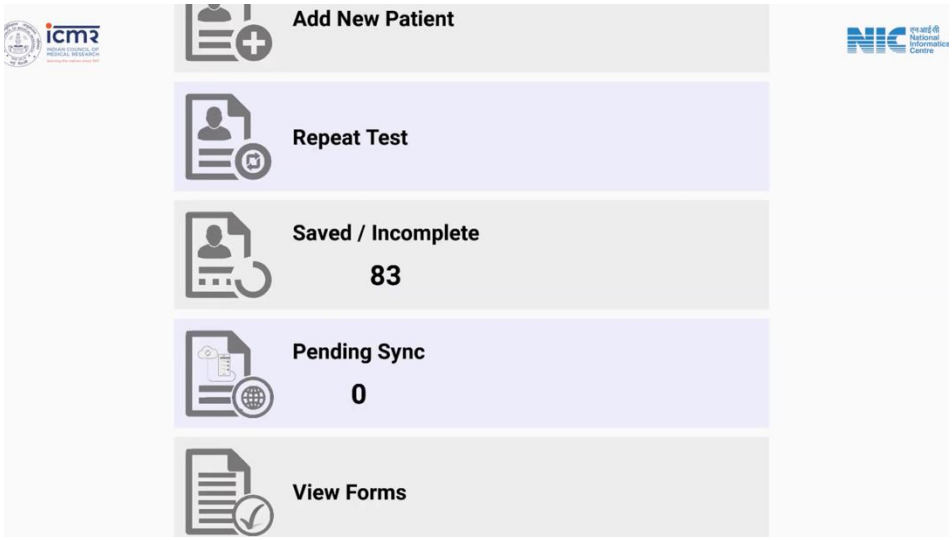
6. **STEP 6:** You can edit/continue. You can now also preview your form and proceed with submit/save/edit/delete options.



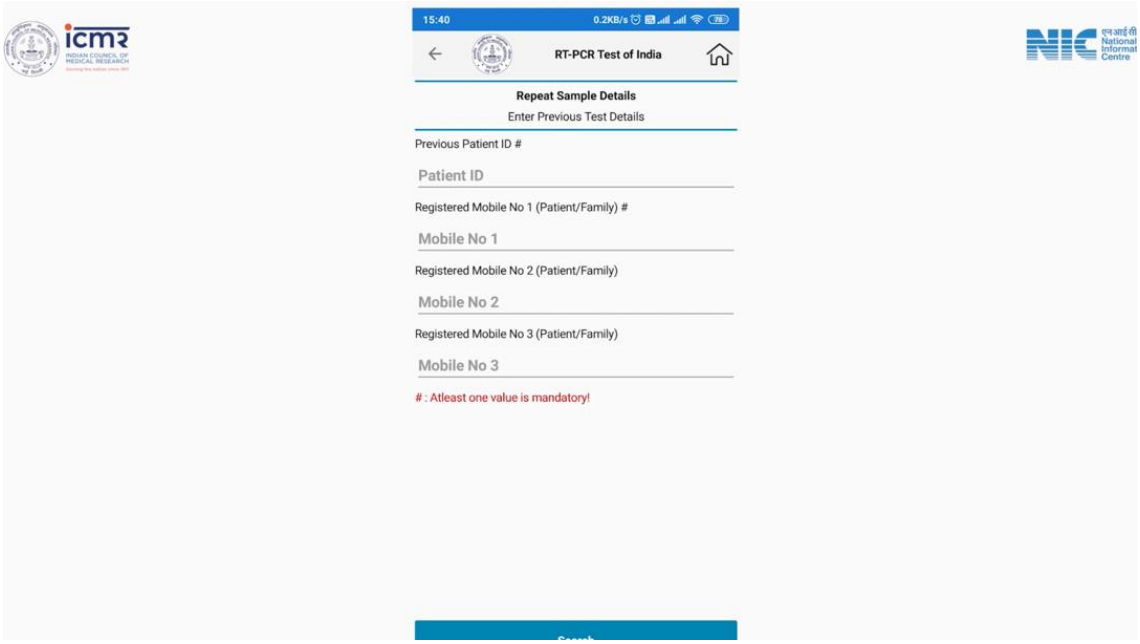
7. **STEP 7:**Once submitted, patient will receive an SMS to view report



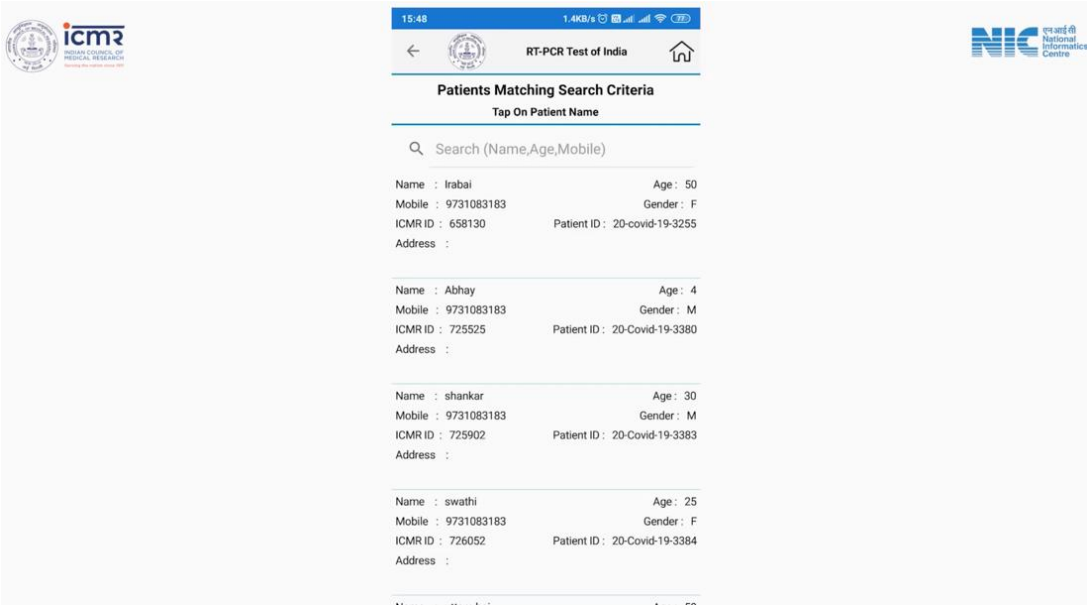
PROTOCOL FOR REPEAT TEST



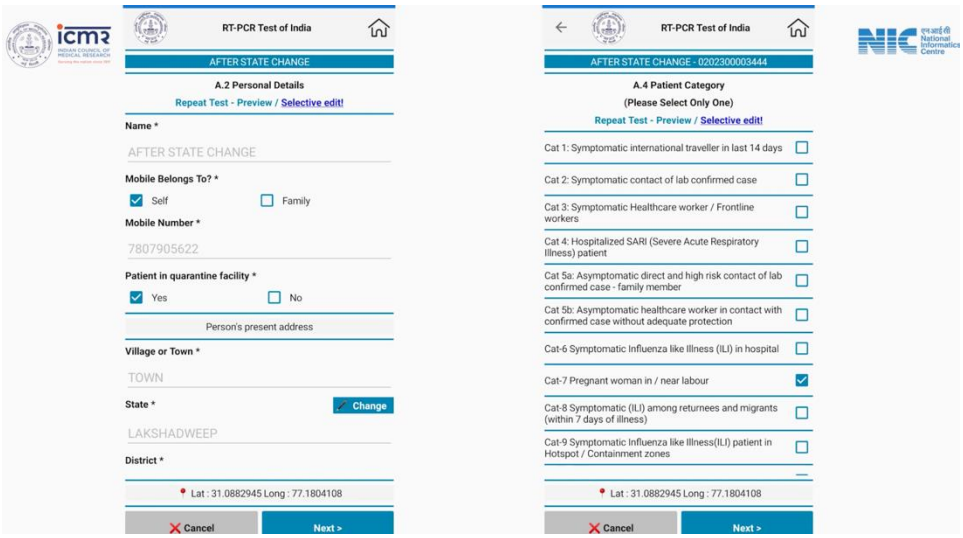
STEP 1: After clicking on Repeat test menu- the screen will ask for the parameters as shown in the image below and at least 1 value is mandatory.



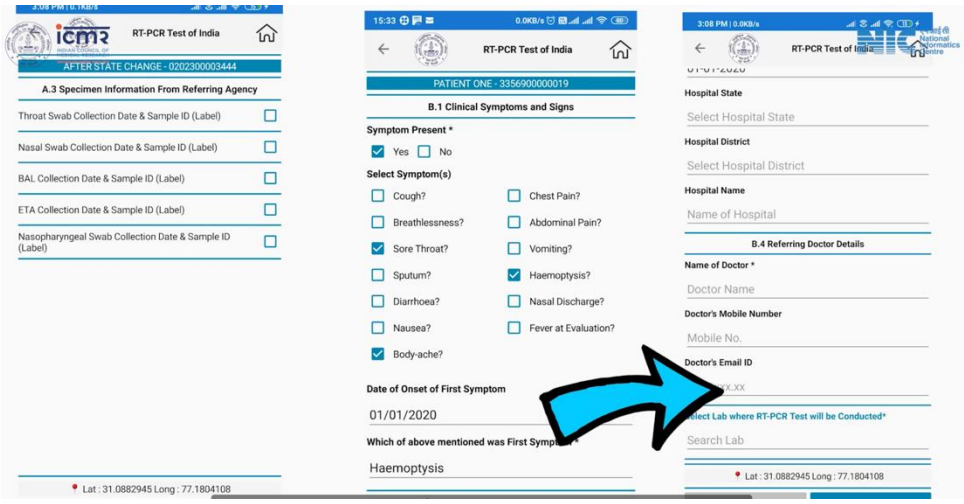
Right after entering mandatory field/, you will be able to view patient matching search criteria



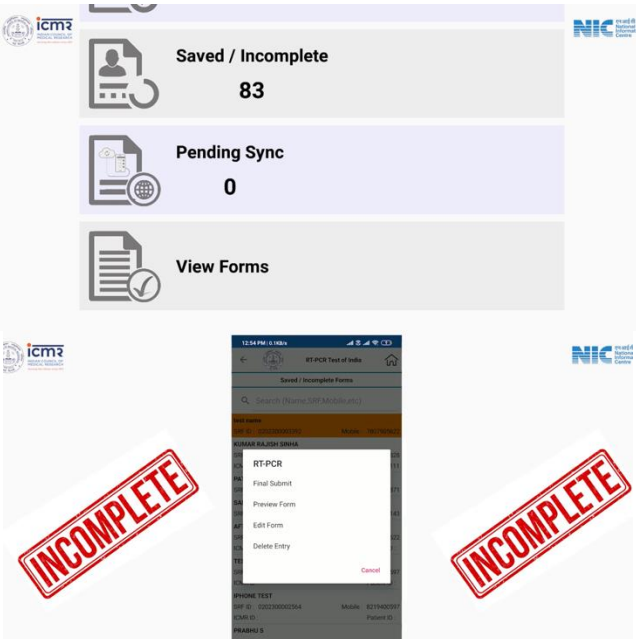
STEP 2: Tapping the desired patient record, the app will populate A2 and A4 section with limited editing privileges.



Sections A3 and B1 to B4 may be entered.

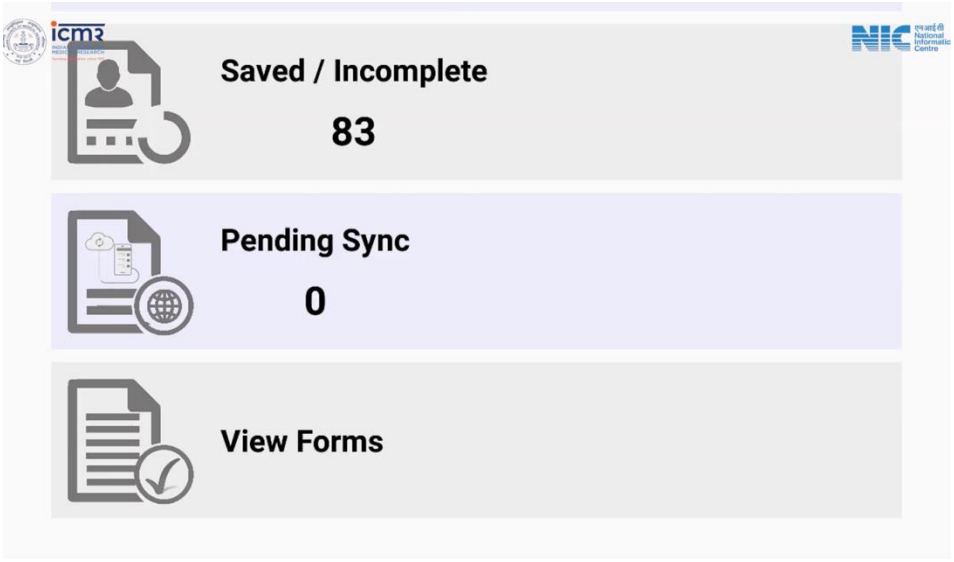


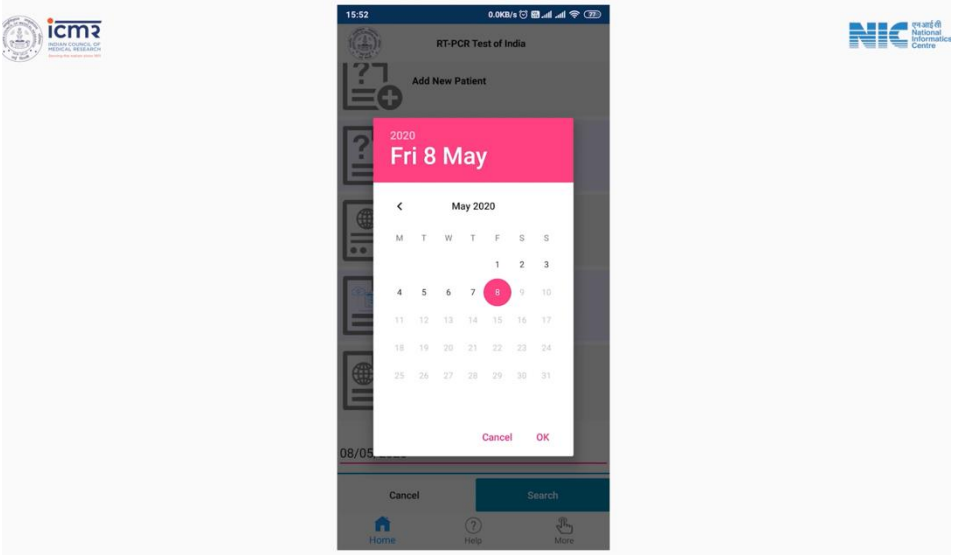
A. Protocol for saved/incomplete registration forms and pending sync

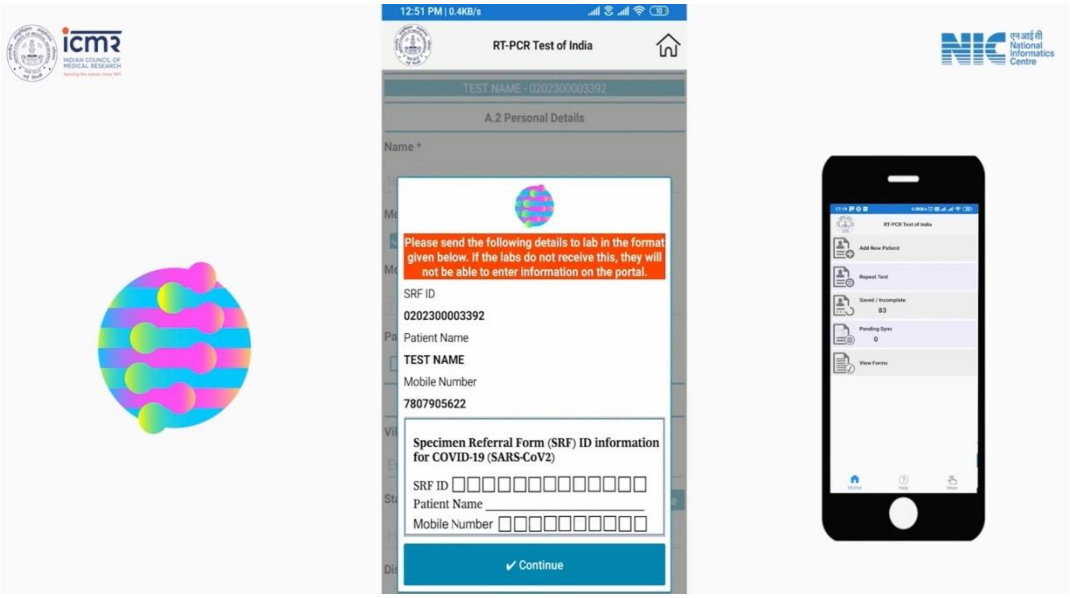


Complete registration of all pending forms and submit information.

.**VIEW FORMS**-There is also an option of VIEW FORMS menu. You can select a date to view SRF forms submitted by you. Tap on SRF to view PDF.







Descriptive Video available on: https://youtu.be/CXTmT6JIXII

3. SPECIMEN ACCESSIONING IN LAB INFORMATION MANAGEMENT SYSTEM (Electronic/Manual)

1. Accessioning of samples in Laboratory Information Management System (Electronic/Manual) shall be done for ALL samples.

2. Samples shall be accessioned ONLY after collection is done through RTPCR app by authorized users (refer the manual issued from time to time).

3. The SRF ID generated through RTPCR app shall be included in LIMS registration, along with other details as mandated by in-house SOP on sample accessioning. Note: Wherever required, such as for international travelers, passport number is mentioned during accessioning as per travel requirements.

4. All records shall be maintained for the sample collected including necessary Govt recognized ID such as Aadhar card, PAN card etc for positive identification of patients.

5. The compliance to all mandated requirements is ensured from collection till reporting and record keeping. The lab reference number is the sample ID that is uniquely generated through LIMS as well as the SRF ID has to be used for the ICMR data entry. The testing batches are maintained in google excel/manual registers (whichever is applicable) to allow information exchange for samples tested in a batch, their kits/methodology/ time trail etc.

6. The records such as Sample IDs are maintained in soft format along with other information in LIMS. The data is regularly backed up as per lab's established procedures.

7. All sample collected shall be accessioned in LIMS and all shall be updated on ICMR in a real time manner to ensure compliance to government guidelines.

Data Retention Policy

8. Laboratories should retain record of test results for five years either electronically or hardcopies.

9. If data are maintained electronically, data should be backed up on a regular basis and stored in a separate location from the original data, if possible.

10. All data recording should be checked by the laboratory supervisor for correctness and completeness, and each entry should be checked for accuracy of transcription.

11. All these data storage must be password protected with limited access to protect the patient information.

For authenticity of Reports and traceability

As per a mandate issued by NABL on 19.5.2021 on provision of QR Code on the Medical Test Reports is as follows:_

- Medical laboratories may note that, in a memorandum issued on 13.05.2021 by the Ministry of Civil Aviation, Government of India, it is mandatory for travellers flying abroad to carry their negative RT-PCR test report having QR codes linking to the original report.

- Medical Laboratories should provide QR code on all test reports issued which can be scanned using any QR scanning application available on mobile / any devices to authenticate and reproduce the test report online. This will prevent the manipulation of test results & the usage of fake test reports.

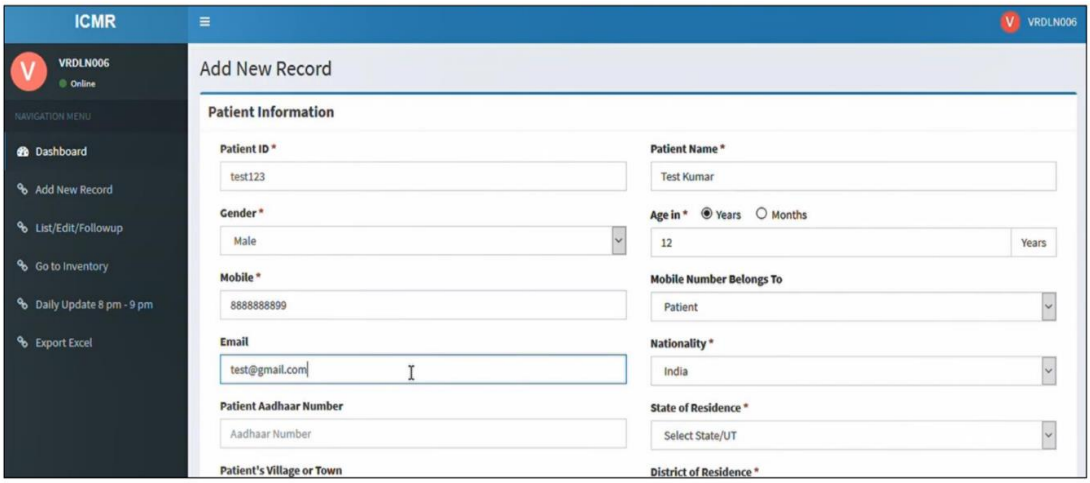
- Laboratories to ensure that all the requirements of ISO 15189, NABL, regulators are met. Authorized test results in a tamper-proof and non-editable test report will build trust in laboratory results.

4. Data Upload on ICMR portal

All COVID-19 tests conducted through RT-PCR, TrueNat and CBNAAT are reported on ICMR data entry portal which helps in drawing the National estimates on numbers of tests conducted, numbers of positives, tests conducted per million population etc. This data portal is the single National source of data entry which is accessed by all relevant Ministries / Departments for defining National strategies for COVID-19. ICMR urges all the laboratories to continue entering data into the ICMR portal https://cvstatus.icmr.gov.in/login.php to help in guiding the National strategies appropriately.

Step 1: To enter data into the ICMR Coronavirus status portal, enter login credentials and click sign in

Page to add new record will open with two sections, patient information and clinical data



Step 2: Personal and Clinical data entry

• All the fields marked in asterisk are mandatory

• Add all the personal details in the fields

• In the patient category, select one option

• Add date of arrival in India (if applicable)

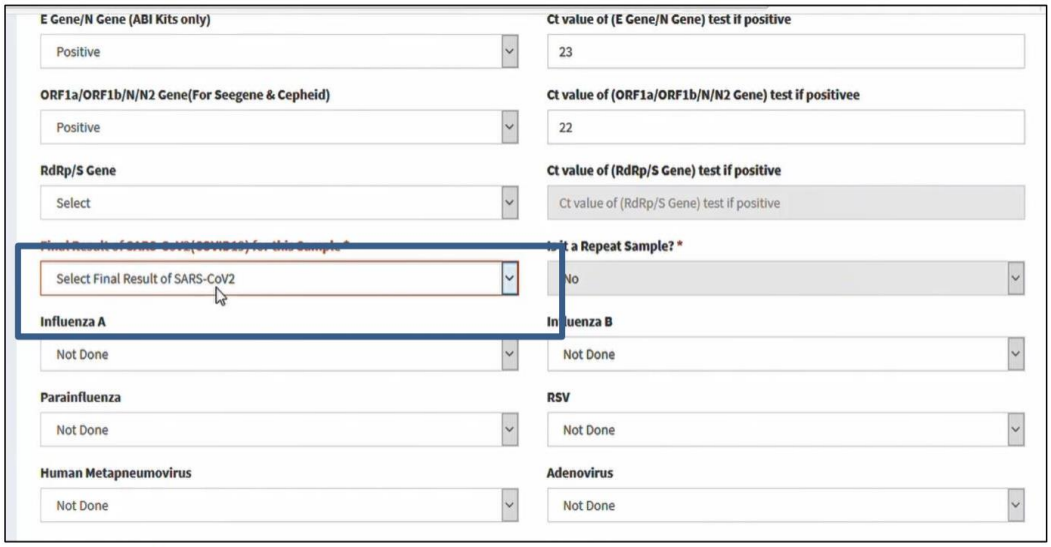
In the clinical information section –

• You can select multiple underlying medical condition and symptoms

• In the contact history with lab confirmed patient, enter details of the contact

• For every positive test result enter the Ct value Most important field: Enter the final result of SARS-CoV2 (COVID19) for this sample

Note- Once entered the value for this field CANNOT BE EDITED. Please fill this very carefully. Click submit at the end of the page to submit the record. On successful submission, the record can be viewed in the List option



5. OBSERVATION COMPLIANCE

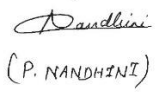
The laboratory must ensure periodic audits of accessioning data and record any mismatch/incomplete information in the occurrence log book with corrective and preventive action.

6. REFERENCES:

https://www.nic.in/products/covid19-sample-collection-management-system/

ICMR Guidelines on Data entry..\..\Downloads\Data_Entry.pdf

FIND Because diagnosis matters	Specimen Accessioning, Aliquoting & Long-term storage of Clinical Specimens			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-003	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
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Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
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SOP Annual Review	Name, Title	Signature	Date
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1. PURPOSE

To describe the method for receiving the specimen box containing clinical specimens, their aliquoting and storage.

2. INTRODUCTION

Detection of viral RNA from clinical specimens is key to the diagnosis of SARS-CoV-2 infection. Recommended upper and lower respiratory specimens obtained from suspected SARS-CoV-2 infected individuals are collected in suitable medium and transported to the diagnostic laboratory. Once received by the laboratory, the specimens must be evaluated for acceptance criteria and aliquoted for further use by the specimen processing team. Proper recording of the specimen receipt and appropriate storage of specimens till the completion test procedure, is the key for a successful test result.

Specimen collection details:
(Adapted from the WHO guidelines on 2019-nCoV):

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container*	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container*	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Not applicable
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	Autopsy sample collection preferably to be avoided
Serum (2 samples – acute and convalescent)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later

**For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens.*

Image ICMR-NIV SOP for specimen, collection & transport (REFERENCE 1)

3. PRINCIPLE

Specimens collected from suspected cases of SARS-CoV-2 infection are transported in a triple layer packing to the laboratory at 2-8°C (REFERENCE 1). All specimens received in the laboratory should be considered as "potentially infectious" and handled appropriately. The specimen box is opened in a Bio Safety Level 2 (BSL-2) equivalent laboratory inside a certified biosafety cabinet (BSC). The specimen must pass acceptance criteria in order to be eligible for receiving by the laboratory and for further processing. Specimen must be processed within the recommended time frame when stored at 2-8°C (As shown in the image above). They should be aliquoted and frozen at -70/ 80°C for long-term storage (REFERENCE 2, 3).

3.1 Specimen acceptance criteria

- Specimen is properly labeled with unique identification number and date
- Specimen label matches (unique identification number and date) with the request form/ online details received
- Specimen is received at recommended temperature (2-8°C)

3.2 Specimen rejection criteria

- Viral transport media (VTM) tube content has leaked
- The Specimen Referral Form is soiled with leaked content
- Unique patient identifiers missing/mismatching on specimen tube and Specimen Referral Form
- Specimens not transported with ice packs or the ice packs not cold (2-8°C)

4. PERSONNEL QUALIFICATIONS

4.1 Medical fitness

- All personnel involved in specimen receipt and handling should be tested for COVID-19 beforehand. Only those who test negative should be involved in performing the test procedure.
- Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered.
- If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

4.2 Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure
- Hygiene requirements
- Donning and Doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials
- Laboratory design, including airflow conditions

- Use of biological safety cabinets (operation, identification of malfunctions and maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators, (operation, identification of malfunctions and maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)
- Good laboratory practice and good microbiological techniques
- Workflow in laboratory
- Procedure to be performed
- Waste handling
- Importance of laboratory results for individual patient and COVID pandemic management

5. 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 the procedure or unable to carry it out as described are responsible for seeking advice from their supervisor.

6. CROSS REFERENCE

SOP#001 Specimen Collection and Transport of Clinical Specimens

7. EQUIPMENT & MATERIALS

7.1 Equipment

1.1.1 Certified Biosafety Cabinet Class II Type A2

1.1.2 Vortex mixer

1.1.3 -70°C/ -80°C (with free space for sample storage)

1.1.4 2 – 8 °C Refrigerator

1.1.5 Tabletop refrigerated centrifuge

7.2 Consumables

7.2.1 Transfer pipettes sterile

7.2.2 Absorbent liner

7.2.3 Sterile tweezers /forceps

7.2.4 Cryovials

7.2.5 Biohazard bags

7.2.6 Markers

7.2.7 Twist tag (to tie the bags)

7.2.8 Personal Protecting Equipment

- Coverall/ Gown
- Powder free nitrile Gloves, N95 masks,
- Goggles
- Shoe covers
- Head cover/Hair cover
- Face shield

7.3 Disinfectants

7.3.1 Ethanol/Isopropyl alcohol (Annexure.1)

7.3.2 Sodium Hypochlorite stock solution (Annexure.2)

8. PROCEDURE

Detailed Instructions:

Specimen box containing specimens is opened inside a certified biosafety cabinet (BSC Class II Type A2) in a BSL2 equivalent laboratory. (Note: *Class II BSC to be used for handling of COVID-19 should be tested and certified by a trained professional prior to use.)*

8.1 Don the PPE (cover all/ Lab gown, N95 mask, head cover, goggles, outer shoe cover, and two pairs of gloves) required for the procedure.

8.2 Review the checklist and ensure the availability of required consumables, worksheets, disinfectants and biohazards bags in the sample processing room.

8.3 After donning the PPE, transfer the specimen box from cold room/ sample reception area on a trolley to the specimen processing area.

8.4 If the PASS BOX is available, labeled cryo vials*, specimen reception log and specimen box should be taken inside the negative pressure room through the PASS BOX. Switched on the PASS BOX UV for 15 minutes.

8.5 All tubes should be labelled correctly and legibly with a permanent marker.

8.6 Turn on the BSC, let it run for 10 min and ensure the functionality check.

8.7 Turn on the refrigerated centrifuge.

8.8 Wipe the BSC work area with freshly prepared 1% Sodium hypochlorite and give a contact time of 15 minutes. To remove hypochlorite residue that may corrode the BSC surfaces, thoroughly clean the surfaces with 70% ethanol. Turn on the UV for 15-20 min.

(Note: Do not spray 1% sodium hypochlorite inside the BSC, instead soak the paper towel outside the BSC and use them to wipe the surfaces.

8.9 Spread the absorbent liner inside the biosafety cabinet and prepare the work area. Bring in the biohazard bags for solid waste and container for liquid waste. Bring in a container with appropriate amount of 1% sodium hypochlorite for rinsing the Pasteur pipettes and VTM tubes. Bring in the other required items on your checklist.

8.10 Arrange items in such a way that work area is divided into a clean area, central work area and dirty area.

8.11 Bring the specimen box to BSC.

8.12 Open the specimen box and take out all the VTM tubes containing the specimen and place on a stand.

8.13 Disinfect the inner side of the specimen box with freshly prepared 1% sodium hypochlorite solution and take it out of the BSC.

8.14 Carefully wipe the outer side of the VTM tubes with 1% sodium hypochlorite.

8.15 Arrange the VTM tubes as per the order in the specimen reception log.

8.16 Verify the specimen labels with the specimen reception log sheet.

8.17 Apply the specimen acceptance and rejection criteria (Given above)

8.18 Mark the RNA ID (Lab ID) of the specimen on top of the VTM tubes. Place the tubes on a tube rack in serial order.

8.19 Vortex the VTM tubes for 30 seconds to ensure release of specimen from the swab into the VTM.

8.20 Bring in the centrifuge safety cups inside the BSC and load the VTM tubes. Make sure the VTM tube caps are tightly secured. Wipe the centrifuge cups with disinfectant and take them out to the centrifuge for spin.

8.21 Spin the VTM tubes in refrigerated centrifuge at 1500g (~1000 rpm) for 10 minutes.

8.22 This step will ensure the settling down of cellular material in the VTM tube, to ensure cell free viral suspension.

8.23 Arrange the RNA ID labeled cryovials on the tube rack.

8.24 Bring in the centrifuge safety cups inside the BSC and arrange VTM tubes on tube rack. Wipe the safety cups with 1% sodium hypochlorite, followed by 70% alcohol and take them out of the BSC. Aliquot specimen (~1.0 ml) in pre-labelled cryo vials using a sterile pasteur pipette.

8.25 After aliquoting the specimen from a VTM tube, rinse the tube with 1% sodium hypochlorite using the same pasteur pipette. Discard the rinse in the liquid waste container. Close the cap of the VTM tube and discard both the pasteur pipette and VTM tube in the bio-hazard waste bag inside the BSC.

8.26 Store one set of vials at -70/ -80°C for later use. The other set can be stored at 4 °C for RNA extraction if to be used on the same day.

8.27 Remember the specimen should be processed as soon as possible on arrival. If this is not possible, follow WHO & ICMR guidelines for specimen storage at 4°C (REFERENCE 1). Transfer the aliquots to -70/-80°C freezer for long-term storage.

8.28 Long-term storage of specimen is also useful for preparation of External Quality Controls (EQC), for ILC with designated referral lab and also for sending for Whole genome sequencing (WGS) to Regional Genome Sequencing Laboratory(RGSL). Refer to ICMR guidelines on the long-term storage of specimens (REFERENCE 3)

8.29 After completing the work tie the biohazard bag inside the BSC and bring it out of the BSC. Place it in the biohazard bin. The waste should be handed over to the autoclave team.

8.30 Liquid waste should be discarded as per lab policy after appropriate contact time.

8.31 The BSC should be cleaned as above.

8.32 Leave the BSL-2 lab and doff the PPE.

9. QUALITY CONTROL

9.1 Only the specimen that pass the acceptation criteria should be considered for processing.

9.2 Specimen should always be stored at 2 – 8 °C upon arrival.

9.3 Specimen should be aliquoted and stored at –70/-80°C for long-term storage.

10. SPILL MANAGEMENT

Refer to SOP# 004 on spill management for handling the infectious spills.

11. BIOHAZARD WASTE DISPOSAL

11.1 All solid waste (tips, gloves, packaging, etc) collected in the specimen processing room should be discarded only in labelled biohazard bags (Labelled as COVID-19 WASTE) inside the biosafety cabinet. Filled Biohazard Bags should be tied inside the biosafety cabinet with tag.

11.2 Removed PPE should be discarded in marked designated bins. Bags should be tied and labelled.

11.3 Tied and labelled biohazard bags should be autoclaved at 121°C and 15 psi for 60 minutes (gravity flow) and 45 minutes in vacuum autoclave.

Note: Waste containing sodium hypochlorite should never be autoclaved.

11.4 Autoclaved waste should be weighed and clearly labeled as "COVID-19 waste" and handed over to Housekeeping Staff.

11.5 Housekeeping Staff should take the autoclaved waste to designated area for pickup and incineration

11.6 Any incidents including spills, mechanical breakdowns, failure in biocontainment or any other maintenance problem should be reported immediately to the biosafety officer.

11.7 Any incidence of exposure to personnel should be reported to the officer in charge.

11.8 Refer to Ref: SOP# 005 on Biomedical Waste Management.

12. REFERENCES

1. ICMR NIV Sample transportation SOP https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20%202019-nCoV.pdf

2. WHO Sample Storage Recommendations (Annexure I): https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

3. ICMR long term storage of specimen: https://www.icmr.gov.in/pdf/covid/labs/Govt_labs_sample_retention_advisory_25062020.pdf

ANNEXURES

ANNEXURE – 1: Disinfectant- Sodium Hypochlorite

Formula for preparing working solution of Sodium Hypochlorite from the stock:

Amount of stock solution required (ml)	Concentration of stock solution available	Working solution concentration required for use	Working solution volume required (ml)
100	40	4	1000

Water Required = Working solution volume required − Amount of stock solution required

For 1L of 4% Sodium Hypochlorite solution= 100ml of stock (40%) +900ml of water

Preparation of different concentrations of Sodium Hypochlorite Solution.

Required Strength (Available solution of chlorine)	Stock/commercially available Sodium Hypochlorite		
	4% (40g/L); dilute	5% (50g/L); dilute	6% (60g/L); dilute
1% (10 g/L)	1:3*	1:4	1:5

**parts of stock solution: parts of water*

1% Working Solution from **4%** Stock Solution of **Sodium Hypochlorite**

Required Volume of Working Solution(ml)	**Quantity of Stock Sodium Hypochlorite (ml)**	**Quantity of Water**
250 ml	62.5 ml	187.5 ml
500 ml	125 ml	375 ml
1000 ml	250 ml	750 ml
2000 ml	500 ml	1500 ml

NOTE: Kindly see the concentration of commercially available Stock solution before Dilution

ANNEXURE – 2: Disinfectant-70% Alcohol

Preparation of 70% Ethanol or 70% IPA (Isopropyl Alcohol)

1) Measure the required quantity of desired alcohol to be used using a clean measuring cylinder.

2) Use freshly collected distilled water for preparation of 70% alcohol solution.

3) Prepare solution in the proportion of 70:30, alcohol: water.

Calculate the quantity of stock Ethanol solution required for preparation of desired amount of 70% Ethanol solution by using the formula given below:

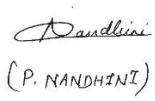
$$\text{Volume (ml) of Ethanol (Stock)} = \frac{\text{Concentration of Ethanol required (70\%)} \times \text{Total Volume (ml)}}{\text{Concentration of Ethanol (Stock)}}$$

4) Transfer the prepared IPA to a sterilized glass bottle.

5) Affix the label on the bottle with following information.

- Name of reagent
- Strength
- Date of preparation
- Use before date
- Prepared by

FIND Because diagnosis matters	RNA extraction for SARS-CoV-2 testing using PATHKIT			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-008	1.0	

	Name, Title		Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead			7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist			13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager			21 Jan 2022
Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)	
	Nil			
SOP Annual Review	Name, Title	Signature	Date	
	Nil			
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1. PURPOSE

To provide the procedure for the isolation of viral RNA from clinical samples obtained from suspected cases of COVID-19.

2. INTRODUCTION

Detection of viral RNA from clinical specimens is key to the diagnosis of SARS-CoV-2 infection. Recommended upper and lower respiratory specimens are obtained from suspected SARS-CoV-2 infected individuals, collected in suitable transport medium and transported to diagnostics laboratory. Specimens are lysed to extract the RNA. Total RNA is either extracted manually or by automated systems. RNA is subsequently used for reverse transcription and real time PCR amplification. Detection of viral target genes and presence of clinical symptoms are the key criteria for the diagnosis of COVID-19..

3. PRINCIPLE

RNA is a single stranded nucleic acid, which is highly unstable with a very short half -life, once extracted out from its source. Another reason for its instability is the RNAses which are present everywhere, in the cells, tissues, bacteria and environment. RNases degrade RNA, and/ are tough enzymes to inactivate. RNases resist heat inactivation and thus need strong denaturants for their elimination. Therefore, during RNA extraction, specimens are lysed under strong denaturing conditions, in a RNAse free environment to ensure isolation of intact RNA. Three common methods of RNA extraction are:

3.1 Treating the specimens with reagents such as TRizol, RNAzol followed by precipitation with isopropanol.

3.2 Silica based column purification such as in Qiagen -QIAMP Viral RNA extraction method

3.3 Magnetic beads mediated purification such as in MagMAX Viral RNA Isolation method

4. PERSONNEL QUALIFICATIONS

4.1 Medical fitness

All personnel involved in specimen processing should be tested for COVID-19 beforehand. Only those who test negative should perform specimen processing. Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered. If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

4.2 Education and training

The lab personnel must have received education and training on the following topics before performing this procedure:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure

- Hygiene requirement
- Use of protective equipment and clothing
- Laboratory biosafety, specifically handling of potentially infectious materials
- Laboratory design, including airflow conditions
- Use of biological safety cabinets (operation, identification of malfunctions and maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators (operation, identification of malfunctions and maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)
- Good laboratory practice and good microbiological techniques
- Workflow in laboratory & procedures
- Waste handling
- Importance of laboratory results for patient management
- Importance of laboratory results for COVID pandemic management

5. 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 or unable to carry it out as described are responsible for seeking advice from their supervisor.

6. CROSS REFERENCE

SOP#001 Sample Collection and Transport of Clinical Specimens

7. EQUIPMENT & MATERIALS

7.1 Equipment & tools

7.1.1 Certified Biosafety Cabinet Class II Type A2

7.1.2 Vortex mixer

7.1.3 Micropipettes (2 or 10 µL, 200 µL and 1000 µL)

7.1.4 Refrigerated microcentrifuge (with rotor for 1.5 ml centrifuge tubes)

7.1.5 -70/ -80°C (with free space for sample storage)

7.1.6 2 – 8 °C Refrigerator

7.2 Consumables

7.2.1 Nuclease free RNA storage cryovials

7.2.2 Nuclease free filter tips (aerosol resistant (ART))

7.2.3 1.5 ml/ 2 ml centrifuge tube

7.2.4 Tube racks

7.2.5 Markers

7.2.6 Absorbent liners

7.2.7 Paper Towels

7.2.8 Biohazard bags Red &Yellow

7.3 Reagents & Disinfectants

7.3.1 RNA Zap

7.3.2 PATHKITs RNA Extraction kit

7.3.3 Nuclease free Water

7.3.4 Reagent grade Ethanol and Isopropanol

7.3.5 Sodium Hypochlorite

7.4 Personal Protective Equipment (PPE)

7.4.1 Lab gowns

7.4.2 N95 masks

7.4.3 Googles

7.4.4 Gloves

7.4.5 Shoe Covers

7.4.6 Head/ Hair Covers

8. PROCEDURE

RNA quality and quantity are the key to the success testing, therefore:

- Specimen should be stored and processed within the recommended time frame.
- RNase free reagents should be used and work surfaces should be RNase free.

- Extracted RNA is very unstable, it should always be stored at on ice for short term or at 20°C. If this is not possible store the RNA at -70/-80°C till further use. Repeated freeze thaw of RNA specimen should be avoided.

8.1 RNA Extraction is carried out in a BSL 2 equivalent facility inside a certified biosafety cabinet (BSC Class II Type A2).

8.1.1 PPE required for the procedure: COVER ALL/Lab gown, N95 mask, head cover, goggles, outer shoe cover or Lab specific shoe and two pairs of gloves.

8.1.2 Review the checklist, ensure the availability of required consumables, worksheets, disinfectants and biohazards bags in the sample processing room.

8.1.3 After donning the PPE, enter into the RNA extraction area.

8.1.4 Turn on the biosafety cabinet (BSC), let it run for at least 10 minutes and carry out functionality check. Smoke test should be done at least weekly to check efficiency of BSC.

8.1.5 Wipe the work surface area inside the BSC with paper towel soaked in freshly prepared 1% Sodium hypochlorite. Give a contact time of 10-15 minutes. Wipe off the surface area thoroughly with 70% ethanol. This will ensure removal of residual sodium hypochlorite salts.

Note: (sodium hypochlorite is corrosive, soak the towel outside the BSC. Do not spray.

8.1.6 Spread the absorbent liner inside the biosafety cabinet and prepare the work area. Bring in the discard containers lined with biohazard bags, liquid waste container with sodium hypochlorite and other required items.

8.1.7 Ensure tip boxes, micropipettes, tube rack surfaces are RNase free. Use cleaning agent such as RNase ZAP to remove RNases.

8.1.8 Bring in the specimens to be processed to the BSC.

8.2 Carry out RNA extraction as per manufacturer's instructions

8.2.1 Thaw all the reagents from the extraction kit to room temperature

8.2.2 The Carrier RNA is supplied in lyophilsed form and the same has to be resuspended using the carrier RNA solubilization buffer (provided in the kit). The carrier RNA has to be aliquoted appropriately to avoid multiple freeze thaw.

8.2.3 If the RT -PCR kit has an indigenous internal control (IC), then the IC has to be added after the addition of the carrier RNA (step

8.2.4 The Wash buffer is supplied as a concentrate (10 mL in 50 mL Bottle), and before using for the first time, 40 mL of ethanol (96-100%) has to be added to each bottle. Wash Buffer is stable for 1 year when stored closed at room temperature, but only until the kit expiration date.

8.2.5 Transfer 200µl of viral transport media (after vortexing) into a fresh Micro Centrifuge Tube (MCT), provided with the kit.

8.2.6 400µl of Lysis Buffer followed by 10 µl of carrier RNA into the MCT and the same is mixed thoroughly by pipetting. (please note that the carrier RNA has to be added after the lysis buffer in order to avoid the lysis of the same)

8.2.7 400µl of Isopropyl Alcohol (IPA) is added to the samples and mixed well. Transfer 500µl of the total solution to the spin column in a 2 ml collection tube. Close the cap and centrifuge at 8000 rpm for 1 min. Repeat with the remaining 500µl of the total solution.

8.2.8 Carefully open the spin column and add 500µl of Wash Buffer, and centrifuge at 8000rpm for 1 min and discard the filtrate.

8.2.9 Repeat step 8.2.8 with 500µl of Wash Buffer

8.2.10 Centrifuge (dry spin) at full speed for 1 min.

8.2.11 Now place the column in a clean 1.5ml RNase Free Micro Centrifuge Tube (not provided with kit) and add 60µl of elution buffer and centrifuge at 8000 rpm for 1 min

8.2.12 Store the eluted RNA in -20°C until further processing

8.2.13 Return back all the reagents to the appropriate storage boxes after use.

8.2.14 Always adhere to manufacturer's instructions, when using a RNA extraction kit. The extracted RNA should always be stored in the -70/-80°C freezer available. Only use RNase/Nuclease free reagents and water for handling RNA.

8.2.15 Sample RNA extraction should not be delayed.

8.2.16 Make sure you take all the precautions to avoid RNAse contamination.

8.2.17 RNA extraction reagents should not be contaminated. Whenever necessary aliquot the reagents for use. Remember, PCR is a highly sensitive process and small contamination will negatively impact test results. Contaminated reagents must be discarded.

8.2.18 Always keep extracted RNA at 4°C for immediate use. For long term storage use properly labeled sterile/ nuclease free cryovials and freeze at -70/-80°C.

8.2.19 After completing the work, the biohazard bag with RNA extraction waste along with tips and tubes should be tied and autoclaved.

8.2.20 The BSC should be cleaned as described above, and switch on UV after completing experiment.

8.2.21 Leave the BSL-II lab and doff the PPE.

9. QUALITY CONTROL

9.1 Nuclease-free Water (not DEPC-Treated) must be used as the Negative Control for the extraction reaction.

9.2 A known positive sample must be included along with the unknown sample as a quality control procedure during every batch of the testing.

10. BIOHAZARD WASTE DISPOSAL

10.1 All solid waste collected in the BSL2 laboratory should be discarded only in labelled biohazard bags (Labelled as COVID-19 WASTE) inside the biosafety cabinet. Filled Biohazard Bags should be tied inside the biosafety cabinet with tag.

10.2 Removed PPE should be discarded in marked designated bins. Bags should be tied and labelled.

10.3 The tied and labelled biohazard bags should be autoclaved at 121°C and 15 psi for 60 minutes (gravity flow) and 45 minutes in vacuum autoclave.

10.4 Autoclaved waste should be weighed and clearly labeled as "COVID-19 waste" and handed over to Housekeeping Staff.

10.5 Housekeeping Staff should take the autoclaved waste to designated area for pickup and incineration

10.6 Liquid waste from RNA isolation should be decontaminated by placing it for 30 minutes in 1% sodium hypochlorite in a glass container, neutralised after which the decontaminated solution should be drained in the designated sink in the Laboratory which is connected with the pre-treatment plant

10.7 Any incidents including spills, mechanical breakdowns, failure in bio-containment or any other maintenance problem should be reported immediately to the biosafety officer.

10.8 Any incidence of exposure to personnel should be reported to the officer in charge.

11. SPILL MANAGEMENT

Refer to SOP #004 on spill management.

12. REFERENCES:

12.1 Diagnostic testing for SARS-CoV-2: interim guidance, 11 September 2020: https://apps.who.int/iris/handle/10665/334254

12.2 DNA, RNA, and protein extraction: the past and the present. Tan SC, Yiap BC. J Biomed Biotechnol. 2009; 574398. doi: 10.1155/2009/574398. Erratum in: J Biomed Biotechnol. 2013;628968. PMID: 20011662; PMCID: PMC2789530. https://pubmed.ncbi.nlm.nih.gov/20011662/

12.3 PATHKITS Viral RNA Extraction Kit Insert (10.0. (https://pathkits.com/rna-extraction-kit.html)

ANNEXURES:

ANNEXURE – 1: Disinfectant- Sodium Hypochlorite

Formula for preparing working solution of Sodium Hypochlorite from the stock:

Amount of stock required (ml)	Concentration of stock	Working concentration	Working volume required
100	40	4	1000

Water Required = Working solution volume required − Amount of stock solution required

Preparation of different concentrations of Sodium Hypochlorite Solution.

Required Strength (Available solution of chlorine)	Stock/commercially available Sodium Hypochlorite		
	4% (40g/L); dilute	5% (50g/L); dilute	6% (60g/L); dilute
1% (10 g/L)	1:3*	1:4	1:5

**parts of stock solution: parts of water*

1% Working Solution from **4%** Stock Solution of **Sodium Hypochlorite**

Required Volume of Working Solution(ml)	**Quantity of Sodium Hypochlorite (ml)**	**Quantity of Water**
250 ml	62.5 ml	187.5 ml
500 ml	125 ml	375 ml
1000 ml	250 ml	750 ml
2000 ml	500 ml	1500 ml

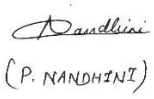
ANNEXURE – 2 Disinfectant-70% Alcohol

Preparation of 70% Ethanol or 70% IPA (Isopropyl Alcohol)

- Measure the required quantity of desired alcohol to be used using a clean measuring cylinder.
- Use freshly collected distilled water for preparation of 70% alcohol solution.
- Prepare solution in the proportion of 70:30, alcohol: water.

- Transfer the prepared IPA to a sterilized glass bottle.
- Affix the label on the bottle with following information.
    - Name of reagent
    - Strength
    - Volume Prepared
    - Date of preparation
    - Use before date
    - Prepared by

FIND Because diagnosis matters	RT-PCR testing for SARS-CoV-2 using COVIPATH kit			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-009	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist		13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager		21 Jan 2022

Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
	Nil		

SOP Annual Review	Name, Title	Signature	Date
	Nil		

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- **PURPOSE**

To provide instructions for performing real-time reverse transcription polymerase chain reaction (rRT-PCR) for the detection of SARS CoV-2 target genes in the RNA isolated from the clinical specimens using CoviPath COVID-19 RT-PCR Kit (an ICMR approved kit)

1. INTRODUCTION

This SOP describes rRT-PCR method to detect SARS-CoV-2 from the clinical specimen. rRT-PCR, considered as the gold standard for the diagnosis of SARS-CoV-2 infection, is highly sensitive and has very low limit of detection (1,2). The procedure is carried out after viral RNA has been extracted from ICMR approved specimen types, in a BSL 2 equivalent facility. Key steps in the procedure need to be performed in physically separated areas in the laboratory. Adherence to good laboratory practices is critical for quality test results.

2. 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.

In a one-step rRT-PCR process, where reverse transcription and PCR reaction occur in the same PCR tube, the reaction mix contains following key ingredients:

- Reverse transcriptase that converts RNA to cDNA.
- Primers and probes that specifically bind to SARS-CoV-2 target gene of interest.
- Each probe has a fluorophore at the 5' end and a quencher at the 3' end. Fluorescence from the fluorophore is inhibited by the presence of quencher in the vicinity, therefore the intact probe does not fluoresce (3).
- Taq polymerase, dNTPs, buffer for the PCR.

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,

- **CROSS REFERENCES**

1.1 SOP for RNA extraction (SOP -008)

1.2 Verification data for RT-PCR kit to be used

- **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.

- **EQUIPMENT & MATERIALS**

1.3 Equipment & Tools:

a. Real time PCR machine
b. Biosafety cabinet (level II)
c. PCR hood or Laminar flow hood
d. Vortex mixer
e. Calibrated micropipettes for:

1.3.e.1 Master Mix preparation (10 µL, 200 µL and 1000 µL)

1.3.e.2 RNA addition (10 µL,)

1.3.e.3 Positive control addition (10 µL,)

f. Refrigerated microcentrifuge (with rotor for 1.5 ml centrifuge tube)

g. -70/ -80°C and -20°C freezers

h. 4°C Refrigerators

i. Benchtop minifuge

j. 96 well plate spinner

k. Mini Spinner

l. Tube racks

m. PCR tube rack

1.4 Consumables:

a. PCR 96 well plates

b. Plate covers

c. Plate sealers

d. PCR tubes/strips

e. Aluminum foil

f. Cool racks

g. Nuclease free tips

h. Tube racks

i. Markers

j. Absorbent liners

k. Paper towels

l. Nuclease free Gloves

1.5 Reagents & Disinfectants

a. RNAse Zap

b. CoviPath Covid -19 RT- PCR Kit that contains

- Covipath COVID 19 Assay Multiplex (A50780)
- Covipath 1- step Multiplex Master Mix (No ROX)
- Covipath COVID-19 Control
- 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.

c. Nuclease free Water

d. Reagent grade Ethanol and Isopropanol

e. Absolute alcohol

f. Sodium Hypochlorite

g. Biohazard bags Red & Yellow

1.6 Personal Protective Equipment (PPE)

a. Lab gowns

b. Surgical/medical masks/

c. N95 Mask

d. Gloves

e. Shoe Covers

f. Hair Covers

- **PROCEDURE**

This procedure consists of following main steps:

- Master mix preparation
- RNA addition
- Positive and Negative Control addition
- PCR machine set up
- Data collection
- Disposal of used PCR plates/PCR tubes/Strips

For uninterrupted workflow, review your checklist and ensure all the necessary items are available at the site where you are going to be working. Maintenance of unidirectional workflow is

important. Do not enter in master mix preparation room after entering into the RNA extraction room.

Note: It is better to have designated personnel to work in master mix area so as to minimize contamination. All sections such as pre PCR, and amplification area must have dedicated equipment and consumables.

- i. Master mix preparation

  Important:

  Master mix preparation should be carried out in a RNA/ DNA free area, (preferably in a PCR hood or laminar flow cabinet). PPE, tools and consumables used in this area should not be used elsewhere. Minimize RNase contamination by frequently changing gloves, using RNase free water, molecular biology grade tips and tubes.

  PPE to be worn during this procedure: Lab coat, gloves, hair cap, shoe covers, surgical mask.

  a. Before starting this step calculate the amount of reagents needed by using a master mix calculation work sheet. (Annexure 1)

i. Determine the number of reactions (N) to set up per assay. Add the reactions for following controls:

- No template control (NTC)
- Extraction negative control (ENC)
- Positive control (PC)
- Internal Control (IC; If recommended by manufacturer)

ii. Add extra number of reaction/s to account for pipetting error.

- If number of samples (n) including controls = 1 to 10, then N = n + 1
- If number of samples (n) including controls = more than 10, then N = n + 2

iii. Carry out the calculations on Mastermix Calculation worksheet.

iv. In addition, prepare a layout of your sample reactions tubes or 96 well plate/384 well plate.(Annexure 2)

v. Carry the worksheet, sample list and sample layout with you to the master mix preparation area.

b. Cleaning the Reagent Preparation Room

i. Prepare sufficient amount of 1% sodium hypochlorite solution and 70% ethanol for cleaning. Ensure availability of waste collection bags and bins.

ii. Clean the work surface work area, micro pipettes, tip boxes with freshly prepared 1% sodium hypochlorite. After 15 minutes of contact time, wipe off with 70% ethanol. Turn on UV (if available) in the hood for at least 20 minutes before use.

iii. Place an absorbent liner. Place all necessary items - pipettes, tips, 70% alcohol, tissue roll, tube rack, waste collection bag in the hood. To avoid RNase contamination, frequently change gloves.

iv. Take out the master mix reagents from the freezer and allow them to thaw on a cooler rack. Keep them protected from light by covering with aluminum foil if required. Meanwhile, label the master mix tube and reaction tubes or plates.

v. Gently tap the reagent tubes on the sides to mix the salts and then spin down the reagent tubes in a mini spin for 5 to 10 sec (do not spin the reagents for too long).

vi. Pipette the calculated amount of reagents into the master mix tubes as per the table below:-

Master mix contents	96 well		384 well	
	1X	N reaction	1X	N reaction
Multiplex Master Mix	6.25µl	6.25*N	5 µl	5*N
RT PCR assay multiplex	1.25 µl	1.25*N	1 µl	1*N
NFW	7.5 µl	7.5*N	4 µl	4*N
Total reaction volume	15 µl		10 µl	

vii. Mix the reagents by gently pipetting up and down. Do not vortex. Spin the master mix tubes for few seconds to collect contents at bottom, and then place the tubes in cold rack.

viii. Set up reaction strip tubes or plates in 96-well cooler rack. Dispense appropriate volume of master mix into each well as per the plate set up.

ix. Before moving the plate to the RNA addition area, pipette appropriate volume of the nuclease free water into negative control tubes (NTC)/ wells. Cap NTC tubes/wells. Cover the plate with aluminum foil to protect it from light.

x. Clean the work surface area with 1% sodium hypochlorite and 70% alcohol as described above.

- ii. RNA addition (in Template Addition Room)

  i. A separate PCR hood or laminar flow hood should be used for RNA addition. If that is not available, wear the appropriate PPE (COVER ALL/Lab gown, N95 mask, head cover, goggles, outer shoe cover, and two pairs of gloves).

  ii. Clean the biosafety cabinet used for RNA preparation thoroughly with 1% sodium hypochlorite, giving a contact time of 15 minutes, followed by wiping with 70% alcohol.

  iii. Items such as micro-pipettes, tips, tube racks used here should not be used elsewhere and they should be cleaned with cleaning agent such as RNAse ZAP to get rid of RNases.

  iv. Take out the RNA samples from the freezer on cold rack and bring them inside the BSC. Let them thaw. Gently tap on the sides of the tubes to mix the content and vortex for approximately 5 seconds.

  v. Place the tubes back in the cold rack.

  vi. Add 15 µl (as recommended in kit manual) of RNA to each tube and the same volume of mock extraction control into the respective wells as per the set up. Change the tips after each addition.

  vii. Cap the tubes/cover the plate with aluminum foil to which the samples and mock control has been added.

- iii. Positive control addition

  - Positive control should be added in a positive control addition area or if the separate area is not available, it should be added after addition of sample RNA. The dilution for the control is as below:-

    Positive control preparation-

    Dilution 1 - 95µl of TE buffer + 5ul of Covipath Covid19 control (mix & centrifuge).

    Dilution 2 - Take 75ul of dilution buffer + 25ul of dilution 1 (Mix &Centrifuge).

    10 µl of this diluted positive control (dilution 2) will be used as a template in RT-PCR

    Note: - The volume of the RNA template and the positive control will vary depending on the PCR plate that will be used,

  i. If 96 well plate is used, seal the plate with optical sealer. Centrifuge the plate / tubes for10 seconds. Make sure that bubbles are eliminated from the bottom of the reaction tubes.

  ii. The kit contents included the controls should be stored at -20°C immediately

iii. Now the PCR plate is ready to be transported to the Amplification room. Place it in a disinfected cold rack and cover with clean aluminum foil. Remember not to apply any disinfectant on the PCR plate or PCR tubes.

iv. Discard the solid waste in the appropriate waste bin outside the BSC. Clean the work surface area with 1% sodium hypochlorite, followed by 70% ethanol.

v. Exit the RNA extraction area with PCR plate on cold rack and remove the PPE.

- iv. PCR machine set up (Amplification Room)

i. Don the Lab coat and gloves and enter the Amplification room.

ii. For real time PCR set up follow the instructions given by the Real-time PCR instrument manual for plate set up. Save your plate setup.

iii. Program the run method as per instructions provided in the RT-PCR kit:

STAGE	TEMPERATURE	TIME	CYCLES
1	25°	2 MIN	1
2	53°	10 MIN	1
3	95°	2 MIN	1
4	95°	3 SEC	40
	60°	30 SEC (Data collection)	

Passive Reference Dye-None

After the protocol of amplification is done, remove PCR Plate from the thermal cycler and discard them in a sealable plastic bag for autoclave and decontamination.

iv. Fluorescent Channels

Reporter/Quencher	Target
FAM/None	ORF1ab
VIC/None	N gene
JUN/None	RNaseP

Detection of the Positive control in fluorescence channel FAM and VIC.

Detection of the internal control (IC) in fluorescence channel JUN in NTC.

Note: Set the threshold above the maximum level of No template control curve (random noise curve), then analyze the results.

- v. Data Collection
  - i. After completion of the run, save the result file. Ensure a proper backup of the data.
  - ii. Remove the PCR tubes/ plates, inspect the wells for any visible signs of reaction mix evaporation. Note down the details.
- vi. Discard plates

- in the amplification room in a waste bin lined with red biohazard bag.

- **QUALITY CONTROL**

In accordance with manufacturer instructions, following internal controls should be run with every RT-PCR reaction batch

a. No template Control

b. Sample Extraction Control (Nuclease free water that is extracted along with the samples)

c. Positive Control that is provided in the kit

d. Known positive and negative samples

- **RESULT & INTERPRETATION**

- The negative control reactions for probe/primer sets should not exhibit fluorescence growth curves (FAM and VIC) 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:

Channels	Positive control	Expected Ct values
FAM	ORF1ab	≤37
VIC	N gene	≤37
JUN	RNase P	≤35

- All clinical samples should exhibit RNase P reaction curves that cross the threshold line at or before 35 cycles.

- the below table gives the expected Ct values for the controls and the clinical specimens

Sample or Control	Target	C_q cutoff (QuantStudio™ 5 Real-Time PCR Instrument)
Positive Control	N gene	Valid C_q values are ≤37
	ORF1ab	Valid C_q values are ≤37
Negative Control	Viral targets	Valid C_q values are >37
	RNase P	Valid C_q values are >35
Clinical samples	Viral targets	Positive C_q values are ≤37
	RNase P	Valid C_q values are ≤35

- The below table provides interpretation of Test Results for the Clinical Specimens

ORF1ab	N gene	RNase P	Status	Result[1]	Action
Negative	Negative	Negative	Invalid	NA	Repeat the test. If the repeat result remains invalid, consider collecting a new specimen.
Negative	Negative	Positive	Valid	SARS-CoV-2 not detected	Report results.
Positive	Negative	Positive	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Negative	Positive	Positive	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Positive	Positive	Positive	Valid	SARS-CoV-2 detected	Report results.
At least one positive		Negative	Valid	SARS-CoV-2 inconclusive	Repeat the test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.

- 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

- ICMR Advisory on cutoff for the Ct value for RT-PCR Assays

A single cut off of 35 with good sigmoidal real time RT-PCR curve is acceptable.

- All patients with a CT value of less than or equal to 35 may be considered positive while those with more than 35 may be considered negative.
- All samples of CT value of less than or equal to 35 with poor sigmoidal curve should be essentially retested.

(Ref : ICMR Letter dated 3/4/2021)

- Limitations

- One should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.
- A false negative result may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.
- A false negative result may occur if an excess of template is present in the reaction due to competitive inhibition.
- A false negative result may occur if RNA is degraded or is contaminated with RNase
- Not achieving desired Ct value for PC/ inappropriate test results may occur if pipetting is not being done properly.
- Handling by trained staff and timely calibration of pipettes would minimize these issues.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or Imuno suppressant drugs have not been evaluated. The Covipath™ COVID-19 RT-PCR Kit cannot rule out diseases caused by other bacterial or viral Pathogens.

- WASTE MANAGEMENT, CONTAMINATION CONTROL AND OTHER SAFETY PRECAUTIONS

Waste management:

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

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

Contamination Control

- Tools and instruments used for master mix preparation area should be labelled accordingly and should not be used elsewhere. Never take anything from this site to RNA extraction/addition area.
- Add RNA to PCR tubes in a PCR or laminar flow hood. If these facilities are not available, clean and decontaminate the RNA extraction BSC and use it for sample addition.

- Positive controls should be handled very carefully. Dedicate a separate area and PCR Hood for positive control addition.

- Change gloves frequently and especially if they get contaminated with RNA sample

- Clean micropipettes, racks, instruments and the work area with freshly diluted 1% bleach (20 min), followed by 70% alcohol after every use

- Use area-dedicated spray flasks or beakers (separate beakers for surface cleaning and instruments)

- Do not take anything from one dedicated area either to other area of the lab

- REFERENCES:

1. SARS-CoV2 Testing: The Limit of Detection Matters. Arnaout R, Lee RA, Lee GR, et al. Preprint. bioRxiv. 2020;2020.06.02.131144. Published 2020 Jun 4. Doi:10.1101/2020.06.02.131144. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7302192/
2. Diagnostic testing for SARS-CoV-2: interim guidance document WHO, 11 September 2020: https://apps.who.int/iris/handle/10665/334254
3. 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/
4. Phases of Real-Time PCR: https://www.labce.com/spg1913034_phases_of_real_time_pcr.aspx
5. CoviPath™ COVID-19 RT-PCR Kit – USER GUIDE

SOP CHANGE HISTORY

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

- **ANNEXURES**

  a. ANNEXURE 1: MASTER-MIX CALCULATION WORKSHEET (96 reaction well plate)

Date:

Components	Volume required per reaction	Volume required for N reaction	
Multiplex Master Mix	6.25µl	6.25*N	
RT PCR assay multiplex	1.25 µl	1.25*N	
NFW	7.5 µl	7.5*N	
Total reaction volume	15 µl		

Kit:

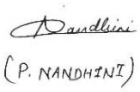
b. ANNEXURE-2: AMPLIFICATION WORKSHEET

Master mix prepared by:_____Template addition by:_____

PCR run by:_____ PCR Checked by: _____

RT-PCR batch log												
PCR machine name:				Batch no.:.....				Date:				
Name of rt-PCR kit:.....				Lot no.:				Expiry:				
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												
Remark :												
Results -												
No. of positive results -												
No. of negative results -												
No. of presumptive positive -												
No. of invalid/indeterminate results -									Signature:			

FIND Because diagnosis matters	Detection of SARS-CoV-2 using CBNAAT			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-006	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist		13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager		21 Jan 2022

Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
	Nil		

SOP Annual Review	Name, Title	Signature	Date
	Nil		

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1. Purpose

The purpose of this SOP is to describe the stepwise procedure for the detection of SARS-CoV-2 using CBNAAT/Xpert Xpress SARS-CoV-2 test.

2. Introduction

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider.

The Xpert Xpress SARS-CoV-2 test is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens (REFERENCE 1).

3. PERSONNEL QUALIFICATIONS

3.1. Medical fitness

- All personnel involved in specimen receipt and handling should be tested for COVID-19 beforehand. Only those who test negative should be involved in performing the test procedure.
- Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered.
- If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

3.2. Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure
- Hygiene requirements
- Donning and Doffing of PPE

- Laboratory biosafety, specifically handling of potentially infectious materials
- Laboratory design, including airflow conditions
- Use of biological safety cabinets (operation, identification of malfunctions and maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators, (operation, identification of malfunctions and maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)
- Good laboratory practice and good microbiological techniques
- Workflow in laboratory
- Procedure to be performed
- Waste handling
- Importance of laboratory results for individual patient and COVID pandemic management

3.3. Responsibilities

All staff members trained to use the GeneXpert System working in the laboratory are responsible for the implementation of this Standard Operating Procedure.

Lab Technician or Consultants who are trained, certified and designated by the laboratory to collect and process infectious specimens.

4. Pre-analytic Procedure

Refer to the following procedures

Specimen collection and transport (SOP001)

Specimen accessioning, aliquoting and long-term storage (SOP003)

Important: Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen

4.1. Equipment

- GeneXpert instrument - Cepheid GeneXpert GX-IV,
- Xpert Xpress SARS-CoV-2 1X10 tests kit per box
- Computer & Barcode scanner - Operator manual

4.2. Materials

The Xpert Xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes	10
· Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
· Lysis Reagent	1.5 mL per cartridge
· Binding Reagent	1.5 mL per cartridge
· Elution Reagent	3.0 mL per cartridge
Disposable Transfer Pipettes	10-12 per kit
CD · Assay Definition File (ADF) · Instructions to import ADF into GeneXpert software	1 per kit
Flyer · Directions to locate the Product Insert on www.cepheid.com	1 per kit

4.3. Storage and Handling

• Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.

• Do not open a cartridge lid until you are ready to perform testing.

• Do not use a cartridge that is wet or has leaked.

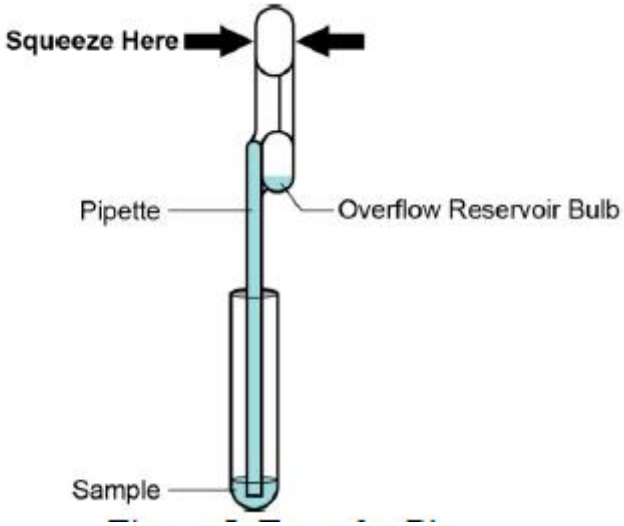
4.4. Calibration Procedures (Metrological Traceability):

Equipment calibration: Once a year

5. Analytic Procedure

5.1. Preparing the Cartridge

- Remove a cartridge from the package.
- Check the specimen transport tube is closed.
- Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the
- specimen transport tube.
- Open the cartridge lid.
- Remove the transfer pipette from the wrapper.



- Squeeze the top bulb of the transfer pipette completely and then place the pipette
- Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (as shown in the above Figure). Check that the pipette does not contain bubbles.
- To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening

Figure 6. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

Note	Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

(Sample Chamber) in the cartridge shown in Figure 6. Dispose of the used pipette.

- Close the cartridge lid.
- Load the cartridge into the GeneXpert Dx instrument or Infinity system.

Important: Start the test within 30 minutes of adding the sample to the cartridge.

5.2. Starting the Test

This section lists the basic steps for running the test.

- Turn on the GeneXpert instrument:
- Log on to the GeneXpert Instrument System software using your user name and password.
- In the GeneXpert System window, click **Create Test** (GeneXpert Dx).
- Type the Patient ID, make sure the Patient ID is typed correctly. The Patient LRN is associated with the test results and is shown in the View Results window.
- In case Sample ID is used then Scan or type in the Sample ID and, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports. The Scan Cartridge dialog box appears.
- Scan the barcode on the Xpert Xpress SARS-CoV-2 Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- Click **Start Test** (GeneXpert Dx). Enter your password, if requested.
- For the GeneXpert Dx Instrument:
- Open the instrument module door with the blinking green light and load the cartridge.
- Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
- Wait until the system releases the door lock before opening the module door and removing the cartridge.
- The used cartridges should be disposed in the appropriate specimen waste containers according to your institution's standard practices.

5.3. Viewing and Printing Results

- Click the **View Results** icon to view results.
- Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

6. Quality Control Procedures:

6.1. Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control

(PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly.

The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

External Controls – External controls, not available in the kit, should be used in accordance with local, state, and federal accrediting organizations' requirements as applicable.

7. Interpretation of Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. · The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting · SPC: NA; SPC is ignored because coronavirus target amplification occurred · Probe Check: PASS; all probe check results pass

Result	Interpretation
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. · The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting · SPC: NA; SPC is ignored because a target amplification has occurred. · Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. · The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting · SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting · Probe Check: PASS; all probe check results pass

Result	Interpretation
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. · SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting · Probe Check – PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. · SARS-CoV-2: NO RESULT · SPC: NO RESULT · Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

8. Retests

8.1. Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to the instructions in Section 14(Table 2, Retest Procedure.

- An INVALID result indicates one or more of the following:

- The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the assay was aborted. Possible causes include: insufficient volume of sample was added, the reaction tube was filled improperly, a reagent probe integrity problem was detected, or the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress, or a power failure occurred.

9. Performance Characteristics:

9.1. Clinical Evaluation:

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium. A total of 45 SARS-CoV-2 positive and 45 SARS-CoV-2 negative NP swab specimens were tested with Xpert Xpress SARS-CoV-2 in a randomized and blinded fashion. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress SARS-CoV-2 test relative to the expected results. Results of these 90 archived clinical NP swab specimens are shown in Table 3. The PPA was 97.8% (95% CI: 88.4% - 99.6%) and the NPA was 95.6% (95% CI: 85.2% - 98.8%).

Table 3. Xpert Xpress SARS-CoV-2 Performance Results

		Expected Results		
		Positive	**Negative**	**Total**
Xpert Xpress SARS-CoV-2	**Positive**	44[a]	2[b]	46
	Negative	1	43	44
	Total	45	45	90
PPA		97.8% (95% CI: 88.4% - 99.6%)		
NPA		95.6% (95% CI: 85.2% - 98.8%)		

a. One specimen was reported as "SARS-CoV-2 Presumptive Pos" in initial testing and yielded a "SARS-CoV-2 Positive" test result upon retesting.
b. The two false positive specimens were collected during the COVID-19 pandemic.

9.2. Analytical Performance

9.2.1. Analytical Sensitivity (Limit of Detection)

The LoD is the lowest concentration (reported as PFU/mL) of live SARS-CoV-2 virus samples that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2 using one lot of reagent and limiting dilutions of live SARS-CoV-2 virus (USA_WA1/2020) prepared in viral transport medium and NP swab clinical matrix. The concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 0.0050 and 0.0200 PFU/mL for the N2 target and E target, respectively (Table 4). Verification of the estimated LoD claim was performed on one reagent lot in replicates of 20 prepared in pooled NP swab clinical matrix. The claimed LoD is 0.0200 PFU/mL (Table 4)

Table 4. LoD Determination using USA-WA1/2020 Strain

Strain	Concentration (PFU/mL)	Total Valid Results	Hit Rate (%) N2 Target	Hit Rate (%) E Target	Mean Ct N2 Target	Mean Ct E Target
SARS-CoV-2 virus (USA_WA1/2020)	0.0200	20	100	95.0	38.3	36.4
	0.0050	22	95.5	68.2	40.5	39.1
	0.0025	22	90.9	36.4	41.5	39.6
	0.0010	22	50.0	18.2	42.0	42.0
	0.0005	22	45.5	18.2	41.7	41.5
	0.0003	22	18.2	4.5	42.1	44.9
	0.0001	22	9.1	0	42.9	N/A
	0	0	0	0	N/A	N/A

9.2.2. Analytical Specificity (Exclusivity)

An in silico analysis for possible cross-reactions with 39 organisms was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2 test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms is expected based on the in silico analysis

10. Spill Management

Refer to SOP# 004 on spill management for handling the infectious spills.

10.1. BIOHAZARD WASTE DISPOSAL

- All solid waste (tips, gloves, packaging, etc) collected in the specimen processing room should be discarded only in labelled biohazard bags (Labelled as COVID-19 WASTE) inside the biosafety cabinet. Filled Biohazard Bags should be tied inside the biosafety cabinet with tag.
- Removed PPE should be discarded in marked designated bins. Bags should be tied and labelled.
- Tied and labelled biohazard bags should be autoclaved at 121°C and 15 psi for 60 minutes (gravity flow) and 45 minutes in vacuum autoclave.
- Note: Waste containing sodium hypochlorite should never be autoclaved.
- Autoclaved waste should be weighed and clearly labeled as "COVID-19 waste" and handed over to Housekeeping Staff.
- Housekeeping Staff should take the autoclaved waste to designated area for pickup and incineration

- Any incidents including spills, mechanical breakdowns, failure in bio-containment or any other maintenance problem should be reported immediately to the biosafety officer.
- Any incidence of exposure to personnel should be reported to the officer in charge.

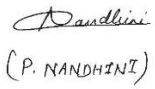
Refer to Ref: SOP# 005 on Biomedical Waste Management.

References:

1. Xpert® Xpress SARS-CoV-2. REF- XPRSARS-COV2-10., Kit Insert, October 2020.

https://www.cepheid.com/Package%20Insert%20Files/Xpert%20Xpress%20SARS-CoV-2%20Assay%20ENGLISH%20Package%20Insert%20302-3787%20Rev.%20B.pdf

FIND Because diagnosis matters	Detection of SARS-CoV-2 using Truenat			
Country	India	DOCUMENT NUMBER	VERSION	EFFECTIVE DATE
		SOP-007	1.0	

	Name, Title	Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist		13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager		21 Jan 2022

Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)
	Nil		

SOP Annual Review	Name, Title	Signature	Date
	Nil		

Distributed Copies to	Name (or location)	# of copies	Name (or location)	Date

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.

1. Introduction

Truenat SARS-CoV-2 testing kits work on the same principle on Real-time Reverse Transcription Polymerase Chain Reaction (RTPCR) based on TaqMan chemistry (REFERENCE 1). 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.

2. PERSONNEL QUALIFICATIONS

2.1. Medical fitness

- All personnel involved in specimen receipt and handling should be tested for COVID-19 beforehand. Only those who test negative should be involved in performing the test procedure.
- Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered.
- If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

2.2. Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure
- Hygiene requirements
- Donning and Doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials
- Laboratory design, including airflow conditions
- Use of biological safety cabinets (operation, identification of malfunctions and maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators, (operation, identification of malfunctions and maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)

- Good laboratory practice and good microbiological techniques
- Workflow in laboratory
- Procedure to be performed
- Waste handling
- Importance of laboratory results for individual patient and COVID pandemic management

3. 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.

4. Pre-analytic Procedure

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen

Refer to the following procedures:

1. Specimen collection and transport (SOP001)
2. Specimen accessioning, aliquoting and long-term storage (SOP003)

4.1. EQUIPMENT & MATERIALS

4.1.1. Truelab® Real Time micro PCR Workstation consisting of Trueprep® AUTO/AUTO v2 Sample Prep Device

- Truelab® Uno Dx/Truelab® Duo/Truelab® Quattro Real Time micro PCR Analyzer
- Truelab micro PCR Printer
- Truepet® SPA fixed volume precision micropipette - 6 µl.
- Truelab® Microtube Stand

4.1.2. 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
- or Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit
- Truenat™ Universal Control Kit

4.1.3. Powder free disposable gloves,

4.1.4. Waste disposal container with lid.

4.1.5. Autoclave

4.1.6. Biosafety Cabinet (Optional)

4.1.7. Refrigerators

4.1.7.1. -70/ -80°C, -20°C freezers

4.1.7.2. 4°C Refrigerators

4.1.7.3. Benchtop minifuge

4.1.8. Reagents & Disinfectants

4.1.9. RNAse Zap

4.1.10. Truenat Covid -19 RT- PCR Kit that contains

4.1.10.1. Trueprep Auto Universal Extraction Kit

4.1.10.2. Truenat Covid 19 duplex Detection Kit

4.1.10.3. Universal Control Kit Panel 1

4.1.10.4. 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.

4.1.11. Nuclease free Water

4.1.12. Reagent grade Ethanol and Isopropanol

4.1.13. Absolute alcohol

4.1.14. Sodium Hypochlorite

4.1.15. Biohazard bags Red &Yellow

4.1.16. Personal Protecting Equipment (PPE)

4.1.16.1. Lab gowns

4.1.16.2. Surgical/medical masks/

4.1.16.3. N95 Mask

4.1.16.4. Gloves

4.1.16.5. Shoe Covers

4.1.16.6. Hair Covers

5. Analytic Procedure

5.1.Principle:

Truenat SARS-CoV-2 testing kits work on the same principle on Real-time Reverse Transcription Polymerase Chain Reaction (RTPCR) based on Taqman chemistry. The RNA from the patient sample is first extracted using Trueprep AUTO Universal Cartridge based Sample prep device and prep kit. In the Truelab Real time Quantitative micro PCR Analyzer 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 labelled fluorescent probe in the Truenat 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 an amplification curve on the analyzer screen, on a real-time basis during the test run.

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. The absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run.

Sample processing protocol on Trueprep® AUTO (Fig.1)

1. Wear personal protective equipment (PPE) as per standard protocol

2. Open the cap of the transport medium for the swab specimen tube

3. Transfer 0.5 ml of swab sample into the Lysis Buffer tube using 1 ml Transfer pipette

4. Discard the Transfer pipette

5. Remove the Cartridge from the pouch, label it and place it on the Cartridge stand. Keep the Elute collection tube (ECT), ECT label and elute transfer pipette in the pouch for later use

6. Transfer the entire content of the Lysis Buffer tube to the Sample Chamber (Black Cap) of the Cartridge using a 3 ml transfer pipette. After transferring, discard the pipette.

7. Switch "ON" the Trueprep® AUTO Sample Prep Device and press the "EJECT" button to open, gently pull out the door (cartridge holder)

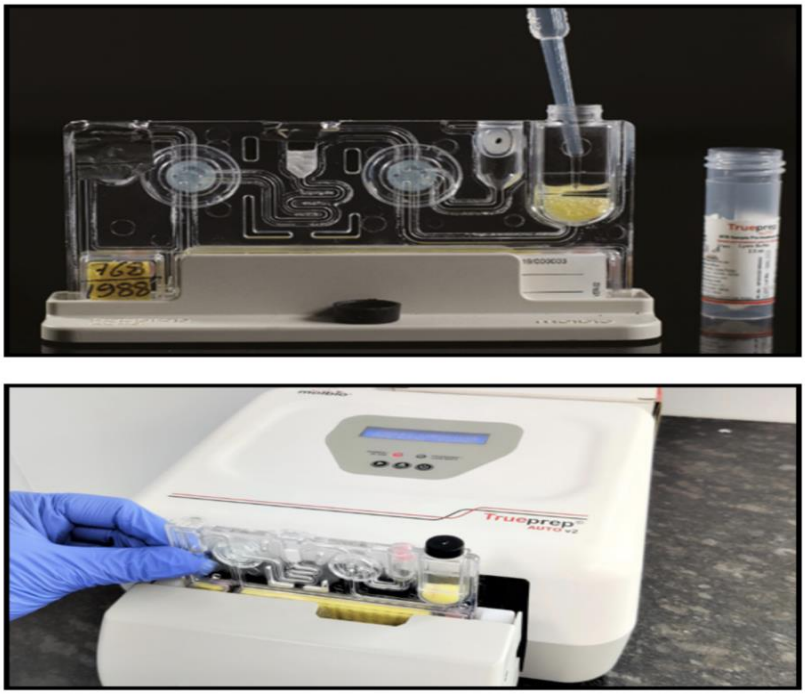
8. Insert the cartridge and gently push the door to close it and press "START"

9. The device will beep at the end of the process (20 minutes) and the Cartridge holder will eject automatically

10. Gently pull out the Cartridge holder, remove the Cartridge, place it on the cartridge stand

11. Pierce the ELUTE CHAMBER with the provided transfer pipette, transfer the entire volume into Elute Collection Tube (ECT), label the ECT tube with patient details. Discard the transfer pipette and Cartridge.

Figure.1 Trueprep® AUTO



5.2. Testing

6.2.1 Switch on the Truelab™ Analyzer

6.2.2 Select User and enter password.

6.2.3 Select the test profile for "H1N1" to be run from the TM Profiles Screen on the Analyzer screen.

6.2.4 Enter the patient details i.e. Patient Name, Patient ID and Age in the Truelab™ Analyzer screen.

6.2.5 Press Start Reaction.

6.2.6 Press the eject button to open the chip tray.

6.2.7 Open a pouch of Truenat™ Covid 19 and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.

6.2.8 Label the tube with the patient ID using a marker pen on the microtube.

6.2.9 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 (Figure 2).

6.2.10 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.

Note:- Do not mix it by tapping, shaking or by reverse pipetting.

6.2.11 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.

6.2.12 Slide the chip tray containing the Truenat™ Covid 19 chipTM based

6.2.13 Real Time PCR test loaded with the sample into the TruelabAnalyzerTM.

6.2.14 Press Done on the "Please Load Sample" Alert message

6.2.15 Read the result from the screen.

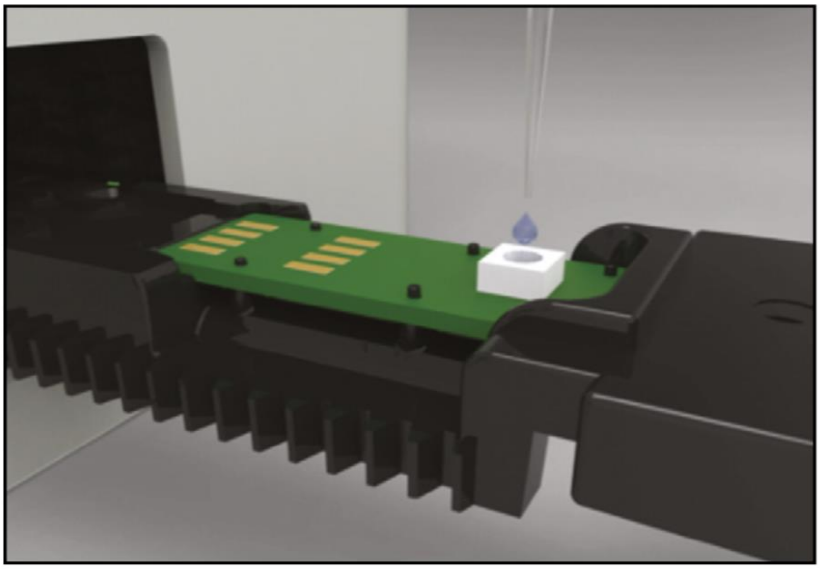
6.2.16 After the reaction is completed, for Truelab Uno Dx, push the Eject button to TM eject the chip tray.

6.2.17 Take out the Truenat™ Covid 19 chip-based Real Time PCR test at end of the test and dispose it off IN 1% hypochlorite.

6.2.18 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.

6.2.19 Switch off the Truelab™Analyzer or repeat steps 3 - 16 to run another sample.

Figure 2: Loading Truelab Analyzer



Transferring the solution to a Truenat chip

5.3. 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.

5.4. 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.

6. 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

Do and Don'ts of Truenat testing should be appropriately followed for producing quality results (Annexure. 2)

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. WASTE MANAGEMENT, CONTAMINATION CONTROL

8.1. 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.

If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

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 (Annexure 1)

II. Establish a regular (e.g. weekly) and thorough laboratory cleaning protocols (floors, doors, walls)

8.2. Contamination Control

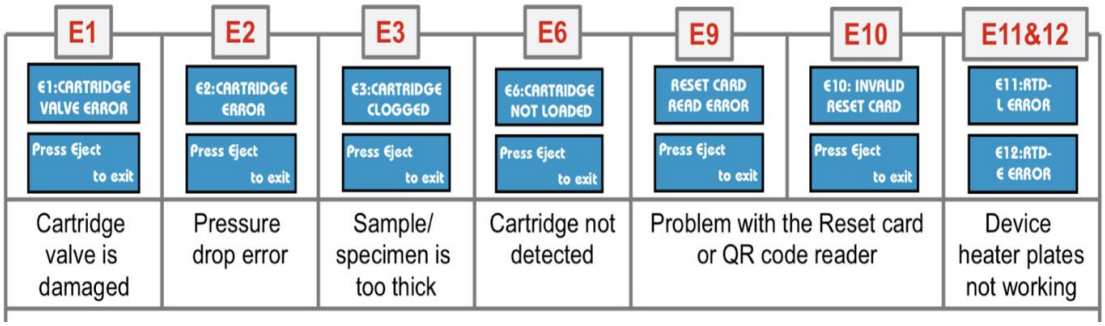
8.2.1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with

disinfectants such as 0.5% freshly prepared Sodium hypochlorite [10 times dilution of 5% Sodium hypochlorite (household bleach)] before continuing work.

8.2.2. 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

9. Troubleshooting

Trueprep Auto v2 Universal Cartridge based Sample Prep Device (REFERENCE 2)



Solution:

E1/E2: The test can be started again by processing the remaining sample in the lysis buffer and can be loaded into a new cartridge.

E3: Sample shall be properly liquefied and made pipettable. The extraction can be repeated with new cartridge or a new sample can be requested for repeat testing

E6: The cartridge shall be loaded properly in correct orientation

E9/10/11/12: The support of the Manufacturer can be taken for resolving these errors

Truelab™ Real Time Quantitative micro PCR Analyzer

ERROR 1	ERROR 2	ERROR 3	ERROR 4	ERROR 5	INVALID
Thermal cycling error	Test stopped manually	Incorrect optical profile	Runtime Error	Probe check Error	Internal Control did not amplify in PCR or improper sample extraction

Solution:

Error1/2/3/4/5: The run shall be repeated using a fresh chip using the remaining elute

Invalid: The same elute can be used to run using another chip. If invalid repeats, the sample can be processed again and this elue can be tested using another chip

Alert/Error Message	Reason	Action
Unable to read chip information	The Analyzer was unable to read chip memory	Tap 'OK' on the Read Error Prompt. Check if chip was loaded properly into the tray. If so, remove the chip and re-select the profile from Status Screen and repeat the steps. If message reappears, load a new chip and re-load the elute again.
Could not initialize. Please try again	The system was unable to establish an internal connection	Please attempt the test again by using a new chip and re-loading the elute again.Contact Molbio support team if the problem persists.
Chip is already used	User loaded a used chip in the Chip Tray	Please use a fresh chip and re-load the elute.
Chip loaded is expired	User loaded an expired chip in the Chip Tray.	Please use a fresh chip and re-load the elute.
Error 1	Thermal cycling error Chip is faulty	Please repeat run with the elute by pressing Repeat button. Contact Molbio's support team if the problem persists.
Error 2	Test stopped Manually. User has manually stopped/aborted run	Repeat run with elute, using another chip
Error 3	Incorrect optical profile. Deviation in the expected optical profile	Please repeat run with the elute by pressing Repeat button. Contact Molbio's support team if the problem persists.
Error 4	Runtime Error run data analysis by machine is incomplete	Please repeat run with the elute by pressing Repeat button. Contact Molbio's support team if the problem persists.

Error 5	Low initial signal due to insufficient mastermix dispensed onto the chip.	Please repeat run with the elute by pressing Repeat button. Contact Molbio's support team if the problem persists
Invalid	Internal Control didn't amplify. Poor sample collection or extraction error	Collect another swab sample from patient, process the sample and repeat run with elute, using another chip

*While IPC will co-amplify in most positive cases also, in some specimens 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.

10. 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.

11. REFERENCES

12.1 Truenat™ Operating Manual

https://www.molbiodiagnostics.com/uploads/product_download/20211115.173628~Truenat-COVID-19-packinsert-VER-04.pdf

12.2 Practical Guide to Implementation of Truenat™ Tests

http://stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf

12. Annexure

12.1. Annexure 1: Truenat Preventive Maintenance Log

Truelab and Trueprep maintenance log																															Version: 1.1 Date:
Compiled by:					Reviewed by:													Approved by:													
Month:																															
	Days of the month																														
Maintenance activity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Daily maintenance																															
Clean work area																															
Discard used chips and cartridges																															
Monthly maintenance																															
Disinfect instrument surfaces	Date: Initials:																														
Clean Truelab bays	Date: Initials:																														
Temperature calibration	Date: Initials:																														
Verification of the fixed 6µl pipette	Date: Initials:																														
As necessary																															
Flush protocol for the Trueprep instrument																															
Spillage tray or linear motion guide tray replacement																															
Slider glass replacement - indicate bay																															

Notes:

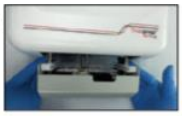
Indicate completion of an activity by writing your initials in the corresponding date box.

- Slider glass should be replaced after running at most 50 tests and/or when related errors occur.
- Temperature calibration of the Truelab should be done on a monthly basis and/or when error related to temperature occurs and/or when temperature curve is abnormal, i.e having blips. Normal values: 3.39-3.42.
- Flush protocol for the Trueprep instrument should be done if no test will be run for the next 10 days and/or when errors relating to extraction process occur.
- Spillage tray and linear motion guide trays should be replaced when there is sample spillage on the trays during extraction

12.2. Annexure 2. Do and Don'ts of Truenat testing

Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

- ✓ Place the device in a dust free environment
- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Replace the cartridge holder tray in case of a spillage in the device



- ✓ If the device is kept idle for more than 10 days, carry out the flushing protocol (Refer manual section 8.2.3) before resuming operation

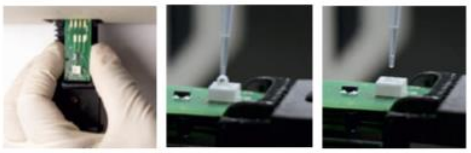
- ✗ Do not leave a used cartridge inside the device. Remove the cartridge after extraction and collect the elute in the ECT
- ✗ Do not keep used material on the work bench and on the device

- ✗ Do not tilt or shake the device while connecting charger, during extraction or while removing cartridge from device
- ✗ Do not touch the nozzle of the liquefaction buffer bottle to the lysis buffer tube during sample preparation

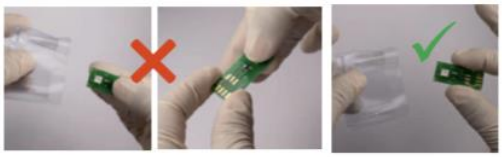
Truelab® Uno Dx/Duo/Quattro Real Time Quantitative micro PCR Analyzer

- ✓ Place the analyzer on a flat surface in a dust free environment, away from direct sunlight and vibration causing instruments

- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Ensure that the elute is loaded in the center of the reaction well



- ✗ Do not move the analyzer when a test is in progress
- ✗ Do not spill water or any other solution on the surface of the analyzer
- ✗ Do not touch the white reaction well or the connector pads of the chip. Hold the chip by the edges only



- ✗ Do not leave a used chip inside the device
- ✗ Do not keep used material on the work bench and on the device

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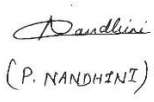
		SOP-004	1.0	

	Name, Title		Signature	Date
Prepared By	Dr. Nandhini Palani, Training Lead			7 Jan 2022
Reviewed By	Dr. Lakshmi Soundarajan, Senior Microbiologist			13 Jan 2022
Approved By	Ms. Preetishirin Fredrick Katapur, Deputy Project Manager			21 Jan 2022
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1. PURPOSE

To provide instructions for cleanup of infectious spills in COVID laboratory.

2. INTRODUCTION

Spill during any testing procedure (pre examination, examination and post examination) can be hazardous to laboratory health professionals. Spill cleanup is performed according to the type, location, and size of the spill. PPE is required for cleanup of an accidental spill. Spill kits should be readily available in the laboratory. Each organization should have management protocols or procedures for hazardous spills, and employees should be familiar with them. All spills must be reported and documented according to the organization's practice (REFERENCE 1).

3. PERSONNEL QUALIFICATIONS

3.1 Medical fitness

All personnel involved in sample collection, receipt and analysis should be tested for COVID-19 beforehand. Only those who test negative should be involved in testing. Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered. If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

3.2 Education and training

The lab personnel must have received education and training on the following topics before performing this procedure:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure
- Hygiene requirement
- Use of protective equipment and clothing
- Handling of potentially infectious materials
- Laboratory design, including airflow conditions
- Use of biological safety cabinets (operation, identification of malfunctions, maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators (operation, identification of malfunctions, maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)
- Good laboratory practice and good microbiological techniques

- Organization of workflow & procedures
- Waste handling
- Importance of laboratory results for patient management
- Importance of laboratory results for COVID pandemic management

4. 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 or unable to carry it out as described are responsible for seeking advice from their supervisor.

5. EQUIPMENT & MATERIALS

5.1 Spill Kit

5.1.1 Sodium Hypochlorite - stock solution

5.1.2 Premarked container for 1% Sodium Hypochlorite

5.1.3 Falcon tube for measuring stock solution

5.1.4 70% Alcohol

5.1.5 Absorbent towels

5.1.6 Biohazard bags (yellow and red)

5.1.7 Shoe cover

5.1.8 N95 masks

5.1.9 Gloves

Note: 1% sodium hypochlorite solution needs to be made fresh. Therefore, a pre-marked disinfectant bottle containing indicated volume of water can be stored in the spill kit. Just before use add required amount of sodium hypochlorite stock solution to the bottle to produce 1% sodium hypochlorite.

5.2 PPE

5.2.1 Lab gowns

5.2.2 N95 mask

5.2.3 Goggles and/or face shield

5.2.4 Gloves

5.2.5 Shoe Cover

5.2.6 Hair Cover

5.3 Spill Incident Logbook

6. PROCEDURE

6.1 Leakage in sample transport box

6.1.1 Notify laboratory staff and people nearby immediately

6.1.2 Cordon off the area and restrict access

6.1.3 Place the leaky transport box in a biohazard bag and close the bag.

6.1.4 Wipe contaminated surfaces with paper towels soaked in freshly prepared 1% sodium hypochlorite

6.1.5 Discard the paper towels and other contaminated items to dispose in the biohazard bag.

6.1.6 Transport these bags to autoclave facility

6.1.7 Document the spill incident.

6.2 Infectious spill inside the biosafety cabinet

6.2.1 Place absorbent tissue over the spill and pour appropriate amount of 1% sodium hypochlorite

6.2.2 Leave affected areas with disinfectant for at least 15 minutes. Do not turn off the BSC.

6.2.3 Carefully collect contaminated material and place in biohazard container for disposal inside the BSC

6.2.4 Wipe up spill, work surfaces, walls, and any equipment in the BSC with paper towels dampened with decontaminant. If using bleach, follow with sterile water and then 70% alcohol wipe to protect metal surfaces from corrosion.

6.2.5 Any equipment or reusable material that has been splashed should be cleaned with the same disinfectant

6.2.6 Remove any contaminated PPE in a manner to avoid cross-contamination

6.3 Infectious spill outside the biosafety cabinet in the specimen processing area

6.3.1 Ask everyone to immediately vacate the affected laboratory area

6.3.2 If the spill has contaminated your gown and shoe cover, spray them with disinfectant before stepping out of the room

6.3.3 Remove contaminated PPE and place in the biohazard bag. Change contaminated clothing items and place them in autoclave bag for decontamination later on. Disinfect your hands and remove N95.

6.3.4 Wear fresh set of PPE

6.3.5 Signs should be posted indicating that entry is forbidden during the clean-up procedure

6.3.6 Laboratory manager should be informed of the incident immediately

6.3.7 Staff must be prevented from re-entering the laboratory for at least 30 minutes hour to allow aerosols to be removed through the laboratory's ventilation system and allow time for heavier particles to settle

6.3.8 Standard operating procedures for spill clean-up MUST be followed

6.3.9 Incident should be documented

6.3.10 Spill cleanup procedure (REFERENCE 2)

6.3.10.1 Put on gloves, a protective laboratory gown and respirator and goggles

6.3.10.2 Re-enter affected area

6.3.10.3 Cover spill with cloth or paper towels to contain it

6.3.10.4 Pour freshly prepared 1% sodium hypochlorite over paper towels and immediate surrounding area

6.3.10.5 Apply disinfectant concentrically, beginning at outer margin of the spill and working towards center

6.3.10.6 Allow sufficient time for the disinfectant to act before clearing away any material for disposal

6.3.10.7 Clean up the contaminated area and place any contaminated material in a biohazard bag for disposal

6.3.10.8 Disinfect the contaminated equipment using 1% sodium hypochlorite. After a contact time of at least 15 minutes, remove the residual salts of sodium hypochlorite by wiping the equipment with water and then 70% ethanol.

6.3.10.9 Remove contaminated PPE before resuming your work.

7. BIOHAZARD WASTE DISPOSAL

7.1 All solid waste (tips, gloves, packaging, etc) collected in the BSL2 laboratory should be discarded only in labelled biohazard bags (Labelled as COVID-19 WASTE) inside the biosafety cabinet. Filled Biohazard Bags should be tied inside the biosafety cabinet with tag.

7.2 Removed PPE should be discarded in marked designated bins. Bags should be tied and labelled.

7.3 The tied and labelled biohazard bags should be autoclaved at 121°C and 15 psi for 60 minutes (gravity flow) and 45 minutes in vacuum autoclave.

7.4 Autoclaved waste should be weighed and clearly labeled as "COVID-19 waste" and handed over to Housekeeping Staff.

7.5 Housekeeping Staff should take the autoclaved waste to designated area for pickup and incineration

7.6 Any incidents including spills, mechanical breakdowns, failure in bio-containment or any other maintenance problem should be reported immediately to the biosafety officer.

7.7 Any incidence of exposure to personnel should be reported to the officer in charge.

7.8 Refer SOP# 005 on Biomedical Waste Management.

8. REFERENCES:

1. Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV) Interim guidance 12 February 2020

   https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf

2. Laboratory biosafety manual, 3rd edition
   https://www.who.int/publications/i/item/9241546506

FIND Because diagnosis matters	Biomedical waste management			
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		SOP-005	1.0	

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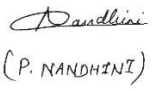
Prepared By	Dr. Nandhini Palani, Training Lead		7 Jan 2022
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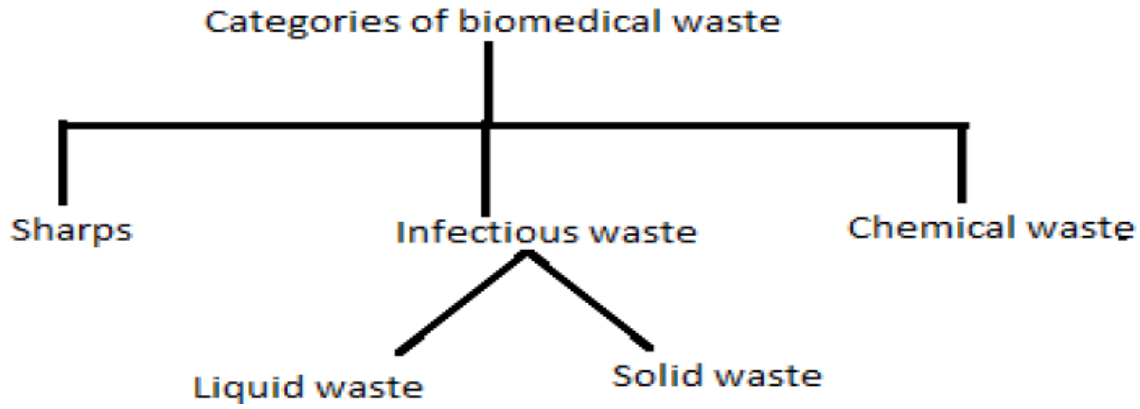
1. PURPOSE

To describe the procedure of decontamination and disposal of the clinical specimens and the other infectious consumables that are generated in the covid-19 lab.

2. INTRODUCTION

Biomedical waste (BMW) is represented by solids, liquids, sharps and laboratory waste, which are generated as the result of healthcare activities for human beings (Fig.1).

Fig.1. Categories of biomedical Waste



Infectious waste is material suspected to contain pathogens (bacteria, viruses, parasites or fungi) in sufficient concentration or quantity to cause disease in susceptible hosts. This category includes liquid waste, contaminated with blood or other body fluids, cultures and stocks of infectious agents from laboratory work, solid waste materials like plastic consumable including vials, tubes, pipette tips, PCR plates, processed specimen or other body fluids which is Infectious because it contains bacteria, viruses, parasites or fungi.

3. PERSONNEL QUALIFICATION

3.1. Medical fitness

All personnel involved in sample receiving should be tested for COVID-19 beforehand.

3.2. Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Hygiene requirements
- Donning and doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials

- Workflow in the laboratory
- Waste handling
- Importance of laboratory results for patient management

4. Responsibilities

It is the responsibility of the laboratory 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

- PPE to be used
- Separate color coded bins/ bags/ container/ trolleys
- Autoclave
- Biological and Chemical Indicators
- Stationeries
- Logbooks
- Disinfectants
    - Ethanol/Isopropyl alcohol
    - Sodium Hypochlorite stock solution

6. PROCEDURE

PPE to be used: Lab coat, N95 mask, gloves and goggles

Detailed Instructions:

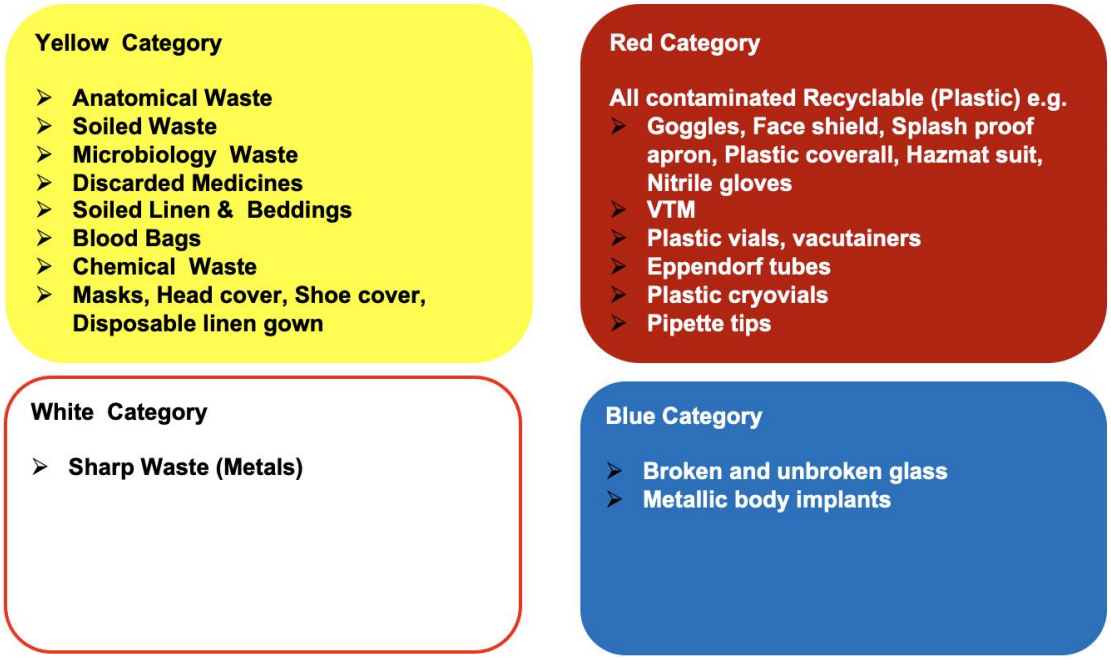
Ensure the availability of the PPE in place and the required materials are all present

The Laboratory personnel has to be supervising the activity of the housekeeping staff during the complete protocol

6.1 Biomedical Waste Segregation

- Keep separate color-coded bins/bags/containers in wards and maintain proper segregation of waste as per BMWM (Biomedical Waste Management) Rules, 2016 as amended and CPCB (Central Pollution Control Board) guidelines for implementation of BMW Management Rules.

- Following color coded bags should be used for disposal of different categories of biomedical waste generated (REFERENCE 1).



Report opening or operation of COVID-19 lab to SPCBs (State Pollution Control Board) and respective CBWTF (Common Bio Medical Waste Treatment Facility) located in the area (REFERENCE 2)

- As precaution double layered bags (using 2 bags) should be used for collection of waste from COVID-19 isolation wards so as to ensure adequate strength and no-leaks.
- It is mandatory for bags/containers used for collecting biomedical waste from COVID-19 labs should be labelled as "COVID-19 Waste".
- Use a dedicated collection bin and trolleys labelled as "COVID-19" to store COVID19 waste and keep separately in temporary storage room prior to handing over to authorized staff and followed by to the Biomedical waste handling agency.
- The (inner and outer) surface of containers/bins/trolleys used for storage of COVID19 waste should be disinfected with 1% sodium hypochlorite solution daily.
- Collect used PPEs such as goggles, face-shield, splash proof apron, Plastic Coverall, Hazmat suit, nitrile gloves into RED bag.
- Collect used masks (including triple layer mask, N95 mask, etc.), head cover/cap, shoe-cover, disposable linen Gown, non-plastic or semi-plastic coverall in YELLOW bags.
- Pre-treat viral transport media, plastic vials, vacutainers, Eppendorf tubes, plastic cryovials, pipette tips with 1% hypo solution and drain away the liquid into the sink connected to the effluent system and then dispose in RED bags.

- PPEs used and other contaminated waste generated from lab waste handlers, and they have to be stored separately in YELLOW bag shall be Pre-treated with Autoclaving / Microwaving before transfer to temporary storage area and then hand over to Common treatment Facility in YELLOW Colored bags with specific marking as "COVID-19 Waste". (Biomedical Waste Agency signed in with the MOU)

6.2 Autoclaving Procedure

The autoclave should be dedicated for the purposes of disinfecting and treating bio-medical waste.

6.2.1 When operating gravity flow autoclave:

- Use a temperature of not less than 121° C and pressure of 15 pounds per square inch (psi) for an autoclave residence time of not less than 60 minutes
- Use a temperature of not less than 135° C and a pressure of 31 psi for an autoclave residence time of not less than 45 minutes
- Use a temperature of not less than 149° C and a pressure of 52 psi for an autoclave residence time of not less than 30 minutes

6.2.2 When operating a vacuum autoclave:

- Biohazard waste shall be subjected to a minimum of three pre-vacuum pulse to purge the autoclave of all air
- The air removed during the pre-vacuum, cycle should be decontaminated by means of HEPA and activated carbon filtration, steam treatment, or any other method to prevent release of pathogen

6.2.3 When operating a vacuum autoclave, the waste shall be subjected to the following:

- A temperature of not less than 121°C and pressure of 15 psi per an autoclave residence time of not less than 45 minutes
- A temperature of not less than 135°C and a pressure of 31 psi for an autoclave residence time of not less than 30 minutes

6.3 Quality control for autoclave

5.3.1 Routine Test:

- ❖ A chemical indicator strip or tape that changes color when a certain temperature is reached
- ❖ Use more than one strip over the waste package at different locations to ensure that the inner content of the package has been adequately autoclaved
- ❖ Records for this test needs to be maintained

5.3.2 Spore testing:

- ❖ Biological indicators in the form of vials or spore Strips; with at least 1X106 spores should be used at least once in every week to validate the autoclaving efficiency of decontamination process

- ❖ Records should be maintained

6.4 Validation test for Autoclave

Procedure

- Four biological indicator strips to be used, one shall be used as a control and left at room temperature, and 3 shall be placed in the approximate center of 3 containers with the waste.

- At least 1 of the containers with a biological indicator should be placed in the most difficult location, generally the bottom centre of the waste pile.

- This test has to be performed 3 consecutive times to define minimum operating conditions. After determining the minimum temperature, pressure and residence time, the operator/occupier shall conduct this test once in 3 months and records in this regard shall be maintained

7. REFERENCES:

7.1. Bio-medical_Waste_Management_Rules_2016

https://dhr.gov.in/sites/default/files/Bio-medical_Waste_Management_Rules_2016.pdf

7.2. Guidelines for Handling, Treatment and Disposal of Waste Generated during Treatment/Diagnosis/ Quarantine of COVID-19 Patients. Revision 4.2020

https://mpcb.gov.in/sites/default/files/biomedical-waste/WasteGeneratedduringTreatmentDiagnosisQuarantineofCOVID1928102020.pdf

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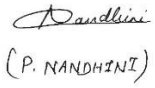
Prepared By	Dr. Nandhini Palani, Training Lead			7 Jan 2022
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1. PURPOSE

The purpose of this SOP is to outline the Internal quality control plan and External quality assurance policy and also to document procedures of the same.

2. SCOPE

This applies to the Covid Testing Lab staff who are participating in the testing process.

Acronymns

- PT - Proficiency Testing
- ILC - Inter Laboratory Comparison
- EQAS -External Quality Assurance Scheme
- QC – Quality Control
- QA – Quality Assurance
- IQC – Internal Quality Control

3. RESPONSIBILITIES

- Laboratory technicians for performing the tests as per Internal Quality Control plan
- Supervisor to verify and approve that daily PCR and other test runs are valid and to troubleshoot and bring to the notice of dept head in case of QC failures.
- For External QA- Lab supervisor to allot the PT samples along with routine samples and ensure EQA plan is complied with.
- Microbiologist responsible for interacting with Mentoring/referral lab for receiving and sending reports as per schedule. QA will be responsible for the communications.

PRECAUTIONS: Routine safety precautions as per protocol.

4. Policies of the laboratory

4.1. Internal Quality Control

With every batch of patient sample, appropriate internal quality controls are to be tested as mentioned below.

- If the results are not within the accepted criteria, the supervisor to be informed for review and troubleshooting.
- No patient sample result to be released if IQC does not pass.

- After troubleshooting and Corrective Action, when samples are re-run, it is advised to pick any 2 samples from the previous run (one positive and one negative) to be included to ensure continuity of valid results.

- The IQC has to be run with every batch of runs.

4.2. Inter Laboratory Comparison – ILQC as per ICMR

- As part of the Inter Laboratory Quality Control (ILQC), all the labs will have to send 5 random positive and 5 random negative samples every 6 months once to the designated QC labs as part of the ILQC protocol as follows.

- 5 negative samples randomly collected over 1 week.

- 5 positive samples randomly collected over 1 week (preferably Ct value between 25-35)

- All testing labs should liaise with the recommended QC labs and will have to ensure regular participation in QC activity.

- All testing labs will have to ensure proper storage of samples at -80°C.

- Proper labelling of the samples as per the instructions given in the ICMR QC portal has to be followed.

- While shipping, the samples should be aliquoted in screw capped vials and proper Biosafety and Biosecurity precautions should be followed as per IATA guidelines.

- All the QC results have to be entered in the QC portal https://covidqcqa.icmr.org.in/login.php and should be shared with the QC lab with the credentials of the individual labs.

- Labs are required to enter the results of only the SARS-CoV-2 target genes along with result of housekeeping gene.

4.3. External Quality Assurance

EQA is a process that allows COVID-19 testing laboratories to assess their performance by comparing their results with results from other laboratories within the network (testing and reference laboratories) via panel testing and retesting. One or more of the following EQA methods can be applied for COVID-19 molecular testing laboratories namely Proficiency testing, or Rechecking or Retesting.

i) Rechecking or retesting—

Samples that have been tested at one laboratory are retested at another laboratory, allowing for inter-laboratory comparison (ILC).

- The laboratory has to participate in an interlaboratory comparison (ILC) program when EQAS is not available, signing an MOU with another NABL accredited lab biannual.

- The laboratory has to integrate the interlaboratory comparison samples into the routine workflow in a manner that follows, as much as possible, the handling of patient samples.

- Interlaboratory comparison samples have to be examined by personnel who routinely examine patient samples using the same procedure as those used for patient samples.

4.4. Proficiency Testing

- An external PT provider sends a set of SARS-CoV-2 positive and negative simulated clinical samples for testing in different laboratories and the results of all laboratories are analyzed, compared, and reported back to the participating laboratories.

- The positive panels contain different genetic lineages of SARS-CoV-2.

- In order to assess quality of COVID-19 laboratory in India, ICMR was provided proficiency testing (PT) panels by World Health Organization-India through the Royal College of Pathologists of Australasia Quality Assurance Programs, Australia.

4.5. Alternative approaches

The laboratory sends samples to other NABL accredited laboratory for interlaboratory comparison for Covid 19 which do not have formal EQAS program.

Interlaboratory comparisons

- The laboratory participates in an interlaboratory comparison (ILC) program when EQAS is not available.

- The laboratory integrates interlaboratory comparison samples into the routine workflow in manner that follows, as much as possible, the handling of patient samples.

- Interlaboratory comparison samples are examined by personnel who routinely examine patient samples using the same procedure as those used for patient samples.

4.6. Split sampling

A known positive and negative sample will be split and given for processing between 2 technicians. The results will be compared and reviewed.

4.7. IQC Failures

- QC failure is an indicator of quality. every month calculate the failure rate as No. of QC runs failed/Total number of QCs run x 100.

- When the results do not correlate, perform troubleshooting systematically using a checklist.

- Based on the analysis, perform retesting/corrective action.

4.8. Analysis

- The analysis for the PT/EQAS, ILC and split sampling has to be the same like analyzing the clinical sample.
- No special consideration will be provided to these samples.
- The routine testing and QC procedure will be followed for analyzing these samples.
- The routine staff who performs the analysis of clinical specimens will perform the analysis of these samples.

4.9. Evaluation of laboratory performance

- The performance in interlaboratory comparisons program is reviewed and discussed with relevant staff.
- When predetermined performance criteria are not fulfilled (i.e. nonconformities are present), staff participates in the implementation and recording of corrective action. The effectiveness of corrective action is monitored.
- The returned results are evaluated for trends that indicate potential nonconformities and preventive action are taken.

4.10. Comparability of examination results

- Laboratory doesn't use different instruments and has dedicated equipment for use; the records of the same are maintained. The laboratory has a defined means of comparing the methods used and establishing the comparability of results for patient samples.
- The laboratory notifies users of any differences in comparability of results and discusses any implications for clinical practice when examination methods are changed.
- The laboratory documents, record and as appropriate, expeditiously acts upon results from the comparisons performed. Problems or deficiencies identified are acted upon and records of actions are documented. The Technical manager monitors the results of all the QA program and appropriate corrective, and preventive actions are implemented and recorded when predetermined performance criteria are not fulfilled.

4.11. Quality Indicators

- The laboratory has established quality indicators to monitor and evaluate performance throughout critical aspects of pre-examination, examination and post-examination processes in the start of every year.
- The process of monitoring quality indicators is planned, which includes establishing the objectives, methodology, interpretation, limits, action plans and duration of measurement.
- The indicators are monthly reviewed for performance evaluation, to ensure their continued appropriateness.

- The following are the quality indicators that are followed in the Covid testing Lab
    - No. of Rejections
    - No. of Repeat cases
    - No. of Internal QC/other failure
    - No. of Amended Reports
    - PT/EQAS Results
    - ILC Results
    - No. of Non- Conformances raised
    - No. of Incident/Accident
    - Equipment Breakdown/calibration

Pre/Post Training Quiz

Instructions for the trainer:

The trainer/instructor will administer the test before the training and collect the response.

The same test will be given to the trainee after the completion of the training.

The difference in scores will be recorded for training evaluation purpose.

Trainee Name: **Date:**

Designation:

Test taken before the training (yes/no):

Test taken after the training (yes/no):

Instructions for the trainee:

The test quiz carries …… questions

Attempt all the questions.

Put a tick mark on the correct option or fill the blanks with True/ False

Please check whether all questions are answered before submitting

Questionnaire

Q1. The most effective strategy to prevent exposure to aerosol in the laboratory is:

a) Use respiratory protection
b) Use certified biosafety cabinet
c) Minimize generation of aerosol by adhering to good microbiological practices
d) All the above

Q2. Arrange the following steps in order when setting up the BSC for specimen aliquoting:

A. Place the biohazard waste collection bag/container
B. Place an absorbent liner on the worksurface
C. Clean the BSC with 1% sodium hypochlorite
D. Carry out the functionality check of the BSC

Correct order:

a) D, C, B, A
b) D, A, C, B
c) C, A, B, D
d) B, A, D, C

Q3. 1% Sodium Hypochlorite solution should be prepared:

a) Weekly
b) Monthly
c) Daily
d) Every 3-4 days

Q4. According to national BMW guidelines autoclaving of biomedical waste should be done in a gravity flow autoclave for:

a) 121 degree Celsius, 15 Psi, for one hour
b) 141 degree Celsius, 15 Psi, for 30 min
c) 149 degree Celsius, 15 Psi, for 45 min
d) 121 degree Celsius, 15 Psi, for 30 min

Q5. True or False:

a) Work surfaces should be thoroughly cleaned with 70% ethanol after disinfecting with 1% sodium hypochlorite to ensure removal of sodium hypochlorite residue _____
b) It is NOT safe to work in the BSC when the magnahelic gauge reading is lower than that provided on certification label. _____

Q6. Choose the correct statement for the extracted RNA:

a) It is quite stable and does not degrade easily
b) It is as stable as DNA
c) It has a very short half-life and susceptible to RNase degradation
d) It is stable at 4°C

Q7. The widely preferred upper respiratory tract specimen for Covid-19 testing is

a) Nasopharyngeal swab
b) Oropharyngeal swab
c) Bronchoalveolar lavage
d) Both a and b

Q8. Which are the targets used for open RT-PCR testing?

a) N gene
b) S Gene
c) ORF1a gene
d) combination of any 2 of the above genes

Q9. The genome size of SARS-CoV-2 is approximately:

a) 27 to 30 kb
b) 1 to 2 kb
c) 5 to 11 kb
d) 12-17 kb

Q10. Truenat Covid- 19 assay is a

a) Chip based real time test
b) Quantitative test
c) Open system RT-PCR test
d) Rapid cartridge based test

Q11. Cycle threshold also known as Ct in real time PCR indicates:

a) Number of amplification cycles at which fluorescence signal is maximum
b) Number of amplification cycles at which fluorescence signal increases beyond the background noise
c) Number of amplification cycles at which fluorescence signal is minimum
d) Cut off time

Q12. A higher Ct value suggests:

a) Sample has more target RNA
b) Sample has less target RNA
c) Patient has severe COVID disease
d) Patient has mild COVID disease

Q13. PCR plates should not be opened after amplification and discarded safely. This is because:

a) Amplicons generated during PCR may get aerosolized and contaminate the work surfaces
b) They contain infectious material
c) Amplicons are a potential biohazard
d) Amplicons can cause toxicity in workers

Q14. It is advisable not to transfer laboratory items from RNA extraction area to reagent preparation area because:

a) RNA extraction is carried out in a high containment area, the items may be contaminated with infectious agent

b) Items may be contaminated with RNA molecules which will lead to reagent cross contamination

c) Both a & b

d) None of the above

Q15. True or false:

Aerosols are generated during micro pipetting, which can cause nucleic acid cross contamination _____

Q16. The decontamination procedure for RNA extraction area is:

a) Wiping surfaces with 1% freshly prepared sodium hypochlorite solution followed by 70% alcohol

b) Wiping surfaces with 0.1% sodium hypochlorite solution followed by 70% alcohol

c) Wiping surfaces with 0.1% bleach solution

d) Wiping surfaces with 100% alcohol

Q17. Choose the best statement. One should not bring worksheets or tools from other sections of the laboratory to master mix room, because:

a) They are not needed

b) They will create more clutter

c) They might carry contaminating nucleic acids and contaminate the master mix

d) They are not autoclaved

Q18. When analysing the data post amplification, irregular signals or signal artefacts may be observed in some wells. This may be due to:

a) Impurities in RNA preparation

b) Air bubbles in the reaction mix

c) Improper sealing, hence evaporation of PCR reagent mix

d) All the above

Q19. True or false:

In a multiplex RT-PCR reaction the user can choose two dyes of the same fluorescent channel _____.

Q20. In a RT-PCR data low Ct values (Example 15 Ct) refers to;

a) Low viral load.

b) High viral load.

c) No Virus.

d) None of these.

Q21. Tick anyone of the Closed systems for Covid -19 Detection

a) Biorad RTPCR

b) Covipath RTPCR

c) CBNAAT

d) None of the above

Q22. When do you repeat a test in CBNAAT assay

a) Result is Invalid

b) Result shows Error

c) Ct values are within the range

d) both a) and b)

Q23. Gowns and Masks are discarded in

a) Red bags

b) Yellow bags

c) White bags

d) Black bags

Q24. The temperature of VTM samples during transport should be

a) 2−8 °C up to 5 days, if >5 days then freeze at −70°C and shipped on dry ice

b) room temperature and transported at 2 to 8°C

c) 15 to 30°C

d) - 20°C

Q25. Biohazard Symbol to be used is

a) 

b) 

c) 

d) 

SARS-CoV-2 detection by rRT-PCR method
Onsite training competency assessment

Intended Use:
This tool is intended to be used by the trainer to assess the success of the training and the resulting improvement in competency of laboratory or health care workers in conducting SARS-CoV-2 rRT-PCR testing. The objective of the competency assessment is to determine whether training has resulted in:

1. Competency in performing the test safely and accurately
2. Competency in result interpretation and reporting
3. Improvement in theoretical knowledge

Evaluation Procedure:

- A pretest, comprising **twenty** multiple-choice questions will be provided to the trainees at the site. Questions will be based on the content presented in the workshop. The scores obtained in this test will be an indicative of theoretical knowledge base of the trainees.

- The trainee will be given blinded/mock (5-10 samples per experiment) specimens for processing. Once they have observed the trainer, they will perform the procedure on their own. The trainer will observe without intervening or correcting mistakes. He/She will score the performance on competency evaluation sheet. Similarly, RT-PCR will be performed either on the RNA extracted from real samples or with the RNA samples given by the training site. Trainer will observe and record the trainee performance.

To obtain a Certificate of Successful Completion, trainees will be required to:

1. Obtain a passing score of **90%** on the practical test in which trainees will be required to demonstrate correctly to perform rRT-PCR test on blinded / mock samples while being observed by a trainer.

2. Obtain a passing score of 80% on the written theoretical test (Section), in which trainees will be required to answer twenty multiple-choice questions (post-test) on content presented in the workshop.

B. Practical Test:

Instructions:

For the Trainer:

- Observe the trainee as he/she performs each step of the processes. The table below is guidance to the requirements of the procedures as indicated in the SOPs, workstation tasks and work instructions.
- For each step performed correctly, place a tick in the YES column. If any step is performed incorrectly, place a tick in the NO column.

For the trainee:

- Review the SOPs and any other work instructions provided to you before starting the procedure
- Perform the procedures according to the SOPs under the observation of the trainer

Date:

Trainer's Name & Designation:

Trainee's Name & Designation:

S.No.	Question	Yes/Y	No/N	Comments
	Specimen Processing			
1.	Did the trainee don the appropriate PPE?			
2.	Did the trainee go over the job aid /instructions before starting the work?			
3.	Did the trainee set up the work area inside the BSC correctly?			
4.	Did the trainee use proper method to aliquot samples from VTM tubes?			
	RNA Extraction			
5.	Did the trainee don the appropriate PPE?			
6.	Did the trainee follow the SOPs/ Kit insert			
	rRT- PCR			
7.	Did the trainee identify correct PPE to be worn during reagent preparation?			
8.	Did the trainee filled required			

S.No.	Question	Yes/Y	No/N	Comments
	information in the assay template			
9.	Did the trainee use correct method to handle PCR reagents?			
10.	Did the trainee use correct pipetting method to dispense PCR reagents into the PCR tubes?			
11.	Did the trainee use correct method to add positive control to PCR tube?			
12.	Did the trainee clean up the workplace after completing the work?			
	Result Interpretation & Reporting			
13.	Was trainee able to identify the amplification status of each well?			
14.	Was trainee able to explain how and when to adjust background?			
15.	Was trainee able to explain how and when to adjust threshold?			
16.	Was trainee able to interpret the Ct value correctly?			
17.	Was trainee able to perform reporting successfully?			

USAID RISE ToT on Molecular Testing for COVID -19
FEEDBACK

Name: State:	Designation:
Training site:	Dates (from and to):

Training Overall

	Excellent: 5	Good: 4	Satisfactory: 3	Fair: 2	Poor: 1
Content was relevant to me					
Material covered was sufficient					
Media were used appropriately which made learning easy					
I am confident of using the concepts covered					
Duration of the training was appropriate					
The training met my expectations					

Faculty

	Excellent: 5	Good: 4	Satisfactory: 3	Fair: 2	Poor: 1
Faculty had a good grasp of the subject					
The concepts were clearly explained					
Faculty involved all participants					
My questions were answered adequately					
Faculty was supportive and encouraging					

Presentations

Sessions	Excellent: 5	Good: 4	Satisfactory: 3	Fair: 2	Poor: 1
Please write appropriate number as your answer to each question					
Donning and Doffing protocol			Content:	Presenter:	Overall:
Specimen collection, receipt, storage, aliquoting, long term storage and sample transport + RNA retention and sample transportation for WGS.			Content:	Presenter:	Overall:
RNA extraction, master mix preparation and setting up PCR			Content:	Presenter:	Overall:
CBNAAT			Content:	Presenter:	Overall:
Truenat			Content:	Presenter:	Overall:
Interpretation of RT test results			Content:	Presenter:	Overall:
CAPA and troubleshooting including troubleshooting exercises; validation verification			Content:	Presenter:	Overall:
Setting up a RT PCR laboratory and biosafety			Content:	Presenter:	Overall:
ICMR testing criteria			Content:	Presenter:	Overall:
Disinfection protocol			Content:	Presenter:	Overall:
Spill management			Content:	Presenter:	Overall:
Biomedical waste management			Content:	Presenter:	Overall:
Risk assessment			Content:	Presenter:	Overall:
GLP/GCLP (Good Clinical Laboratory Practice)			Content:	Presenter:	Overall:
Tool kit Introduction			Content:	Presenter:	Overall:

Hands-on sessions

Sessions	Excellent: 5	Good: 4	Satisfactory: 3	Fair: 2	Poor: 1
Please write appropriate number as your answer to each question					
Donning & Doffing protocol			Content:	Presenter:	Overall:
Specimen collection & triple layer packing			Content:	Presenter:	Overall:
CBNAAT			Content:	Presenter:	Overall:
Truenat			Content:	Presenter:	Overall:
RNA extraction			Content:	Presenter:	Overall:
Master mix preparation and setting up of PCR, interpretation of test results			Content:	Presenter:	Overall:

Facilities at Training Venue

	Excellent: 5	Good: 4	Satisfactory: 3	Fair: 2	Poor: 1
Ambience					
Lighting					
Food and beverage					
Training equipment					

1. **Three most useful aspect of the training program for me were:**

-
-
-

2. **Please write how you are going to use this learning in connection to Below activity:**

Day to day job:

Improvement of my performance:

For my future growth:

3. **Three least useful aspect of the training program for me were:**

-
-
-

4. Please give your suggestions for improving the training program.

5. Would you recommend this training program to your colleagues? YES / NO

Overall Rating of the Training Program

Excellent: 5	**Good: 4**	**Satisfactory: 3**	**Fair: 2**	**Poor: 1**

Signature: Date: