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RISE Reaching Impact, Saturation, and Epidemic Control

Reaching Impact, Saturation, and Epidemic Control (RISE)

A comprehensive manual on SARS-CoV-2 diagnostics

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

February 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 toolkit presented here covers all steps of the diagnostic process. It begins with an overview of COVID-19 diagnostics followed by an in-depth look at workflow processes and, of key importance, biosafety aspects from both staff and environmental perspectives. There are then chapters on sample collection, storage and transport; specific types of molecular tests, including PCR and NAATs; data interpretation; quality assurance; troubleshooting; and equipment management. The final chapter covers audit and accreditation considerations.

This toolkit is a comprehensive resource for all stakeholders involved in the diagnostic laboratory ecosystem, including policymakers, laboratory managers, laboratory technicians, and data analysts.

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Intended Audience(s)
This comprehensive manual is intended for ministries of health, policy formulators and decision makers, including 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

AC	Alternating Current
ACE-2	Angiotensin-Converting Enzyme 2
Ag-RDT	Antigen Rapid Diagnostic Test
AMC	Annual Maintenance Contract
BSC	Biosafety Cabinet
BSL	Biosafety Level
CAB	Conformity Assessment Body
CBNAAT	Cartridge-Based Nucleic Acid Amplification Test
CCD	Charge-coupled device
CCTV	Closed-circuit Television
cDNA	Complementary DNA
CIA	Chemiluminescent Immunoassay
CISF	Central Industrial Security Force
CMC	Comprehensive Maintenance Contract
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Ct	Cycle Threshold
cVNT	Competitive Viral Neutralization Test
DAHUA	The Name of a Manufacturer
DC	Direct Current
DEPC	Diethyl Pyrocarbonate
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
DVR	Digital Video Recorder
E gene	Envelope Gene
ELISA	Enzyme-Linked Immunosorbent Assay
EPABX	Electronic Private Automatic Branch Exchange
EQA	External Quality Assurance
EUL	Emergency Use List
GMPP	Good Microbiological Practices and Procedures
GoI	Government of India
GPM	Gallons Per Minute
GPSP	Galvanized Plain Skin Pass
HIS	Health Information System

IATA	International Air Transport Association
ICMR	Indian Council of Medical Research
IDSP–IHIP	Integrated Disease Surveillance Program–Integrated Health Information Platform
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IGSL	INSACOG Genome Sequencing Laboratory
ILC	Inter-Laboratory Comparison
ILI	Influenza-Like Illness
ILQC	Inter-Laboratory Quality Control
INSACOG	Indian SARS-Cov-2 Genomics Consortium
IPC	Infection Prevention and Control
IR-type	Infrared-Type
LAMP	Loop-Mediated Isothermal Amplification
LAN	Local Area Network
LED	Light-Emitting Diode
LFA	Lateral Flow Assay
LFI	Lateral Flow Immunoassay
LIMS	Laboratory Information Management System
LoD	Limit Of Detection
LRT	Lower Respiratory Tract
M&E	Monitoring and Evaluation
MCB	Miniature Circuit Breakers
MCCB	Molded Case Circuit Breaker
MoHFW	Ministry of Health and Family Welfare
N gene	Nucleocapsid Gene
NAAT	Nucleic Acid Amplification Test
NABL	National Accreditation Board for Testing and Calibration Laboratories
NIC	National Informatics Center
NPV	Negative Predictive Value
OEM	Original Equipment Manufacturer
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PFU	Plaque-Forming Unit

PLHIV	People Living With HIV
POC	Point-Of-Care
PPE	Personal Protective Equipment
PPV	Positive Predictive Value
PRNT	Plaque-Reduction Neutralization Test
PUF	Polyurethane Foam
PVC	Polyvinyl Chloride
pVNT	Pseudovirus Neutralization Test
QMS	Quality Management System
RAT	Rapid Antigen Test
RdRp	RNA-dependent RNA Polymerase
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	Severe Acute Respiratory Syndrome
SOP	Standard Operating Procedure
SRF	Specimen Referral Form
UPS	Uninterruptable Power Supply
URT	Upper Respiratory Tract
UVGI	Ultraviolet Germicidal Irradiation
VNT	Virus Neutralization Test
VTM	Viral Transport Medium
WGS	Whole Genome Sequencing
WHO	World Health Organization

Chapter 1
Introduction to COVID-19 diagnostics

Chapter 1: Introduction to COVID-19 diagnostics

1.1 Background

The World Health Organization (WHO) was first alerted to a cluster of pneumonia of unknown etiology in Wuhan City, Hubei Province of China on 31 December 2019.[1] The virus was initially tentatively named the 2019 novel coronavirus (2019-nCoV). Subsequently, the International Committee of Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2. COVID-19 is the name of the illness caused by SARS-CoV-2.[1] SARS-CoV-2 is classified within the genus Betacoronavirus (subgenus Sarbecovirus) of the family Coronaviridae.[1 2] It is an enveloped, positive-sense, single-stranded ribonucleic acid (RNA) virus with a 30-kb genome.[2] The genome encodes for non-structural proteins (some of these are essential in forming the replicase transcriptase complex), four structural proteins (spike (S), envelope (E), membrane (M), nucleocapsid (N)) and putative accessory proteins.[1 3 4] The virus binds to an angiotensin-converting enzyme 2 (ACE-2) receptor for cell entry.[1] The clinical presentation of SARS-CoV-2 infection can range from asymptomatic infection to severe disease.[1 5-14] Early laboratory diagnosis of a SARS-CoV-2 infection can aid clinical management and outbreak control.

Once an individual has been infected by the SARS-CoV-2 virus, the mean time it takes to develop symptoms (incubation period) is 5 to 6 days following exposure, with a range of between 1 and 14 days.[1 15-19] The virus may be detectable in the upper respiratory tract (URT) 1 to 3 days before the onset of symptoms. The concentration of SARS-CoV-2 in the URT is highest around the time of symptom onset, after which it gradually declines.[1 20] The presence of viral RNA in the lower respiratory tract (LRT), and for a subset of individuals in the faeces, increases during the second week of illness.[20] In some patients, the viral RNA may only be detectable for several days, while in other patients it can be detected for several weeks, possibly months.[1] The prolonged presence of viral RNA does not necessarily signify prolonged infectiousness.

Several studies describe a correlation between reduced infectiousness and the following:[1]

- Increased number of days that have elapsed since symptom onset and resolution.
- Decrease in viral load in respiratory secretions.
- An increase in neutralizing antibodies.

The declaration by WHO of COVID-19 as a global pandemic in March 2020 warranted the urgent need for technologies and tools to be adopted for confirming the diagnosis of suspected cases and to further inform containment and infection prevention responses across nations. Diagnostics thus play an important role in the COVID-19 pandemic response, for three major reasons:[21]

- First, the early clinical presentations of COVID-19 patients tend to be quite non-specific, so a diagnostic test should be used to confirm COVID-19 in symptomatic patients who present for care. This would allow these cases to be appropriately managed and, more importantly, enable public health measures such as isolation or quarantine to be put into place as soon as possible.
- Second, diagnostics should be used to screen all contacts of confirmed COVID-19 cases. We know now that a large number of infected individuals may only have very mild symptoms or no symptoms at all, but they can still shed the virus and transmit infection. Testing all contacts of confirmed cases is also critical in interrupting the chain of transmission of COVID-19 in a community.
- Third, diagnostics provide us with tools to conduct a rapid situation analysis and to carry out surveillance so that we can monitor disease trends over time and assess the effectiveness of control interventions.

COVID-19 testing can be categorized into two main groups:[22] testing for active SARS-CoV-2 infection and testing for past SARS-CoV-2 infection. An active infection indicates that a person has virus that is replicating and that they could infect others. A past infection indicates that an individual has recovered from COVID-19 and has no actively replicating virus.

1.2 Types of diagnostics

There are three major types of diagnostics (Figures 1–3) available for various uses during a pandemic response situation (Table 1):[22]

- Detection of viral RNA, through molecular tests involving manual or automated nucleic acid amplification tests (NAATs), such as reverse-transcription real-time polymerase chain reaction (rRT-PCR).
- Detection of viral antigens through immunodiagnostic techniques, such as lateral flow assays (LFAs), commonly called rapid diagnostic tests or Ag-RDTs.
- Detection of host antibodies through serological techniques, such as LFAs, enzyme-linked immunosorbent assays (ELISAs) or chemiluminescent immunoassays (CLIAs).

To test for active infection, diagnostic antigen or molecular tests are used.[22] These tests can detect whether a patient has a current infection, which can help patients and healthcare providers make decisions about treatment as well as isolation to reduce the spread of infection.[23] Molecular tests can also detect fragments of virus even after an individual is no longer infected.[24] To test for past infection, serology tests are used.[23] The presence of antibodies specific to SARS-CoV-2 indicates that a person has been previously exposed to the virus and is currently seropositive for SARS-CoV-2-specific antibodies.[23]

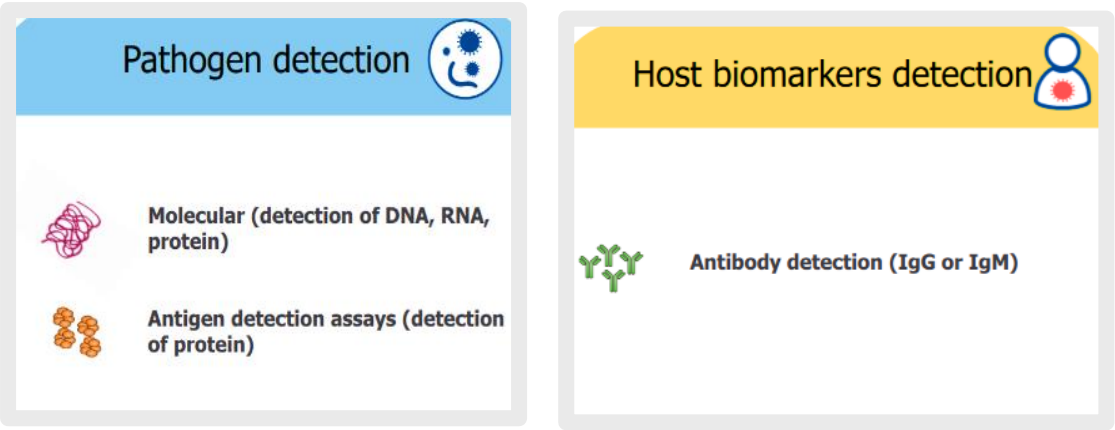

Figure 1: The three major types of diagnostics[25]

1.2.1 Molecular tests

Among the molecular tests, NAAT-based RT-PCR techniques are currently the "gold standard" tests used for routine confirmation of cases of COVID-19.[1 22] In RT-PCR tests, viral genes targeted include the ORF 1ab, N, E, S and RdRP genes. To confirm a positive diagnosis, a validated RT-PCR assay targeting a minimum of two regions on the SARS-CoV-2 genome must be chosen, with one being specific for SARS-CoV-2.[1] Alternatively, the test can include a primer specific for Betacoronaviruses, and the presence of SARS-CoV-2 must be confirmed by sequencing the partial or whole genome of the virus.[1]

There are two types of molecular testing platforms to test for SARS-CoV-2:[1 22] open and closed testing systems. Open systems can accommodate different types of tests from multiple manufacturers. Examples include the BioRad CFX system and the Qiagen Rotor-Gene system. Closed systems are proprietary testing systems that use standardized procedures and cannot be programmed to use test accessories sourced from a different manufacturer. Closed system platforms are validated with an open system RT-PCR test as the gold standard and are approved for use only if they have sensitivity and specificity comparable with that of an open system RT-PCR test.[26] In view of this, some closed system RT-PCR tests, including cartridge-based nucleic acid amplification test (CBNAAT) platforms[27], such as Truenat and GeneXpert, US FDA-approved cartridge-based systems like Abbott ID NOW, and other platforms such as reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assays and CRISPR-based tests, are considered equivalent to open system RT-PCR tests.[26]

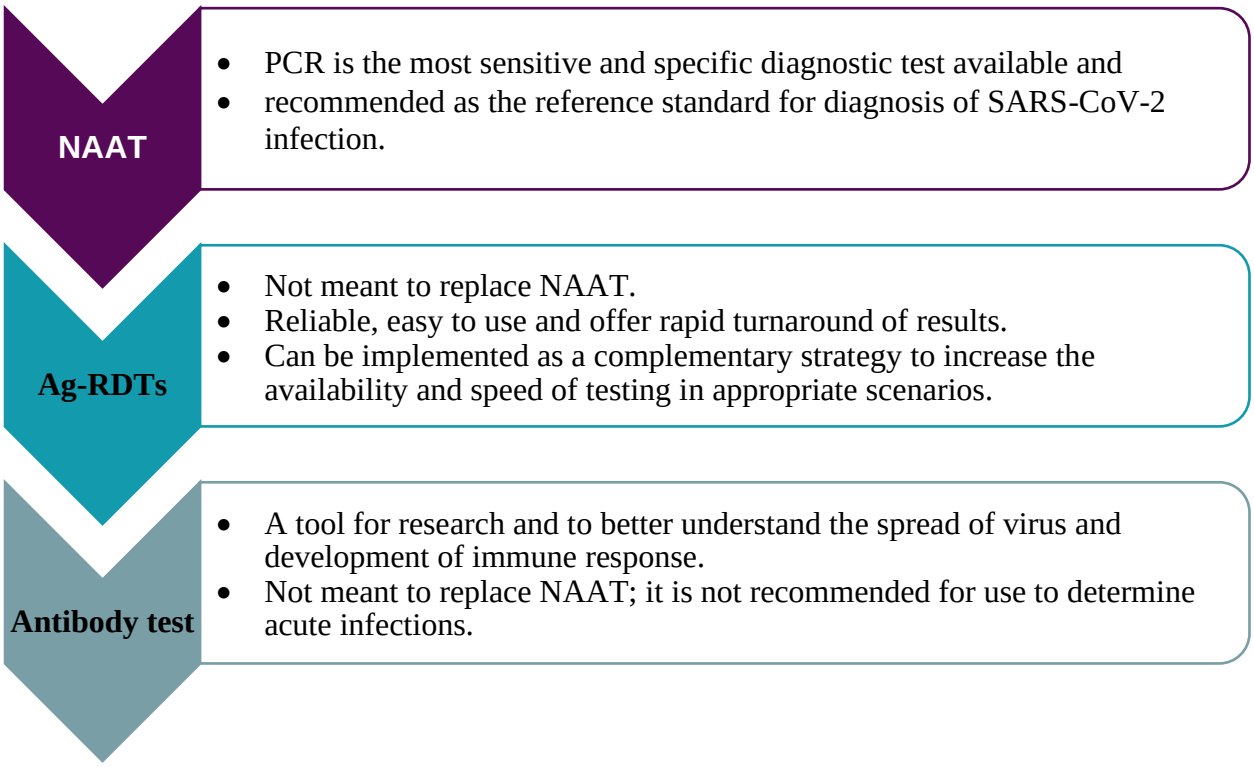

Figure 2: Applications of the three major types of diagnostics (adapted from WHO recommendations[22])

1.2.2 Antigen-based tests

Rapid diagnostic tests (RDTs) that detect the presence of SARS-CoV-2 viral proteins (antigens) in respiratory tract specimens are being developed and commercialized.[1 22 28] Most of these are lateral flow immunoassays (LFIs), which are typically completed within 30 minutes. In contrast to NAATs, there is no amplification of the target that is detected, making antigen tests less sensitive. Additionally, false-positive (indicating that a person is infected when they are not) results may occur if the antibodies on the test strip also recognize antigens of viruses other than SARS-CoV-2, such as other human coronaviruses.[22 29] In June 2020, rapid antigen tests (RATs) were recommended in India for COVID-19 testing.[1] RATs have a short turn-around time, of 15 to 30 minutes, and thus offer the huge advantage of rapid detection of cases and the opportunity to isolate and treat them early to help curb transmission.[22 29]

1.2.3 Antibody-based tests

Serological assays that detect antibodies produced by the human body in response to infection with SARS-CoV-2 can be useful in a variety of settings.[22] For example, serosurveillance studies can be used to support the investigation of an ongoing outbreak and to support the retrospective assessment of the attack rate or the size of an outbreak. As SARS-CoV-2 is a novel pathogen, the understanding of the antibody responses it engenders is still emerging and therefore antibody detection tests should be used with caution and not used to determine the presence of acute infections.[22 29 30] However, antibody testing could play a role in the fight

against COVID-19 by helping healthcare professionals identify individuals who have developed an adaptive immune response to SARS-CoV-2. A rapid antibody test is therefore recommended as a surveillance tool for epidemiological purposes in hot-spot areas and in areas where no cases have emerged so far.[29] [30,31]

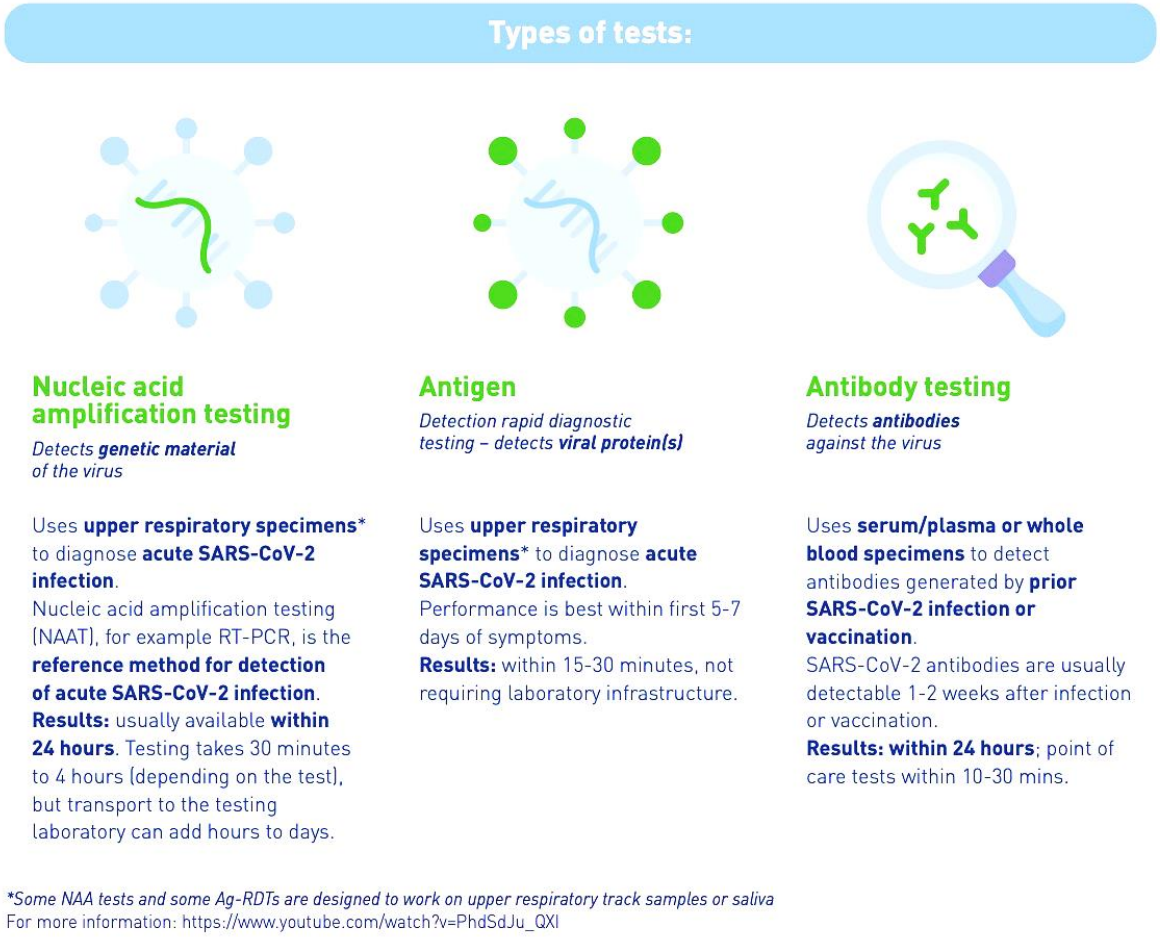

Figure 3: Comparison of NAAT, antigen and antibody tests[32]

It is important to remember that each type of diagnostic test has its own attributes and limitations. The correct test must be used for the correct patient at the correct time in the correct setting.[21] While no test is perfect, tests with both high analytical sensitivity and specificity should ideally be prioritized.[28] Additionally, test performance can be affected by many factors that are independent of the test design, including the prevalence of the disease in the population being tested. Based on the current understanding of COVID-19 and existing clinical/public health guidance, it is best to prioritize tests with a low limit of detection and high clinical sensitivity when selecting a test to diagnose active infection, to reduce the likelihood of missing COVID-19 cases.[28] In this case, clinical sensitivity refers to the ability of a test to correctly identify COVID-19-positive individuals, while clinical specificity refers to the ability of a test to correctly identify COVID-19 negative individuals.

1.3 Testing strategy

Nucleic acid amplification tests (NAATs) are the reference standard for diagnosis of acute SARS-CoV-2 infection. The network of SARS-CoV-2 testing facilities should leverage and build on existing capacities and capabilities, be able to integrate new diagnostic technologies and adapt capacity according to the epidemiological situation, available resources and country specific context. The advisory for the testing strategies for the Indian scenario can be adapted from the ICMR link: https://www.icmr.gov.in/cteststrat.html (Information on Testing Strategies - Molecular based Test (RT-PCR/Truenat/CBNAAT).

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Chapter 2

Workflow process in a molecular testing laboratory for COVID-19

Chapter 2: Workflow process in a molecular testing laboratory for COVID-19

2.1 Introduction

This chapter outlines the physical and infrastructure requirements and describes the general workflow processes in a molecular testing laboratory.

One of the most important aspects that should be taken into consideration when designing a molecular testing laboratory is the creation of unidirectional workflow. Real time PCR, also called as qPCR, quantitative PCR or quantitative real-time PCR, is a technique used to detect and measure the amplification of target nucleic acids as they are being produced. In contrast to conventional PCRs, qPCRs require a fluorescently labelled oligonucleotide probe, and a thermocycler able to measure fluorescence and calculate the cycle threshold (CT) value. Typically, fluorescence increases in direct proportion to the concentration of the PCR product being formed, which allows target quantities to be measured in real-time without opening the tube. Reverse transcription real time PCR (RT-PCR) is used to amplify RNA target sequences, such as messenger RNA and viral RNA genomes. This type of PCR involves an initial incubation of the sample RNA with a reverse transcriptase enzyme and a DNA primer (sequence specific, oligo dT or random hexamer) before real time PCR amplification.

PCR reactions are very sensitive and create a large number of copies of nucleic acids from minute amounts of starting material. This makes them a fundamental and highly effective molecular biology technique. However, because it is prone to amplicon and sample contamination, planning and designing of your PCR lab space will need careful consideration. Minimum aerosolization while opening tubes is necessary to prevent transport of material among samples. All surfaces and equipment must be cleaned with appropriate disinfectants (detailed protocols on decontamination are covered in chapter 3 on Biosafety). This will remove both biologically hazardous substances and any nucleic acids that may be a source of contamination. Reagents should be freshly aliquoted for each test run, as another precaution to prevent contamination. Negative and positive controls should be incorporated in every batch.[1,2]

Nuclease-free water should be used as a negative control to detect and monitor contamination. The negative-control tube should contain all materials necessary for all stages of the test, the same as any other sample. A positive result detected in a negative control indicates the possibility of cross or carryover contamination. All surfaces and equipment should be regularly checked for contamination by taking swab samples, using with damp filter papers, from the laboratory surfaces where tests are carried out. A positive result from a swab sample indicates the presence of contamination.

RNA is more unstable than DNA and is vulnerable to RNases that are present in all cells, therefore the prevention of RNase contamination is very important for RNA-based molecular tests. RNases are resistant to metal chelating agents and can persist even after prolonged boiling or autoclaving. The most common sources of exogenous RNase contamination are

contaminated reagents and automatic pipettors. Laboratory surfaces and glassware may also be contaminated with RNase from laboratory personnel's skin, hair, etc. Precautions to prevent RNase contamination of the laboratory include wearing gloves and changing them frequently during all stages of testing, the use of separate laboratory equipment for RNA-based tests, aliquoting small amounts of reagents, the use of RNase inhibitors such as diethyl pyrocarbonate (DEPC), and the use of RNase-free solutions and tubes. The preparation of a documented action plan in case of contamination is recommended.

The following are essential requirements in a molecular testing laboratory:

- Mechanical barriers to prevent contamination
- Each area should be fitted with adequate requirements.
- Unidirectional workflow
- Maintenance of air pressure
- Temperature and humidity control
- Exhaust ventilation
- Reliable water supply
- Electricity
- Back-up power system

2.2 Physical site

The selection of a physical site will depend on the anticipated testing workload and the efficiency of referral networks. It should also take into consideration the infrastructure requirements, the human resources capacity and running costs. The location of the RT-PCR laboratory itself should be such that it is in a separate building or is separated from the flow of general traffic and accessed via an anteroom/air-lock facility within the same building.3 Even for established COVID-19 molecular testing laboratories, a site assessment should be conducted periodically, to ensure that the size and location of the site meets the requirements of current testing needs and biosafety norms specific to COVID-19 testing.

2.3 Facility design

2.3.1 Building requirements

A BSL-2 level laboratory is mandatory for open RT-PCR system and CBNAAT (cartridge-based nucleic acid amplification test) testing. For Truenat, biosafety requirements are minimal.[4]

2.3.1.1 Requirements for an open RT-PCR system laboratory

A laboratory performing RT-PCR open system testing on diagnostic samples should be divided into four, physically separate rooms.[5]

- 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

The reagent preparation area is cleanest area where reagents for master mix preparation are stored and the process is carried out. This area must be always kept free of extracted RNA and amplicons. To ensure this, we need to follow unidirectional workflow with no movement back from the dirty area to the clean area. There should be a separate anteroom for PCR mix room where PPE for clean area usage can be kept.

The dirty areas are the specimen processing room and the amplification room. The specimen processing room is where specimens are received and stored; it is also where, total nucleic acid is extracted from samples. The amplification room is where the generation of cDNA is performed and further amplification of cDNA and the detection of amplicons occur. No materials or equipment from these areas should be moved back into the clean area without being completely decontaminated, under any circumstances. Gloves and laboratory coats must be removed when leaving the dirty area.[3]

2.3.1.2. Workflow in an open RT-PCR system laboratory

The workflow in a molecular laboratory for open RT-PCR system must be unidirectional, from the clean area to the dirty area. No material should be carried from the dirty room to the clean room. To prevent the movement of personnel from the dirty room to the clean room, it is appropriate to have separate personnel working in each room. The rule of unidirectional workflow, from the clean areas to the dirty areas, must be always followed. Sample preparation should be carried out in a PCR workstation with a UV light. Figure 1 shows a schematic diagram of the unidirectional workflow in an open RT-PCR system laboratory. The guideline for this diagram has been adopted from ICMR and WHO documents.[6 7]

2.3.1.3. Ventilation in an open RT-PCR system laboratory

Air circulating between the various rooms is an important potential source of contamination in laboratories where techniques detecting very tiny quantities of DNA/RNA are used. Each room should be separately ventilated, with the air pressure also adjusted separately. In a room under positive pressure, the air pressure inside the room is higher than the air pressure outside the room, preventing the inward transport of unwanted substances from the outside. In a room under negative pressure, on the other hand, air enters the room and prevents the migration of air from within the room to the surrounding rooms/laboratories. All doors must be kept closed to maintain the negative pressure. Listed below are the air pressures required for each area in a molecular testing laboratory.

- Sample processing area: negative pressure, to keep template nucleic acids inside the room
- Reagent preparation area: positive pressure, to prevent the introduction of contamination
- Template addition area: positive pressure, to keep the template nucleic acids in the room
- Amplification and detection area: positive pressure, to keep amplified nucleic acids in the room [8 9]

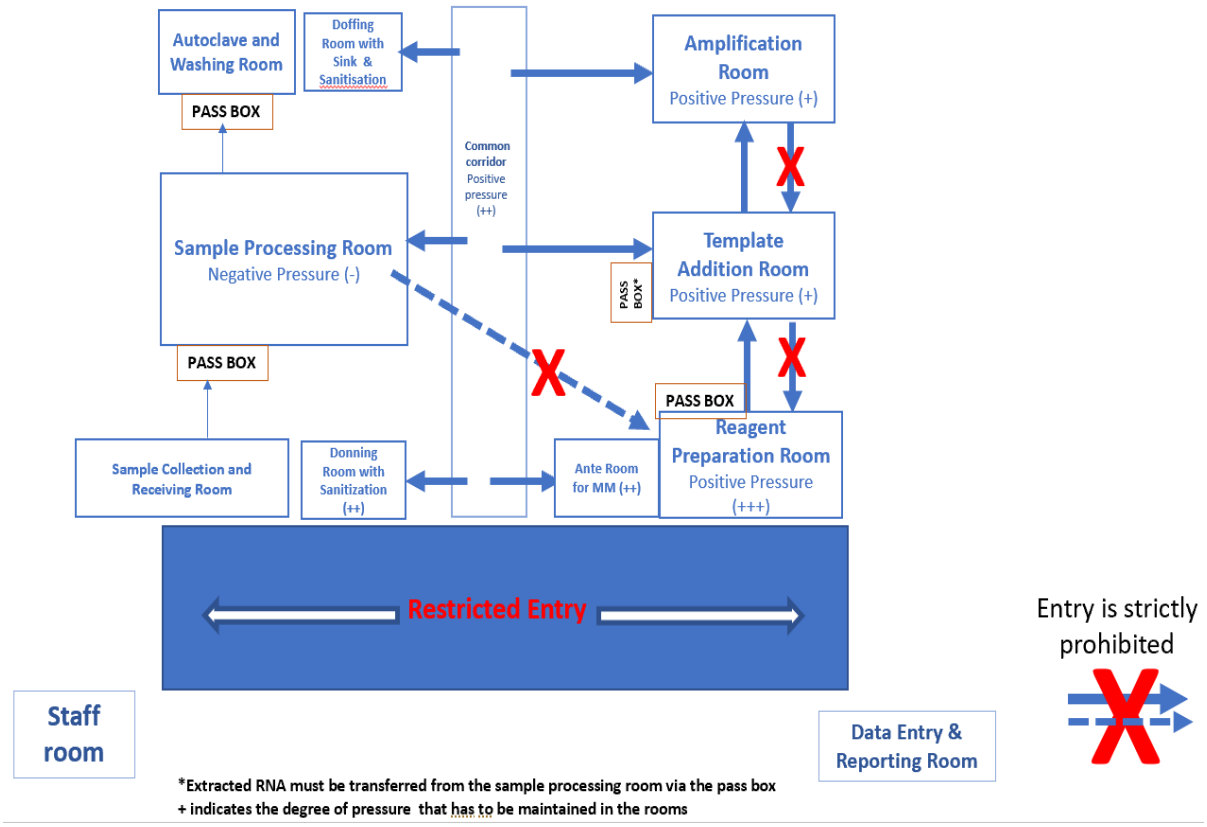


Figure 1: A schematic diagram of the unidirectional workflow in an open RT-PCR system laboratory

2.3.2 Surfaces and finishes [10-12]

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.

Minimum requirements for floors

A single-piece uniform tile flooring material (without pores) is appropriate, and all tile work must be sealed to avoid dirt and other contaminants accumulating in the grouting and seams. Floors must be of sufficient load-bearing capacity to safely support all furnishings, equipment and personnel.

Ideal requirements for floors

Uniform epoxy flooring is ideal. Epoxy flooring should comprise 5 mm (3 mm + 2 mm) of self-levelling industrial epoxy, including screed compound for adhesion. A 3-mm semisolid cladding of epoxy should be applied over a uniform cement floor, followed by a 2-mm semi-liquid epoxy over the 3-mm hardened surface, with a bubble-free, perfectly smooth finish completed in three steps: cementing (uniform flooring), hardening (3 mm epoxy) and smoothening (2 mm epoxy). The epoxy used must be self-levelling and clean-room compatible. The advantages provided by epoxy flooring include:

- Slip-, abrasion- and chemical-resistant flooring
- A seamless, easy to clean surface
- A hard-wearing and durable surface
- A cost-effective flooring solution
- Unaffected by humidity
- Low maintenance costs
- An attractive flooring surface
- Alternatively, uniform monolithic vinyl carpet flooring can be used. The vinyl flooring must be a minimum of 6-mm thick. Flooring joints must be chemically bonded or heat welded, to create a monolithic flooring surface.

2.3.3 Walls and partitions

Minimum requirements for walls and partitions

If existing rooms are permanent brick and mortar structures, then dado tiling should be installed up to the ceiling height, to aid cleaning and disinfection. For partition work, when preparing a

partition wall in sections, these should be half brick partition covered with tiles and then the remainder made using glass and aluminium partitions. The brick section may be up to 4 feet from the floor and the remainder should be the glass and aluminium section. Electrical sockets should be installed in the brick partition wall, with concealed wiring. If the height of the hall is quite low, then glass and aluminium partition walls can be up to the ceiling.

Ideal requirements for walls and partitions

Ideally, PUF wall panels should be designed. Modular PUF panel walls should be suitable for clean-room applications and comprise pre-engineered 40-mm thick PUF panels with GPSP sheets on either side; the PUF insulation should be a of minimum 38 to 40 kg/m^3. Each GPSP sheet should be 0.8-mm thick. The panels must be installed along outer walls, partitions and false ceilings to create an impervious shell that is fully sealed. The panels on each side should be coated with epoxy paint. The panels should be easy to maintain and be aesthetically appealing.

2.3.4 Pass boxes/pass-through windows

Pass boxes and pass-through windows are used for the transfer of materials in contamination-controlled environments. These materials may include samples, reagents and consumables, as well as items to be discarded.

Minimum requirements for pass boxes/pass-through windows

Pass boxes and pass-through windows are usually made from glass and aluminium, with a sliding window and appropriate gasketing. If the partition wall is a permanent brick and cement wall, then an SS 304 stainless steel static pass box should be installed as per specifications. Static type pass boxes should be provided at strategic/required locations for the transfer of samples, chemicals and materials throughout the laboratory (as indicated in the submitted design). In cases requiring two pass boxes, one will receive samples while the second will be used to transfer discarded samples to the autoclave room or for disinfected waste collection. This should be made of SS 304 stainless steel, with an inbuilt UVGI system, and interlock in such a way that both doors cannot be opened simultaneously. It should be panel-mounted, fixed at a height of 750-mm from the floor in the sandwich panel, with dimensions of 610 mm (length) × 610 mm (width) × 610 mm (depth) and a load-bearing capacity of 40 kg. There should be a single door on each side, made of glass and an airtight gasket, with a door latch for each door, and the doors should open outwards. There should be a buzzer to indicate whether either door is open. The handles should be of superior quality, and the viewing glass must be made of polycarbonate or 10-mm thick, tempered glass; hinges should be made of SS304 stainless steel. There should be an LED lamp inside the pass box, which is chemically resistant especially to chemicals such as hypochlorite solution and alcohol. There should be a flange to seal the pass box and the sandwich panel, with lamps on both sides to indicate the status. There

must be a manual on/off switch on both sides of the pass box for both fluorescent and UV lamps. A standard operating procedure (SOP) must be developed for pass box decontamination.

Ideal requirements for pass boxes/pass-through windows

For sample/discard items/reagents/clean consumable flow within in the sections, SS 304 make static pass box to be installed as per specification. A pass box (static type) must be provided at strategic/required locations for transfer of samples, chemicals and materials to and from the laboratories (as indicated in the design submitted). It should be made of SS 304, with an inbuilt UVGI system, with interlocking in such a way that both doors cannot be opened simultaneously; panel mounted, with a buzzer to indicate open status for any door. It should be fixed at a height of 750 mm from floor in a sandwich panel, with dimensions as per site requirement, with load bearing capacity of 40 kg. There should be a single door in each side, with a glass and an air-tight gasket, with a door latch for one door (door opening outwards), with a handle of superior quality. Viewing glass should be made of polycarbonate or 10-mm thick tempered glass. Hinges should be made of SS304, with one LED lamp inside the pass box, that should be chemical resistant especially to hypochlorite solution, alcohol, etc. there should be a flange to seal the pass box and sandwich panel, with indicator lamps on both sides to show the status. There should be a manual on/off switch for both fluorescent and UV lamps on both sides of the pass box. An SOP must be developed for pass-box decontamination.

2.3.5 False ceilings

False ceilings are not mandatory for the molecular laboratory. However, for an ideal setup, this is recommended, and the following requirements can be followed.

Minimum requirements for false ceilings

False ceilings may be made of gypsum or any other material suitable for a clean-room false ceiling and installed at a height of 8’ from the floor so that air-conditioning is feasible.

Ideal requirements for false ceilings

PUF ceiling panels should be designed. A modular PUF panel ceiling suitable for clean-room application should be pre-engineered using 40-mm thick PUF panels covered with GPSP sheets; the PUF insulation should be a minimum of 38 to 40 kg/m^3. Both surfaces should be 0.8-mm thick GPSP sheet and installed on the false ceiling to create an impervious shell that is fully sealed. The panels on either side should be coated with epoxy paint. These panels must be easy to maintain and aesthetically appealing.

2.3.6 Windows

Minimum requirements for windows

Windows should normally be permanently sealed with brick and mortar. If openable, windows must be designed to prevent insects or vermin entering the laboratory, and they should be lockable. Wooden windows are not recommended inside the laboratory facility. In addition to the requirements for windows outlined above, natural lighting should be used optimally wherever possible by ensuring properly sealing of the windows.

2.3.7 Doors

Minimum requirements for doors

Doors should be made of glass and aluminium. They must be of an appropriate size considering the equipment needing to be moved through the doors. All doors should have an automatic door-closure mechanism and a lock and key system. All doors must be compliant with applicable building regulations (for example, fire ratings). In addition, doors should be appropriately labelled; at a minimum they should have labels to indicate:

- International biohazard symbols in areas where biohazardous materials are handled or stored
- Contact details of the responsible person for the laboratory, in case of an emergency
- An indication that access to the area is restricted

Ideal requirements for doors

Modular, PUF-insulated doors should be designed. Flush door finishes should be 45-mm thick, be resistant to chemicals, and have antifungal and antibacterial properties. 1.2 mm thick GPSP sheet suitable to fix on 40-mm thick wall panel with provisions for double-glazing for all doors, with push plates and handles on both sides. PUF panels should comprise GPSP sheets, epoxy-painted on both sides, and have PUF insulation of minimum 38 to 40 kg/m^3. Concealed hardware should be used to fix door frames, e.g., TS-71 door closures, stainless steel hinges, door handles, ball-bearing butt hinges, concealed tower bolts for double doors, with a lock and key arrangement on both sides. Suitable neoprene “Y-seal”-type gaskets should be used between the door jam and door stop. Vision glass for doors should be fixed, vacuumized and insulated with 6-mm-thick toughened glass and should be installed for natural lighting, flush with the surface of the door. All fixings should be flush for ease of cleaning and maintenance. No crevices, joints or sloped profiles are to be used for fixing the glass. This will avoid contamination and dust accumulation.

2.3.8 Requirements for coving

Extruded aluminium anodized R75 clip-on-type (male and female connectors) covings are required for all wall to floor, wall to wall, and wall to ceiling joints. Extruded aluminium double coving should be integrated with the top track of the partition panels. Corner internal and external cove joining pieces should have aluminium anodized finish. Coving should have a similar construction and finish as the walls and be properly sealed with the walls and ceilings using silicon sealant.

2.3.9 Laboratory furniture

Laboratory workstations (number as per the laboratory design)

A laboratory workstation frame should be made up of SS 304 stainless steel, with nylon cushion/bushing for the legs and non-particle shredding material that is chemical resistant, to allow chemical disinfection. It should be strong enough to support the granite top/workbench, as well as equipment placed on the workbench. It should be stable and vibration-free. There must be no drawers or safe in the workstation. There should be space to place a UPS below the work bench.

Garment storage cabinet

One lockable garment storage cabinet should be provided in the changing room/anteroom. It should be made of SS 304 stainless steel with two compartments and shelves for the storage of clean items. It should be of appropriate dimensions to fit in the changing room/anteroom (size to be confirmed with the site in charge).

Coat hangers

Between 8 and 10 individual coat hangers, made of SS30 stainless steel, should be provided to hang gowns/aprons in the changing room/anteroom (in consultation with the site in charge).

Shoe racks (number as per the laboratory design)

A shoe rack should be made of SS 304 stainless steel with five open shelves and wide enough to hold two pairs of on each shelf. It should be able to fit in the available space as per the design.

Wash basins (number as per the laboratory design)

Elbow or foot operated ceramic wash basins should be provided as per the design inside the laboratory. A wall-mounted soap dispenser must be provided for each wash basin unit. A tissue-paper dispenser must be provided near each wash basin for staff to dry their hands. Water lines that penetrate the TB Containment space must be equipped with backflow-prevention

devices. Outlet pipes should be made of PVC, with closures outside of the laboratory made of stainless-steel plate.

Laboratory stools (number as per the laboratory design)

Laboratory-grade, hydraulic, stainless-steel stools of the rotating type with castor wheels at the base, as well as a back support and a footrest, must be provided.

Trolleys (number as per the laboratory design)

Two-tier wheeled trolleys made of SS 304 stainless steel, size 2'x1'6", and with side walls to prevent items falling off, should be provided.

2.3.10 Monitoring mechanisms

The monitoring of crucial parameters in the laboratory will be necessary. CCTV monitoring devices will record footage from various sections of the laboratory. Cameras will continuously monitor activities inside and outside the laboratory via a central CCTV monitor. Five or six camera units should be installed as per site in charge.

Supply, installation, testing and commissioning of the following should be carried out:

- Colour camera 1/3" CCD, IR-type, dome-shaped, 480 TV line resolution, which works in low light
- Six-channel, standalone/network version DVR; make: DAHUA/equivalent reputed OEM
- Hard disk with 1 TB capacity; make: Seagate or equivalent reputed OEM
- Six-channel power supply of reputed make
- Supply laying of co-axial cable with necessary accessories
- Wall-mounted monitor (at least 20-inch LED/LCD), located in the microbiologist's room or as suggested by the site in charge.

2.3.11 Connectivity

Connectivity is essential in a laboratory and must be provided as follows:

- LAN wiring for internet access inside the laboratory, with sockets to be provided at strategic locations (e.g., in the reporting room, the BSL-2 facility and the amplification/PCR room)
- A suitable EPABX system must be provided for the laboratory. Telephones with cables should be installed in the reporting room, the staff room, the BSL-2 room, and any other location as suggested by the site in charge. A telephone with a speaker for hands-free operation should be provided inside the BSL-2 facility.

2.3.12 Split air-conditioners

Wall-mounted, split air-conditioners (ACs) of suitable tonnage (number as per the laboratory design) must be installed. These should be inverter ACs (minimum three star) with a suitable voltage stabilizer. The outdoor units should be appropriately positioned outside of the laboratory, where they are easily accessible but adequately protected from theft. The drainage pipes of the ACs should be sufficiently long to be connected into the drainage system of the institute. ACs should be installed away from the location of any BSCs so that the airflow from BSCs in the BSL-2 lab is not impaired. For the master mix, template addition and amplification rooms, AC units should ideally be installed in front of the door so that positive pressure can be achieved.

2.3.13 Surface disinfectant UV lights

A UVGI system of wavelength 254 nm and adequate capacity must be installed in each section/room of the facility, as per the manufacturer's recommendations. In general, one UVGI fixture covers 200-250 sq ft area. The quantity of UV lights needed will be according to the size of the room.

2.3.14 Electrical system

There are numerous important points to note in relation to the electrical system needed in a molecular testing laboratory, and these are outlined below.

The electrical power requirement (power matrix) for the laboratory should be calculated and provided before the laboratory is designed.

- If earthing is inadequate, the necessary grounding work must be carried out to ensure the entire laboratory has adequate earthing. Earthing should be carried out as per standard for heavy machinery and equipment. The value of the earthing should be less than 5 ohm, and the voltage between E-N should be less than 1 V.
- All electrical fittings and fixtures in the walls in the laboratory areas must be sealed (all conduits and outlets to be sealed with silicon sealant), leak-proof and capable of withstanding chemical exposure during fumigation.
- Lighting should comprise surface-mounted LEDs on the ceilings, arranged as per the layout provided. The LEDs must be of suitable capacity (approximately 18 W) and from a reputable manufacturer. Light fixtures inside the units should have a gasket, or otherwise they should be sealed with silicon. The lighting illuminance should be at 400 to 450 lux.
- Power sockets with covers should be provided for electrical equipment (as per the laboratory layout and design provided). Modular-type power sockets of 5A/15A should be installed at appropriate locations on the wall, as required for laboratory equipment.

The sockets used for UPSs should be labelled as such, for ease of operation and identification.

- Wires and cables should be made of copper wire and be of a standard type from a reputable manufacturer (ISI-marked).
- Electrical-device receptacles, switches and controls should be located so as to minimize their exposure to spilled liquids, e.g., physically above or away from plumbing fittings, cooling-water systems, etc.
- MCCB panel suggesting supply and safety mechanism for different sections of the laboratory should be provided in appropriate places.
- Each circuit and circuit breaker must be sized to carry no less than 100% of the noncontinuous current load plus 125% of the continuous current load for that circuit.
- When designing circuit-breaker panels, at least 20% to 40% more load capacity and circuit-breaker spaces than required by the initial calculations should be provided.
- Each branch circuit should carry its own neutral conductor. Do not use multiwire branch circuits that share a grounded (neutral) conductor.
- Electrical wiring and cabling must be carried out to meet the specific requirements and load consumption of the equipment being used.
- Electrical equipment should be designed and installed such that there is no possibility for a person to make direct contact with any part during normal use.
- Electrical plans and designs should be carefully coordinated with other disciplines to ensure that no conflicts occur.

2.3.15 Emergency preparedness

Emergency preparedness is an essential aspect of planning a laboratory, to ensure the safety of all personnel and the safe and secure operation of the laboratory. All laboratory procedures and activities should be performed according to SOPs that are carried out by staff who are suitably qualified to do so. Regular training should be given in all aspects of laboratory safety. Some specific safety aspects are outlined below.

Personal safety

Personal safety is of utmost importance; essential points to consider are:

- Laboratory coats and other appropriate PPE (gloves, N95 respirators, eye gear etc.) must be worn at all times in the laboratory.
- An eye-wash station must be provided in the doffing section in compliance with ANSI/ISEA Z358.1 guidelines.[13] The water supply for the eye-wash station must be sufficient to supply 0.4 GPM (1.5 liters) for 10 minutes in a low-velocity flow. Eye-wash stations may be sink-mounted or stand-alone.
- All potentially biohazardous waste must be disposed of appropriately.

Fire safety

A fire detection and alarm system (FDA system) and fire extinguishers of type ABC powder (2 kg) should be provided at strategic locations and be in compliance with fire safety guidelines. Training should be provided for the operation of these fire extinguishers.

Electrical safety

All critical electrical equipment must be connected to an online UPS of appropriate capacity and with battery backup. The main input source should ideally be connected to a green energy source for backup or a diesel generator.

2.3.16 Plumbing

All plumbing in a laboratory must be installed correctly to ensure the safety of staff and the wider public. Some specific points to note are as follows.

All liquid drains leaving the laboratory must be connected to a single drainpipe with a back-flow prevention mechanism. This should in turn be connected to the local effluent treatment plant on the hospital campus, if available. The internal drainage system should be connected to the main drainage system as far down stream as possible to ensure maximum dilution. Any penetrations made in walls and floors must be properly sealed. Ensure that all pipes and connections are leak-proof, to avoid flooding behind modular walls. All drains must be equipped with P-traps, and the drainage system should allow easy access for inspection and maintenance.

2.4 Laboratory floor plans

COVID-19 molecular testing laboratories can be divided into six types, according to the type of molecular testing platform used:

- Laboratories using Truenat only
- Laboratories using CBNAAT (GeneXpert system) only
- Laboratories using an open RT-PCR system
- Laboratories using a combination of any of the above
- Mobile laboratories
- Laboratories using large, closed RT-PCR systems (this type of laboratory is beyond the scope of this chapter)

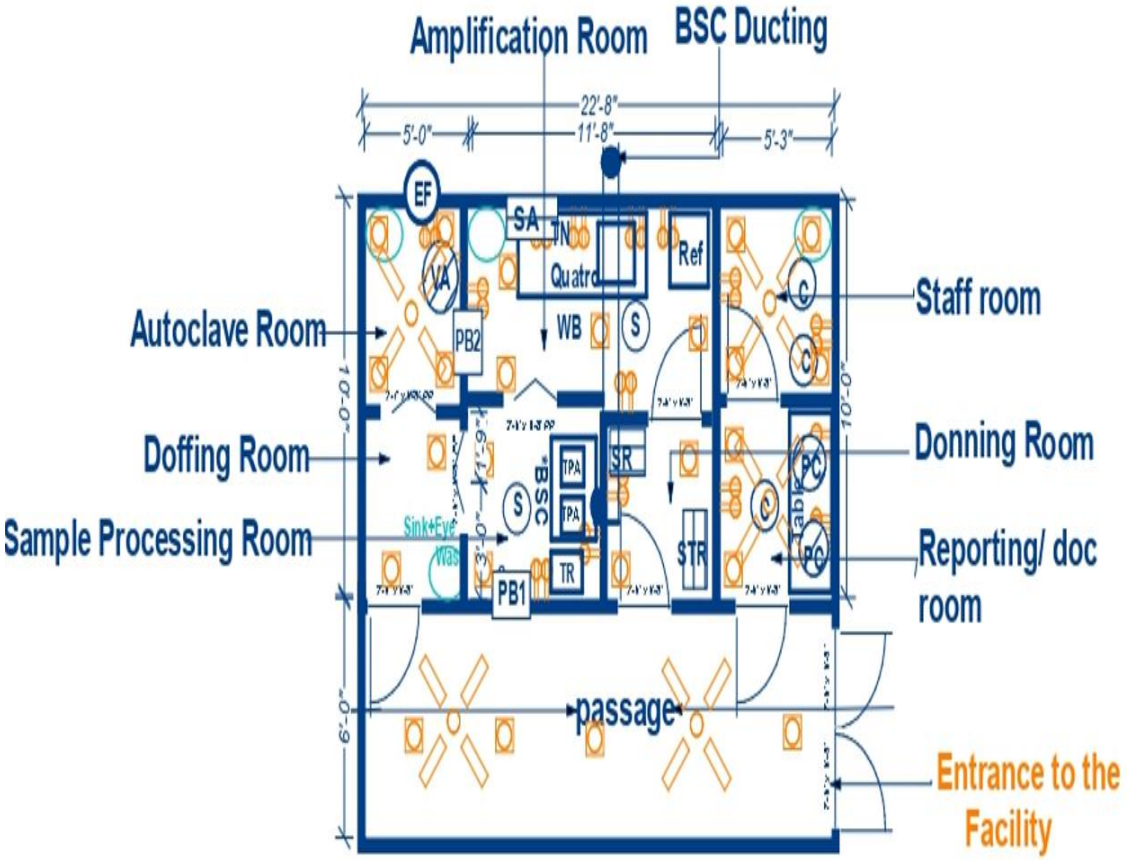
Table 1 shows the properties of the various laboratory models, based on the type of molecular testing platform used and the testing capacities of these laboratories.

Table 1: 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

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. The layouts for the various types of laboratories for different sample loads are shown in Figures 2 to 8. The layouts for type 1a and 1b RT-PCR laboratories have been provided as a single layout (Type 1); similarly, for types 2a and 2b, a single layout has been provided (Type 2).

Figure 2: Floorplan of a laboratory for COVID-19 testing using the Truenat 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 equipment Dimensions (In Feet(')& Inches(")(LengthxWidthxHeight)):

BSC: Class II A2 Biosafety Cabinet (2'x2'x4')
***BSC is not mandatory but desirable**
Ref: Refrigerator(300Ltrs.) (2'x2'3"x5'6")
TN Quatro: TrueLab TM Quattro workstation(11"x1'x1')
TPA:True Prep Auto (2 nos)(7"x7"x6")
VA: Vertical Autoclave(70 Ltrs.)(2'6"x2'2"x4'7")
PC: Personal Computer(2'3"x2'9")
SA: Split AC(1TR)(3'6"x2'x2'5")

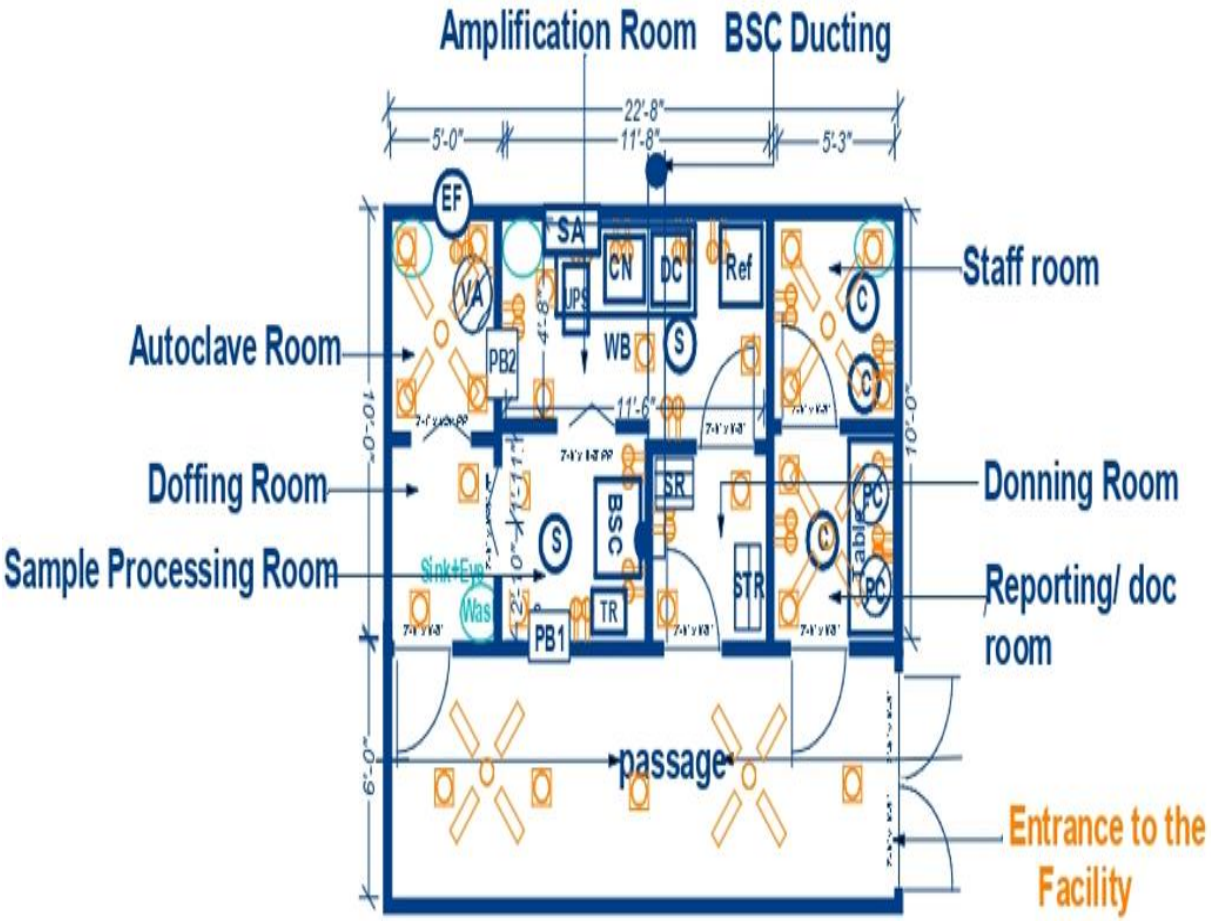
Others:

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

Figure 3: 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

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

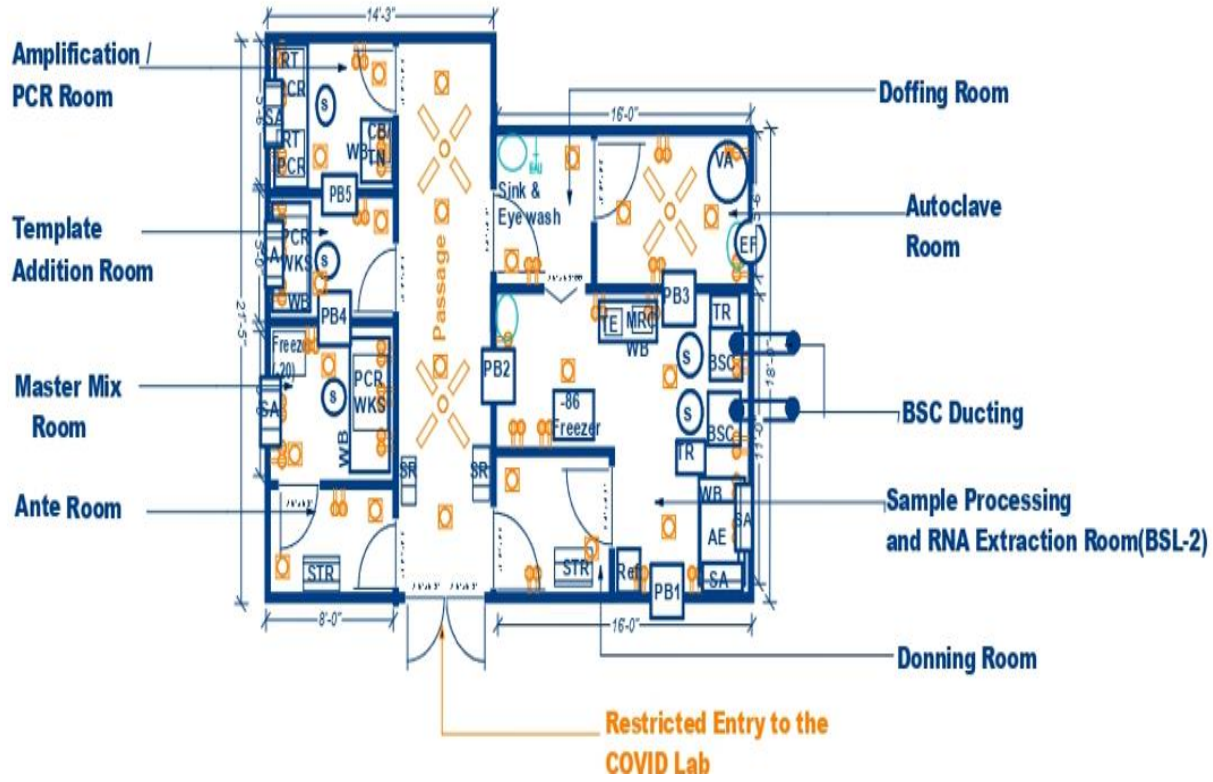
Equipment Details and dimensions
(In Feet(')& Inches(")(LengthxWidthxHeight)):

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"x4'7")
PC: Personal Computer(2'3"x2'9")
SA: Split AC(1TR)(3'6"x2'x2'5")

Legends:

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

Figure 4: Floorplan of an open RT-PCR system laboratory for COVID-19 testing – type 1 (less than 500 samples)



Area Required (in SQFT)

Total area: 650-700

1.For Sample Processing and Automatic RNA Extraction Room: 130
2.Donning Room: 35
3.Doffing Room:35
4.Autoclave Room:50
5.Template addition Room:40
6.Amplification/PCR Room:45
7.Master Mix Room:40
8.Ante Room of MMX Room:30

Equipment Details and Equipment Dimensions (In Feet & Inches (Length x Width x Height)):

BSC: Class II A2 Biosafety Cabinet (2'x2'x7')
Ref: Refrigerator(300Ltrs.) (2'x2'3"x5'6")
CN/TN: CBNAAT(4 Module)/TrueNat Machine (11"x1'x1')
TE: TrueNAT Extractor (11"x1'x1')
VA: Vertical Autoclave(70 Ltrs.)(2'6"x2'2"x4'7")
AE: Automatic RNA Extraction machine (2'6"x2'6"x3')
MRC: Microliter Refrigerated Centrifuge (1' x1'6"x 1')
-86 Freezer (2'6"x2'6"x6')
-20 Freezer(2'x2'x5')
PCR WKS: PCR Workstation/hood(2'x1'6"x2'3")
RT-PCR: Real Time PCR(2'x1'6"x2')
SA: Split AC(3'6"x2'x2'5")

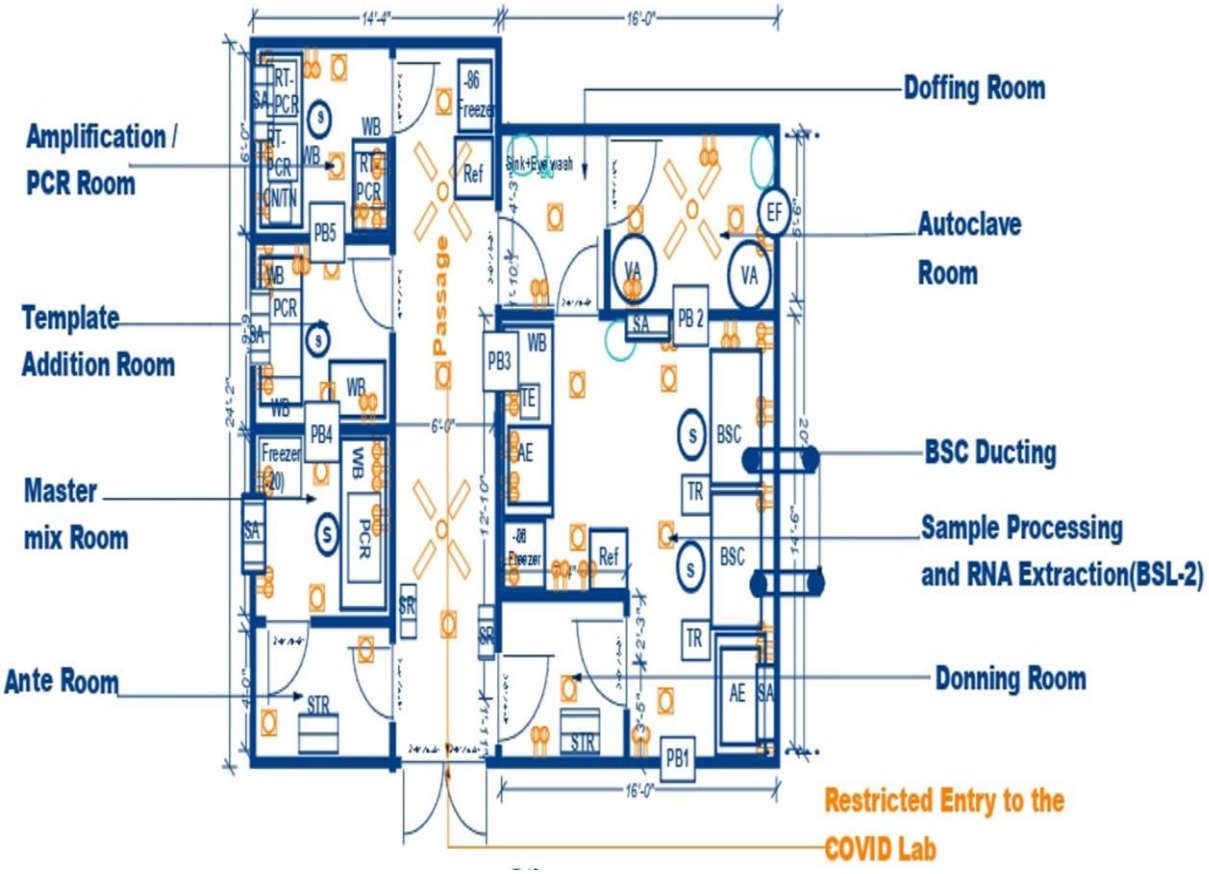
Others:

WB: Work bench
C: Laboratory chair
S: Laboratory Stool
TR: Trolley
EF: Exhaust Fan
STR: Storage Rack
SR: Shoe Rack
PB1: Pass box for passage of sample
PB2: Pass box for passage of clean consumables
PB3: Pass box for passage of waste discard
PB4: Pass box for passage of reagent from MMX to Template addition room
PB5: Pass box for passage from Template room to AMP room

Legends:

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

Figure 5: 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")

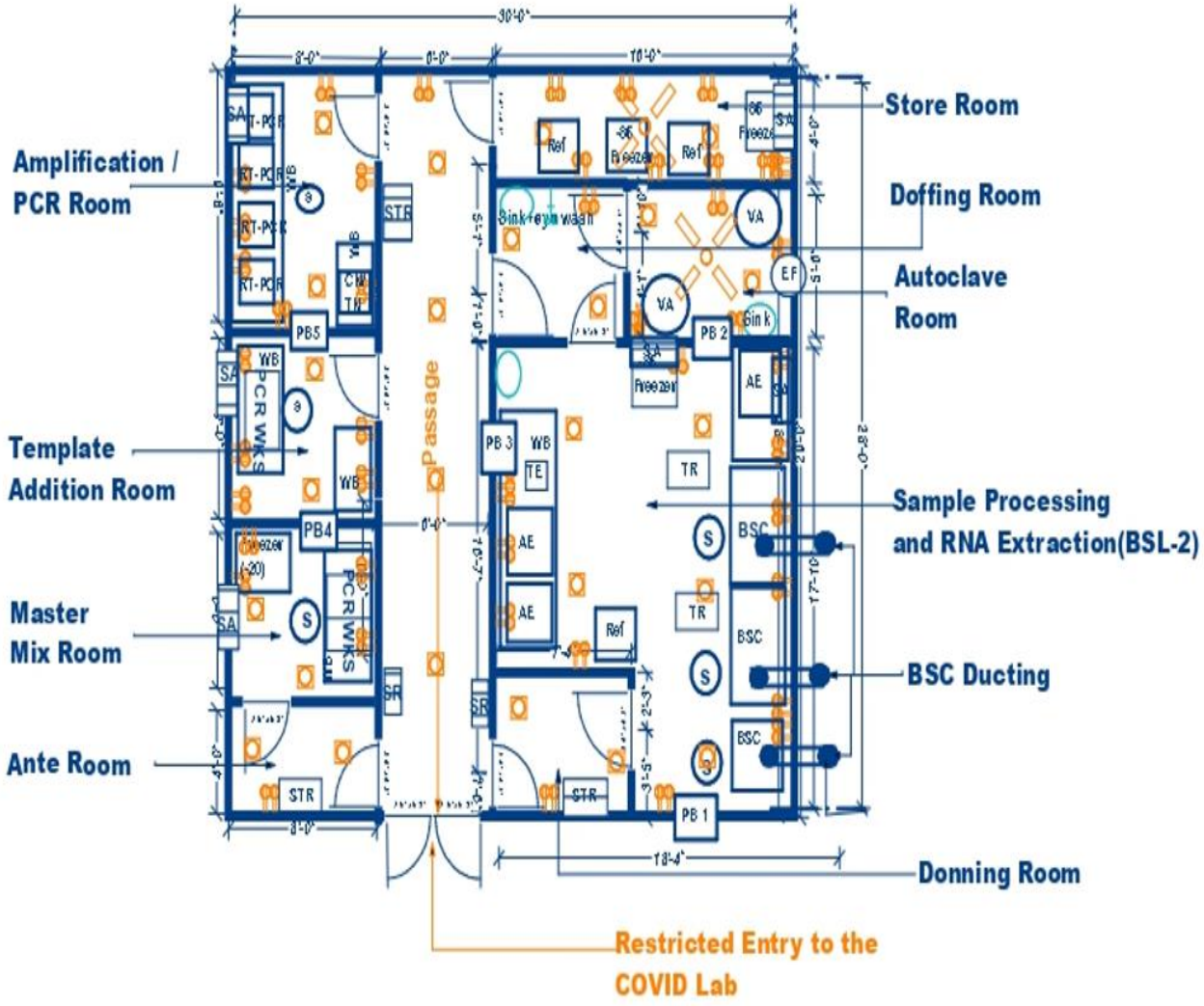
Others:

WB: Work bench
C: Laboratory chair
S: Laboratory Stool
EF: Exhaust Fan
TR: Trolley
STR: Storage Rack
SR: Shoe Rack
PB1: Pass box for passage of sample
PB2: Pass box for passage of waste discard
PB3: Pass box for passage of clean consumables
PB4: Pass box for passage of reagent from MMX to Template addition room
PB5: Pass box for passage from Template room to AMP room

Legends:

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

Figure 6: Floorplan of an open RT-PCR system laboratory for COVID-19 testing – type 3 (2000–5000 samples)



Area Required (in SQFT)

Total area: 870-900
1.For Sample Processing and Automatic RNA Extraction Room:250-300
2.Donning Room:40
3.Doffing Room:40
4.Autoclave Room:50
5.Template addition Room:60
6.Amplification/PCR Room:80
7.Master Mix Room:50
8.Ante Room of MMX Room:30
9.Store Room:60

Others:

WB: Work bench
C: Laboratory chair
S: Laboratory Stool
STR: Storage Rack
SR: Shoe Rack
EF:Exhaust Fan
TR:Trolley
PB1: Pass box for passage of sample
PB2: Pass box for passage of waste discard
PB3: Pass box for passage of clean consumables
PB4: Pass box for passage of reagent from MMX to Template addition room
PB5: Pass box for passage from Template room to AMP room

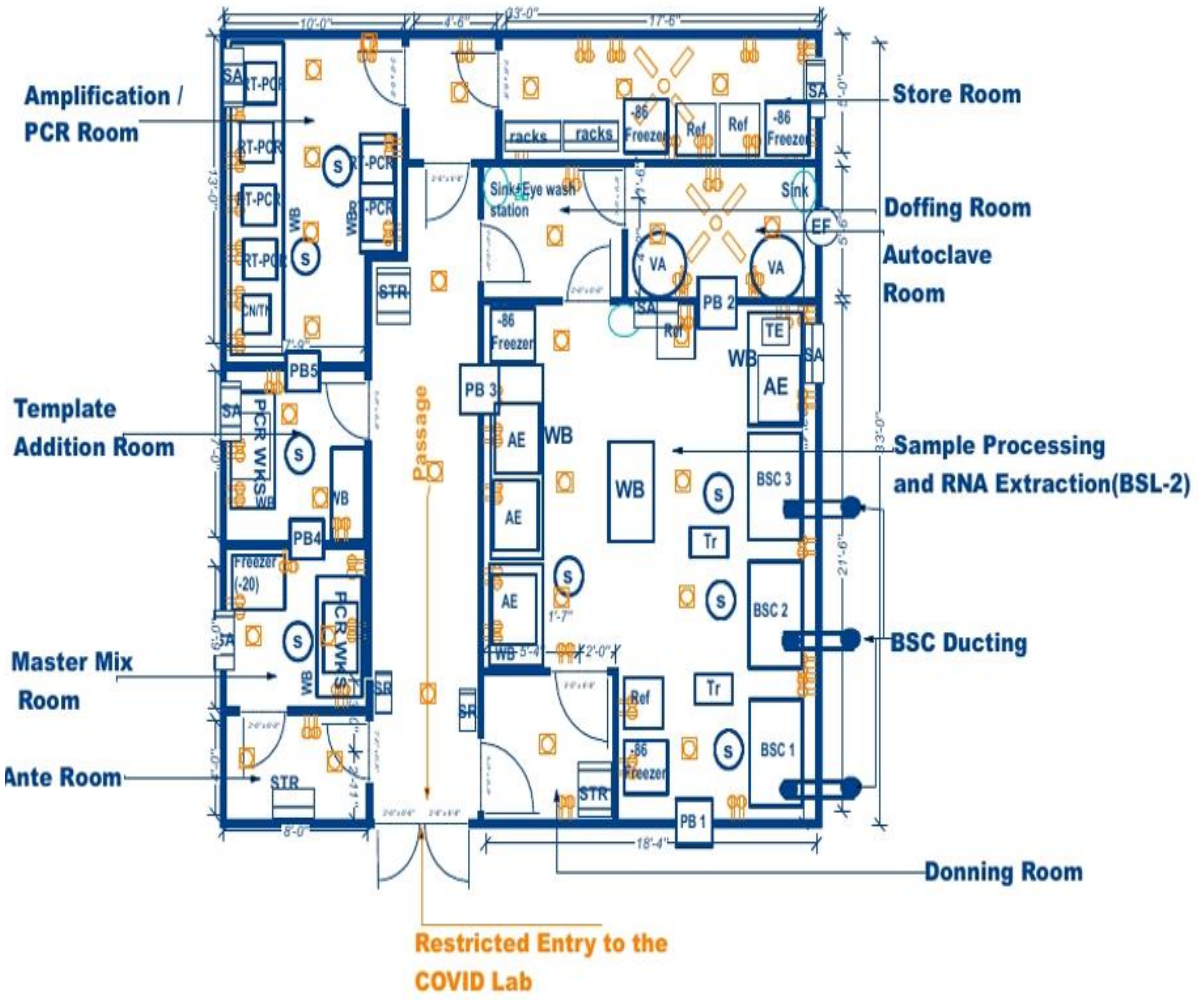
Equipment Details & Dimensions (In Feet(')& Inches(")(LengthxWidthxHeight)):

BSC: Class 2 A2 Biosafety Cabinet (4'x2'6"x7')&(2'x2'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 WKS: PCR Workstation/hood(2'x1'6"x2'3")
RT-PCR: Real Time PCR(2'x1'6"x1'6")
Ref: Refrigerator(2'x2'3"x5'6")
SA: Split AC(3'6"x2'x2'5")
CN/TN: CBNAAT /Truenat (11"x1'x1')
TE: TrueNAT Extractor (11"x1'x1')

Legends:

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

Figure 7: Floorplan of an open RT-PCR system laboratory for COVID-19 testing – type 4 (5000–10 000 samples)



Area Required (in SQFT)

Total area:1050-1100

1.For Sample Processing and Automatic RNA Extraction:320-350
2.Donning Room: 50
3.Doffing Room:45
4.Autoclave Room:60
5.Store Room: 90
6.Master Mix Room:60
7.Ante Room of MMX Room:30
8.Template addition Room:60
9.Amplification/PCR Room:130

Others:

WB: Work bench
C: Laboratory chair
S: Laboratory Stool
STR: Storage Rack
SR: Shoe Rack
Tr:Trolley
EF:Exhaust fan
PB1: Pass box for passage of sample
PB2: Pass box for passage of waste discard
PB3: Pass box for passage of clean consumables
PB4: Pass box for passage of reagent from MMX to Template room
PB5: Pass box for passage from Template room to AMP room

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')
TE: True Nat Extractor(11'x1'x1')
VA: Vertical Autoclave(2'6"x2'2"x4'7")
-86 Freezer(2'6"x2'6"x6')
-20 Freezer(2'x2'x5')
PCR WKS: PCR Workstation/hood(2'x1'6"x2'3")
RT-PCR: Real Time PCR(2'x1'6"x1'6")
CN/TN:CBNAAT/TrueNat(11"x1'x1')
Ref: Refrigerator(2'x2'3"x5'6")
SA: Split AC(3'6"x2'x2'5")

Legends:

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

2.5 Equipment, reagents and consumables

2.5.1 Equipment

The following equipment is recommended for a molecular testing laboratory for COVID-19.

- Level II BSC (preferably type A2)
- PCR workstation
- Vortex mixer
- Micropipettes (each room should have its own separate set of micropipettes)
- Mini-centrifuge (6000 rpm)
- Refrigerated centrifuge (15,000 rpm)
- Two freezers (-20°C and -80°C)
    - -20°C deep-freezer with a UPS, for storage of reagents (primers/probes/positive controls)
    - -80°C deep-freezer with UPS, for storage of aliquoted samples/viral RNA, stored in cryovials
- Two refrigerators (2–8°C), for storage of viral transport medium and for short-term storage of samples and extracted RNA
- UPS (two units, 2 kVA each, with 2 hours back-up, for RT-PCR instruments and nucleic acid extraction systems - if not available, then to be carried); and confirm about power backup for the two deep freezers (check about duration of power outages, if any)
- 96-well-plate shaker
- RT-PCR machine with laptop
- PCR analyser
- Truenat instrument system
- CBNAAT GeneXpert instrument system
- Desktop computers
- Biowaste disposal containers (yellow, red, blue, sharps container)
- Autoclave

The types and quantities of each type of equipment required for each type of laboratory are provided in Tables 2a and 2b. The technical specifications for each type of equipment are provided in Annexure 1.

Table 2a: 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	+/-	+/-
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.

[$] Vertical, for size details, please refer to the laboratory designs.
**For manual extraction, a refrigerated centrifuge is required.
***For all open RT-PCR system laboratories (types 1 to 4), two PCR hoods/laminar flow cabinets will be required.
**** For mobile laboratories, a small refrigerator is recommended.
***** The cost of equipment for each type of laboratory is shown in Annexure 2.

Pipettes

Each laboratory will require a complete set of single-channel and/or a complete set of multichannel pipettes.

Single-channel pipettes: a complete set of single-channel pipettes would comprise P2, P10/P20, P200 and P1000. The volumes of individual pipettes are provided in Table 2b.

Table 2b: Volumes of pipettes required for a COVID-19 testing laboratory

P2	0.2–2 µL
P10	1–10 µL
P20	2–20 µL
P100	20–100 µL
P200	20–200 µL
P1000	100–1000 µL

Multichannel pipettes: Multichannel pipettes are available with 8 or 12 channels; 8-channel pipettes are preferable. Multichannel pipettes of the following three volumes are required:

- 5–50 µL
- 10–100 µL
- 30–300 µL

The quantity of pipettes required for each type of molecular testing laboratory is shown in Table 3.

Table 3: Pipettes required for a COVID-19 testing laboratory

Type of laboratory	**Testing capacity of the laboratory**	**Minimum requirement for pipettes**
Truenat laboratory (one system - Quattro)	Up to 32 samples	One complete set of single-channel pipettes
CBNAAT laboratory (one system with 4 modules)	Up to 32 samples	One complete set of single-channel pipettes
RT-PCR open-system laboratory ± Truenat or CBNAAT		
Type 1a	Up to 200 samples	Three complete sets of single-channel pipettes
Type 1b	200–500 samples	Three complete sets of single-channel pipettes and one complete set of multichannel pipettes
Type 2a	500–1000 samples	Three complete sets of single-channel pipettes and one complete set of multichannel pipettes
Type 2b	1000–2000 samples	Four complete sets of single-channel pipettes and two complete sets of multichannel pipettes
Type 3	2000–5000 samples	Six complete sets of single-channel pipettes and three complete sets of multichannel pipettes
Type 4 (Referral laboratory)	5000–10 000 samples	Seven complete sets of single-channel pipettes and four complete sets of multichannel pipettes
Mobile laboratory (Truenat system)	4 samples per hour	Two complete sets of single-channel pipettes

Annexure 2 provides the costings for setting up each type of COVID-19 molecular testing laboratory. The cost sheets provide both infrastructure and equipment costs for each type of laboratory.

2.5.2 Reagents and consumables

There are various precautions that should be taken to maintain the integrity of all reagents and consumables, as follows.

- Care should be taken to ensure that all reagents are maintained such that they remain contamination-free.

- All reagents should be clearly labelled with the name, expiration date and relevant safety information.
- The date of opening, expiration date and the initials of the individual who opened the reagent must be present on each reagent that is opened for use.
- Reagents with different lot numbers should not be used interchangeably without prior functional verification.
- All reagents from new lots should be tested to ensure that they work correctly, by running a PCR positive-control using the new reagents. If the PCR positive-control fails, the new lots should be tested against the old lots.
- A copy of all manufacturers' specifications and procedures should be kept in the laboratory's SOP binder for every commercially available kit used in the laboratory.
- Logbooks should be maintained for all reagents and kits and should document all pertinent information needed to identify possible sources of contamination, including the following: product name, manufacturer, product number, lot number, all associated dates (receipt, preparation, opening, expiration, when passed internal laboratory assessments, etc.), receiving person or test analyst name and initials, storage location and location of components.
- A list of consumables and reagents required for open and closed system laboratories is provided in the respective chapters.

2.6 Human resources

2.6.1 Personnel

For a COVID-19 molecular laboratory, the minimum staff requirements (for 8-hour shifts/day) are as follows:[14]

- Medical microbiologist: one or more, with experience of working in molecular virology
- Laboratory technician: depending on lab workload, at least two to three per shift, with relevant experience of working in molecular virology
- Multitasking staff: one or more per shift for washing/cleaning

It should be noted, however, that the total staff requirements will be based on the number of shifts, the sample load of the laboratory and also on the type of platform used for RNA extraction. (Automated platforms will require less personnel compared to manual.) Desired expertise the staff should possess includes:

- A good understanding of laboratory biosafety and biosecurity
- Trained for handling respiratory samples for viral diagnosis, RNA extraction and RT-PCR
- Experience of work in virology and handling clinical specimens, especially respiratory samples

Dedicated laboratory technicians should be allocated for:

- Sample processing
- Master mix preparation/template addition/amplification

2.6.2 Sample registration and data entry

Dedicated staff for sample registration and data entry should be allocated as appropriate for the testing capacity of the laboratory.

2.6.3 Housekeeping staff

Separate housekeeping staff must be allocated and trained properly to take care of the clean and dirty areas of the laboratory. Clean areas should be cleaned first. Material/accessories used for cleaning of clean area should be kept separate in clean area only. Similarly, material/accessories used to clean dirty areas should never be taken inside the clean areas of the laboratory.

Table 4 provides an estimate of the human resources required for each type of laboratory.

Table 4: Staff requirements according to the laboratory model

Laboratory model	**Testing capacity of the laboratory (8-hour shift per day) ***	**Human resources requirements**	
		Number of technicians	Number of authorized signatories
A. Truenat laboratory	Up to 32 samples	2	1
B. CBNAAT laboratory	Up to 32 samples	2	1
C. RT-PCR open-system laboratory +/- CBNAAT/Truenat			
Type 1a	up to 200 samples	2	1
Type 1b	200–500 samples	3	1
Type 2a	500–1000 samples	5	2
Type 2b	1000–2000 samples	6	2
Type 3	2000–5000 samples	7	2
Type 4 (Referral laboratory)	5000–10 000 samples	8	3
D. Mobile laboratory (Quattro Truenat)	4 samples per hour	2	1

2.6.4 Training

There is a variety of staff training requirements:

- Adequate staff training and implementation of a competency assessment is essential prior to starting any COVID-19 testing in a laboratory, as shown in Table 5.
- All laboratory staff should undergo induction training of 4-days duration, followed by 6-monthly refresher training
- Competency assessments of laboratory technicians should be performed before and after training, which should include an assessment of the knowledge and skills for performing each of the tasks involved in a diagnostic test; passing criteria should be defined for the same.
- Housekeeping staff must be trained about which areas, surfaces and objects in the facility must be cleaned and disinfected and how often. Also, before using cleaning and disinfectant products, the instructions should be followed, the safety data sheet (SDS) reviewed, and appropriate PPE used.

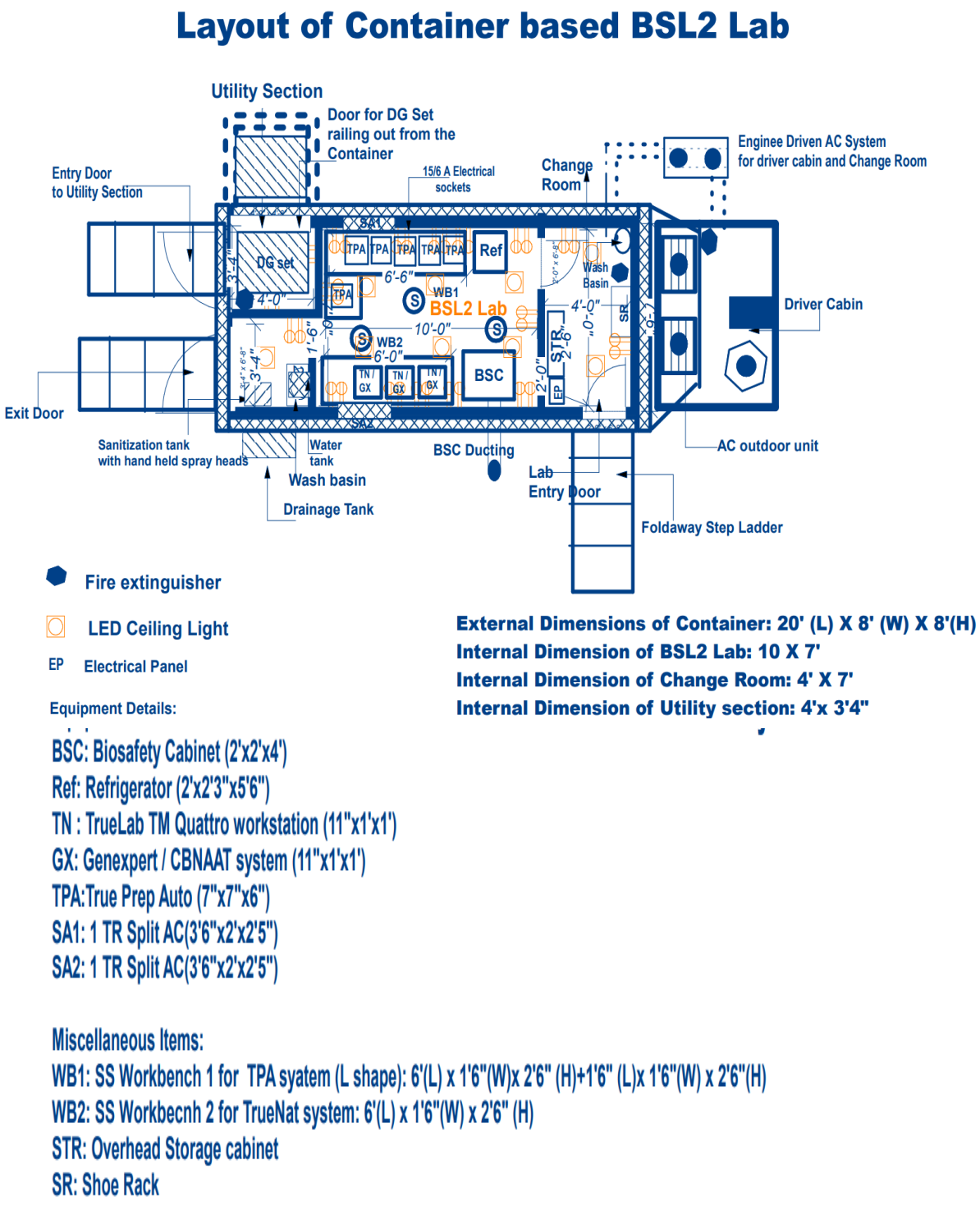
Table 5: Key training areas for laboratory and support staff[15]

Sample collection personnel	**Sample collection personnel and couriers**	**Laboratory personnel/technicians**
• Specimen collection, identification, packaging and storage of samples • Appropriate sample collection techniques • Correct sample identification, packaging and storage	• Specimen packaging and transport	• Assay-specific training sessions • COVID-19 testing techniques and quality assurance, including proficiency testing (PT), external quality assessment (EQA) and reporting of results • Biosafety • Equipment management • Operation and maintenance of laboratory equipment • Stock management • Laboratory inventory management cycle

Data management personnel	**Quality assurance officer**	**Biosafety officer**
• Data management for COVID-19 • Analysis and interpretation of COVID-19 testing data	• Laboratory quality assurance • Implementation of essential elements of quality assurance in the laboratory	• Biosafety • Effective implementation of safety guidelines in the laboratory

The specifications for mobile COVID-19 testing laboratories are provided in Annexures 3 to 5 below.[9]

Figure 8: Layout of a mobile laboratory for COVID-19 testing



Annexure 1: Technical Specifications of the equipment's used in COVID- 19 molecular testing laboratory.

1. Biosafety cabinet

S. No.	Technical specifications
1.	**Main specifications** - The BSC to certify of the class IIA2 NSF 49 or class II EN 12469 or equivalent standard; specifically, with regard to inward airflow (≥0.40 m/s according to EN 12469:2000 or ≥0.50 m/s according to NSF 49:2004) - External height ≤2200 mm including support stand, allowing an available space of at least 400 mm from the top of the BSC to the ceiling. Higher versions may be accepted, provided the 400 mm over the BSC is available to measure air velocity above the exhaust filter, and to have enough space for changing the filter and for ducting and/ or a thimble connection to outlet.
2.	**Internal working area (approximate):** - For a BSC of 120 cm (4 ft): width 1150 mm × depth 630 mm × height 650–750 mm. - A BSC of 60 cm (2 ft): width 800 mm × depth 630 mm × height 650–750 mm. - Inside finish: stainless steel, high quality (e.g., grade 304). - External housing, including screws, made of stainless steel or equivalent resistant galvanized (zinc-coated) sheet steel, subsequently powder coated and thermally hardened; minimum 80 µm thick, or other material that is hard-wearing, resistant to disinfectants and chemicals used in a TB laboratory, and abrasion resistant. - Vertically adjustable sliding window: aerosol-tight, sliding, safety glass (laminated multilayer safety glass only), thickness ≥6.7 mm, counterbalanced. - High optical transmission, but absorption of UV light; minimal reflection. - Working aperture: ≥170 mm measured from work surface to the bottom of the sash window. - Maximal lifting height of front window: 500 mm. - Ability to lock the window hermetically for gaseous disinfection for filter decontamination. - Single-piece working surface with integrated (V-shaped) front air grill. - Alternative: Working surface as segments. - Noise pressure level: ≤60 dbA.
3.	**Internal fittings** Two plugs, 230 ± 10 V, AC, 50 Hz, protected with separate T5A (slow blow) fuse. Voltage and plugs adapted to those used in the country. The line cord / Power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards.

	Warning: Plugs inside the BSC may differ from the main connection to the electricity network. Flicker-free, low-glare, warm-coloured light, >1000 lux.
4.	**Control display on the front of the BSC.** - Electrical control or indicators. - Electronic fan control. - Flow meter for air inflow velocity. - Flow indicator or meter for air down flow velocity. - Operating hours indicator (counter). - Optional: UV light timer. - Filter and flow conditions ultraviolet C (UVC) light (253.7 nm wavelength); 30 W with hour counter; with interlock with white light so that the UVC light can be switched on only when the white light source is switched off. Optional: (if a safety gas burner will be used): Gas tap with solenoid valve, optional right or left side. For a laboratory located in a seismic area, gas pipes are not recommended; small gas containers (approximately 200–400 ml) with butane gas directly fixed to the burner to be used instead.Not necessary when a micro-incinerator is used. Pre filter construction preferred; easily accessible, filter change without tools preferred. High-efficiency particulate air (HEPA) filter (exhaust air filter); classification at least H14; conforming with EN 1822; metal framed.
5.	**Air down flow velocity:** - NSF 49–2002: Requires compliance with the manufacturer's set points, or down flow velocity with a deviation of <0.025 m/s from a nominal set point. - EN 12469: Airflow velocity should be between 0.25 and 0.50 m/s and is defined by the manufacturer according to the construction. Additionally, no individual measurement should differ by more than 20% of the value requested by the manufacturer within the limits given.
6.	**Air circulation volume flow (Modify according to the BSC dimensions):** -For a BSC of 120 cm (4 ft): 700–1200 m^3/h.
7.	**Influx air velocity:** -According to NSF 49, the average airflow velocity at front aperture should be 0.51 m/s for class A2. - EN 12469 does not sub classify within class II BSC. The average airflow velocity at front aperture should be at least 0.4 m/s, according to the manufacturer's specifications.
8.	**Exhaust volume airflow/fresh airflow inward**: - For a BSC of 120 cm (4 ft): 300–600 m^3/h. Blower system to be able to maintain the airflow within a minimum window (narrow limits) on voltage fluctuations.

	Alarms, visible and/or audible, for any unsafe condition of the BSC (e.g. airflow, window position, hardware or software errors). Possibility to shut down alarm for cleaning and maintenance.
9.	**Electricity requirements** Supply voltage: 230 ± 10 V, AC, 50/60 Hz. Voltage and plugs to be adapted to meet the country requirements. The line cord / Power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. Lead fuse T16A (slow blow) or circuit breaker B16. The electrical regulations valid in the country of use as well as the relevant connection conditions are required.
10.	**Power consumption (approximate): Modify according to the BSC dimensions** - For a BSC of 4 ft or 2 ft: 600 W. - Power consumption for plugs inside: Approximately 1000 W. Note: In areas with frequent breakdown of electricity supply, BSCs with low energy consumption can be an advantage; a UPS with lower capacity can be used. - Conform to electrical safety standards IEC 60601–1, UL 61010–1, EN 61010–1. - Protection class (in accordance with EN 60529). - Designed not to interfere with circuit radio (in accordance with EN 55014).
11.	**Documentation** **Manufacturer's certificate** -The manufacturer must have a management system certified to ISO 9001. -The manufacturer to individually test each BSC before shipment. The test report to be provided to the customer, with a duplicate fixed to the BSC. The tests to be performed with research-grade instruments for valid calibration according to test methods outlined in EN 12469 or NSF 49. The test report to contain at least data on: - inflow air velocity - downflow air velocity - filter leak scan for both filters to document filters' efficiency and integrity.
12.	**Accessories** **Table or support frame (support stand)** for a working height of 78 ± 2 cm, adjustable at least at three points (feet) to level. **A telescopic support stand** is advisable for a flexible use. **Ergonomic laboratory chair**, designed for infectious laboratory areas: - adjustable height to suit different users, seat range approximately 400–490 mm - adjustable-angle back rest (no arm rest)

	- caster wheels (five) - all metal parts chrome plated - disinfectable with alcohol-containing disinfectants.
13.	**Thimble Ducting**: Air duct construction with thimble to exhaust air from the BSC. The air duct to be made for the BSC offered and fit precisely. A thimble connection (see WHO TB Biosafety guidelines 2012 page 33) is used with Class II type A2 BSC that is ducted to the outside. The thimble fits over the cabinet's exhaust housing, sucking the air expelled from the cabinet into ducts that lead outside. A small opening (usually 5 cm Page 16 of 62wide) is maintained between the thimble and the cabinet's exhaust housing. This opening enables room air to be drawn into the exhaust ducting system. The thimble must be removable or be designed to allow for operational testing of the cabinet. The power of the external extraction fan installed at the end of the ducting should exceed the volumetric flow rate of each BSC by 30–50%, and should be controllable and connected to an uninterrupted power supply. The air from the BSC should be ducted with ventilation pipes that have a diameter exceed 20 cm. The extractor fan assembly must be easily accessible and preferably kept at the end of ducting with stable fitting. Ducting design should be straight, and the number of bends should be minimal; bends should be round-shaped (sharp/square bends should not be used). Ducting should have adjustable balancing dampers with easily accessibility so that flow can be controlled as and when required. All standard accessories, consumables and parts required for the proper installation, operation and maintenance of the BSC to be included in the offer by the supplier and to be specified and quantified.
14.	**Operation, maintenance and installation** **Operation and maintenance manual** - At least one set of operation, maintenance and service manuals, written in United Nations languages (or at least in English) and preferably also in the official national language of the country requesting the BSC.
15.	**Installation and maintenance** - The bidder must arrange for the equipment to be installed by certified or qualified personnel; any prerequisites for installation to be communicated to the purchaser in advance, in detail. - The bidder to also provide user training (including how to use and maintain the equipment) and a comprehensive maintenance plan. The cost of the maintenance plan to be defined and guaranteed over the period of warranty. - The supplier to provide an after-sale service that covers the whole country. The service to have competent staff, adequate infrastructure and sufficient spare parts to be able to respond to any complaints and to repair or replace the BSC within 14 days.

	- Initial on-site testing (aerosol leak test, recirculating air filter, exhaust air filter, airflow measurements inside the BSC and inward/exhaust airflow) to be carried out by a certified expert and certified compliant for satisfactory installation and safe operation. Measurement results to be printed out for documentation in the maintenance record.
16.	**Standard maintenance tools** All standard accessories, consumables and parts required to operate the equipment, including all standard tools and cleaning equipment, to be included in the offer. Bidders to specify the quantity of every item included in their offer (including items not specified above). If special tools are needed (e.g. to change filters), they must be provided.
17.	**Spare parts** - Each assembled BSC to be accompanied by an authorized list of accessories and spare parts. - At least one, and preferably two, additional sets of HEPA filters as specified above.

2. Deep freezer (-20°C)

S. No.	Technical specifications
1.	**Main specifications** - 100% CFC- free. - One-door freezer, to be used as free-standing freezer. - Capacity (gross): 200-litre vertical type - Cooling system, static. - Defrosting of freezing compartment, initiated manually. - Temperature range of freezer compartment: –9 °C to – 25 °C which can be set by user for specific temperature - Housing material and door: steel, coated, white. - Polyurethane foam “PUF” insulation - Door hinges right or left as desired, reversible. - Fungus-resistant door gasket. - Door with key lock. - Adjustable feet for levelling. - feet standard (optional casters) - Interior container made of white or any colour plastic with 6-9 shelves. - External digital temperature display for freezer compartment with high accuracy sensor mounted inside the chamber suspended in air. - Control panel at the top of cabinet with thermometer Alarms for-Voltage, Over heat, Over cool as well as for under temperature, power failure, Door ajar conditions

	- Refrigerant: CFC Free.
2.	**Electricity requirements** - Supply voltage: 230 ± 10 V, AC, 50/60 Hz. Voltage and plugs to be adapted to meet the country requirements. The line cord / Power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. - Conform to Indian Electrical Safety standards or international standards like IEC 60601–1, UL 61010–1, EN 61010–1. - Protection class (in accordance with EN 60529).
3.	**Documentation** **Manufacturer's certificate** - The manufacturer must have a management system certified to ISO 9001. - Declaration of conformity to the requirements of standards and regulations of the directives that apply to the product, including energy classification, gas used as refrigerant, climate class. - One certificate to state that the freezer has been calibrated at the factory. Quality and safety standards met by the product must be listed.
4.	**Operation, maintenance and installation** **Operation and maintenance manual** At least one set of operation, maintenance and service manuals for the freezer, written in United Nations languages (or at least in English) and preferably also in the official national language of the country requesting the freezer.
5.	**Installation and maintenance** Any prerequisites for installation to be communicated to the purchaser in advance, in detail. The supplier to provide an after-sale service that covers the whole country. The service to have competent staff, adequate infrastructure and sufficient spare parts to be able to respond to any complaints and to repair or replace the freezer within 14 days.
6.	**Standard maintenance tools** All standard accessories, consumables and parts required to operate the equipment, including all standard tools and cleaning material, to be included in the offer. Bidders to specify the quantity of every item included in their offer (including items not specified above).
7.	**Spare parts** Each freezer to be accompanied by an authorized list of accessories and spare parts.

3. Ultra-low freezer (-86°C)

S. No.	Technical specifications
1.	**Main specifications:**

	Upright model: CFC-free, high-efficiency double-refrigeration system for cooling and freezing filled in the bottom. Temperature: 0°C to -86°C +/- - 0.5°Cc (to work in -70 to -86°C range)
2.	**Temperature control:** - Digital temperature controller (including display at suitable eye level) - Microprocessor Control/Microcontroller-for temperature setting - Alarms for-Voltage, Over heat, Over cool as well as for under temperature, power fail, Door ajar conditions
3.	**Size:** 450-litres with minimum of six stainless-steel, rust-free shelves. Fixed on casters for easy manoeuvrability. Polystyrene-insulated inner doors for the compartments. Pre-coated metal body to prevent environmental damage
4.	**Electricity**: 230 V AC, 50 Hz single phase. The line cord/power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards.
5.	**Refrigeration system:** Heavy-duty maintenance free refrigeration system with hermetically sealed refrigeration compressors and reliable cascaded refrigeration to minimize noise and vibration. Air-cooled with security lock to prevent unintentional switch off. Short cooling time of 4 to 5 hours at 40°C ambient temperature. The equipment should be of continuous duty and frost- free. Convenient Air Filter Grill allows easy access for cleaning and changing. Access port for CO_2 back up. It shall be fitted with 24x7 temperature recorders / data loggers which allows for a minimum of 3 GB data storage and the data must be downloadable via a USB port. Deep freezer shall not have an automated defrosting system without a manual override. Audiovisual electronic alarm system independent of power supply and Remote alarm contact in case of equipment failure/ power failure Electrically heated doors for quick opening of frozen doors.
6.	**Accessories** to include suitable boxes and racks for storage of specimen deposits/ DNA extracts/culture isolates in cryo-vials (16 in No. of suitable dimension for 2-mLcryo-vials). Cryo-gloves (four sets, wrist length 12") to be provided as part of accessories.

4. Refrigerator

S. No.	Technical specifications
1.	Vertical, capacity 300 litres or more (up to 450 litres), frost-free, CFC-free, single door. Household refrigerator. Equipment quoted should comply with Indian Standards Institutions Guidelines or any other National or International Guidelines. Supply voltage:

	230 ± 10 V, AC, 50/60 Hz. Voltage and plugs to be adapted to meet the country requirements. The line cord/power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. Voltage regulator of appropriate rating to be included to cope with 160-260 V.

5. Single-channel, variable volume microlitre pipettes

<table>
<tr><th>S. No.</th><th>Technical specifications</th></tr>
<tr><td>1.</td><td>Description of function and use: microlitre pipettes are used for molecular biology procedures; a set with different volumes is required, such as 0.5–10 µL, 2–20 µL, 20–200 µL and 100–1000 µL</td></tr>
<tr><td>2.</td><td>Main specifications
Single channel microlitre pipettes.
Fully autoclavable (121°C); UV-resistant material.
<table>
<tr><th>Pipette for Range</th><th>Increment</th><th>Accuracy</th><th>Precision</th></tr>
<tr><td>0.5 to 10 µL</td><td>0.1 µL</td><td>At least ±5.0–1.0%</td><td>At least 3.0–0.</td></tr>
<tr><td>2 to 20 µL</td><td>0.1 µL</td><td>±3.0-1.0%</td><td>2.5-0.4%</td></tr>
<tr><td>20 to 200 µL</td><td>1 µL</td><td>±1.8-0.6%</td><td>0.7 to 0.2%</td></tr>
<tr><td>20 to 200 µL</td><td>5 µL</td><td>±1.0-0.6%</td><td>0.7 to 0.2%</td></tr>
</table>
In accuracy, the first value applies to smallest volume and the last one to the largest volume in the stated range
In precision, the first value applies to smallest volume and the last one to the largest volume in the stated range
Three defined stops (single-button operation preferred):
- take-up from the first stop
- dispensing and blow out
- tip ejection.
Easy and safe tip ejection mechanism.
Fixation of adjusted volume.
Slim pipette shaft.
Cone for standard tips.</td></tr>
<tr><td>3.</td><td>Documentation
Manufacturer’s certificate
The manufacturer must have a management system certified to ISO 9001.
One certificate to state that the pipette has been calibrated at the factory.
Quality and safety standards met by the product must be listed.</td></tr>
<tr><td>4.</td><td>Operation, maintenance and installation
Operation and maintenance manual
At least one set of operation, maintenance and service manuals for each microlitre pipette, written in UN languages (or at least in English) and</td></tr>
</table>

	preferably also in the official national language of the country requesting the microliter pipette.
5.	**Installation and maintenance** The supplier to provide an after-sale service that covers the whole country. The service to have competent staff, adequate infrastructure and sufficient spare parts to be able to respond to any complaints and to repair or replace the microliter pipette within 14 days.
6.	**Standard maintenance tools** All standard accessories, consumables and parts required to operate the equipment, including all standard tools and cleaning and lubrication material, to be included in the offer. Bidders to specify the quantity of every item included in their offer (including items not specified above). A maintenance kit, with full documentation and tools for in-laboratory calibration according to ISO 9000, are part of the procurement. - Spare parts - Gaskets. - Lubricants. - Each microlitre pipette to be accompanied by an authorized list of accessories and spare parts

6. Electronic multichannel pipettes

S. No.	Technical specifications
1.	**8-channel, 30-300 µL, variable volume micropipette** Volume (µL) / Systematic error / Random error 30 µL / ± 0.9 µL / ± 0.3 µL 150 µL / ± 1.5 µL / ± 0.75 µL 300 µL / ± 1.8 µL / ± 0.9 µL - Operating mode- manual - Pipetting type- Aircushion - Ultra-light in weight - Spring loaded tip cone for minimal tip attachment forces. - Soft eject- low tip ejection force. - Fully Autoclavable - Large 4-digit volume display - Load multi-channel tips quickly and securely without a need for rocking - Extremely consistent sample pickup across all channels **8-channel, 10-100 µL, variable volume micropipette**

	<table><tr><td>Volume (µL)</td><td>Systematic error</td><td>Random error</td></tr><tr><td>10 µL</td><td>± 0.3 µL</td><td>± 0.2 µL</td></tr><tr><td>50 µL</td><td>± 0.5 µL</td><td>± 0.4 µL</td></tr><tr><td>100 µL</td><td>± 0.8 µL</td><td>± 0.3 µL</td></tr></table> - Operating mode - manual - Pipetting type - Aircushion - Ultra-light in weight - Spring-loaded tip cone for minimal tip attachment forces. - Soft eject- low tip ejection force. - Fully autoclavable - Large 4-digit volume display - Load multi-channel tips quickly and securely without a need for rocking - Extremely consistent sample pickup across all channels Needs to be filled for other ranges as per requirements

7. Microlitre refrigerated centrifuge

S. No.	Technical specifications
1.	**Main specifications** - Robust metal housing; compact design with chemical-resistant (coated) housing. - Easy-to-clean, smooth rotor chamber that is resistant to acids, alkalis and disinfectants used in the laboratory. - Microprocessor-controlled centrifuge. - Cooling capacity at maximum speed at +4°C. - Standard rotor with a capacity of at least 18 positions; aerosol-tight (chemical-resistant coated); exchangeable. - Maintenance-free motor. - Maximal relative centrifuge force: 15,000 G. - Automatic lid lock, starting with and during run of rotor. - Option: Automatic opening at the end of the run. - Emergency unlock for electricity blackout. - LCD display; protected; showing time and relative centrifugal force or speed in rcf or rpm & temperature. - Speed adjustable in 100 rpm steps. - If a keypad is used, it should be foil protected. - Timer for runs between 30 seconds and 30 minutes and an option for continuous operation for longer runs. - Short time operation by pressing a time button for short spin. - Adjustment of running time in steps of 30 seconds.

	- Short acceleration time to maximum rcf in ≤20 seconds. - Short breaking time from maximum rcf in ≤20 seconds. - Noise level: ≤58 dbA
2.	**Electricity requirements** - Supply voltage: 230 ± 10 V, AC, 50/60 Hz. - Voltage and plugs to be adapted to meet the country requirements. The line cord / Power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. - Power consumption: Approximately 500 W. - Conform to electrical safety standards IEC 60601–1, UL 61010–1, EN 61010–1. - Protection class (in accordance with EN 60529). - Designed not to interfere with circuit radio (in accordance with EN 55014).
3.	**Documentation** **Manufacturer's certificate** The manufacturer must have a management system certified to ISO 9001. One certificate to state that the centrifuge has been calibrated at the factory. Quality and safety standards met by the product must be listed. **Accessories** Optional: adapter set; reduction device for smaller tubes to be centrifuged in the standard rotor to maximal rotor capacity, for 0.5 mL and 0.2 mL tubes.
4.	**Operation, maintenance and installation** **Operation and maintenance manual** At least one set of operation, maintenance and service manuals written in UN languages (or at least in English) and preferably also in the official national language of the country requesting the microliter centrifuge.
5.	**Installation and maintenance** The bidder must arrange for the equipment to be installed by certified or qualified personnel; any prerequisites for installation to be communicated to the purchaser in advance, in detail. The bidder to also provide user training (including how to use and maintain the equipment) and a comprehensive maintenance plan. The cost of the maintenance plan to be defined and guaranteed over the period of warranty. The supplier to provide an after-sale service that covers the whole country. The service to have competent staff, adequate infrastructure and sufficient spare parts to be able to respond to any complaints and to repair or replace the microliter centrifuge within 14 days.
6.	**Standard maintenance tools** All standard accessories, consumables and parts required to operate the equipment, including all standard tools and cleaning and lubrication material, to

	be included in the offer. Bidders must specify the quantity of every item included in their offer (including items not specified above).

8. Vertical autoclave

S. No.	Technical specifications
1.	**Main specifications** - Vertical autoclave, universal basic version for microbiological standard laboratory to sterilize liquids, instruments, glassware, plastic articles or general infectious waste. - Triple-walled construction; chamber, basket, door lid, doorframe, bolts made of corrosion-resistant material and able to prevent stress cracking preferably made of high-grade stainless-steel sheet of SS-304 grade. Housing with SS legs - Pressure vessel should be Hydraulic tested at factory with minimum hydrostatic pressure: 2.5 kg/cm sq. (35 psi) - Working Chamber volume: approx. 70 -80 liters. - Electrically heated by immersion type heaters bearing ISI mark. - Fast safety lid lock with silicone gasket, it may be radial locking, automatic locking, single lever locking, fly nut assembly mechanism and with heat resistant/safety handle. - Manual water feed system with water level indicator, pressure gauge, steam release cock, spring loaded safety valve, water inlet and water valves - Accessories to be supplied include stainless steel basket (where 2 fit in autoclave directly plus two spare total 4), stainless steel wire basket (where 2 fit in autoclave directly plus two spare total 4), chemical indicator tape for sterilization (2), Bbiological indicator (100), spare heating elements (two), fuses (10) and silicone gaskets (2). - Automatic Water Cut-off Device – To protect the heaters from running dry and to ensure that the machine is automatically switched off in case the desired water level falls below the prescribed level - Working temperature: 121°C, Maximum operating temperature: 134°C (273°F). - Working pressure: 15 PSI, maximum operating pressure: 2.5 bar or 36 PSI Timer with Alarm System - To regulate the sterilization time of the media to be sterilized with a buzzer, sterilization timer: 1–99 minutes A visual chamber gauge, which easily identifies pressure in the chamber must be accessible to the operator as a backup for reading pressure gauge when no electrical power is available.
2.	**Micro-processor temperature control system** with sensor-with user changeable set temperature and timer option. The microprocessor controls the desired temperature (pressure automatically regulated) by cutting off the current to the heating element automatically & restart the mechanism as required. The control panel to be mounted so that the components sensitive to steam and heat are protected. Large LCD display showing: - Chamber temperature - Sterilization time - Alarm information.
3.	**Alarm:** audible, with display on dysfunction & after completion of sterilization cycle.

	Electrical control box, fitted with toggle switch, indicating neon lamps for autoclave ON/OFF status, heater ON/Off status. Over-temperature and over-pressure protection limiter
4.	**Electrical requirements**: Equipment to work on 230 ± 10 V single phase, 50 Hz, plug type adopted to local country scenario, Voltage regulator of appropriate rating to be included to cope with 160-260 V. The line cord/power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards.

9. PCR workstation

S. No.	Technical specifications
1.	**Main specifications** Exterior dimensions (H × W × D): approximately 700 mm × 750 mm × 600 mm. Interior working area (W × D): approximately 700 mm × 500 mm. Exterior: stainless steel or powder-coated metal. Interior: stable formed stainless steel. Side panels transparent, able to absorb wavelengths below 400 nm. Overhead UV light for DNA decontamination; two lamps, 25 W each. Separate, switchable, UV air-sterilizing circulation unit; UV lamp (25 W). Timer and key lock for UV lamp; timer operates only when key lock is on. Overhead white light; 15 W; at least 800 lux. At least two plug outlets built into the chamber; AC 230 ± 10 V; 50 Hz; 5A fuse.
2.	**Electricity requirements** - Supply voltage: 230 ± 10 V, AC, 50/60 Hz**.** - Voltage and plugs to be adapted to meet the country requirements. The line cord/power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. - Power consumption: depends on the electrical equipment used inside the workstation; maximum 1200 W. - Conform to electrical safety standards IEC 60601–1, UL 61010–1, EN 61010–1. - Protection class (in accordance with EN 60529). - Designed not to interfere with circuit radio (in accordance with EN 55014).
3.	**Documentation** **Manufacturer's certificate** The manufacturer must have a management system certified to ISO 9001 and a type-test certificate of relevant optical and mechanical tests. Quality and safety standards met by the product must be listed.
4.	**Operation, maintenance, and installation** **Operation and maintenance manual** At least one set of operation, maintenance and service manuals written in UN languages (or at least in English) and preferably also in the official national language of the country requesting the workstation.

5.	**Installation and maintenance** The bidder must arrange for the equipment to be installed by certified or qualified personnel; any prerequisites for installation to be communicated to the purchaser in advance, in detail. The bidder to also provide user training (including how to use and maintain the equipment) and a comprehensive maintenance plan. The cost of the maintenance plan to be defined and guaranteed over the period of warranty. The supplier to provide an after-sale service that covers the whole country. The service to have competent staff, adequate infrastructure, and sufficient spare parts to be able to respond to any complaints and to repair or replace the workstation within 14 days

10. Real-time RT-PCR machine

S. No.	Technical specifications
1.	- Table top model - Complete system including basic system, essential accessories, state-of-art computer workstation, acquisition and analysis software, startup kit inclusive of calibration standards etc. - Open system to accommodate Taqman, SYBR green and all other fluorescent dye-based chemistries. - Peltier based 96-well block or (384 well) as per requirement - Standard optical 96-well plates, 0.2 mL strip, 0.2 mL tube compatibility - Min sample value requirement – 5 µL - CCD camera with halogen/LED and at least five excitation and five emission filters - Multiplexing ability up to five dyes in a single run - Calibrated dyes at installation: FAM/SYBR Green, VIC/JOE, NED/TAMRA/Cy3, ROX/Texas Red®, and Cy5, should offer flexibility in dye selection. - Facility to calibrate new dye within the wavelength range without addition of new filters - Passive reference dye ROX or any other calibrated dye and should be optional - Option for melt curve analysis - Temperature range 4°C to 100°C - Sensitivity: Detection of 1 copy of template - Software applications: Comparative Ct, Standard Curve, Relative Standard Curve, Allelic Discrimination / SNP Genotyping, Plus/Minus, dissociation/melt curve - Electrical requirement: 220 V/50 Hz. All accessories - CE mark or equivalent

11. Automated RNA extraction machine

S. No.	Technical specifications
1.	- The instrument should be an open system for assay kits, able to accommodate kit from other manufacturers. - Instrument should be compatible for loading 96 samples. - The principle should be on magnetic bead based, to purify nucleic acids, proteins, cells, bacteria in a convenient, rapid and reproducible manner from different starting materials with high quality and yield. - The processing volume should be flexible for all type of sample volumes from 30–5000 µL. - Entire RNA extraction time on instrument should be <40 min for samples per run. - Instrument should not have liquid transfer step involved to avoid sample cross contaminations. - The instrument should have an option of stand-alone mode and PC controlled mode. - The system should have a memory for 200 internal protocols in stand-alone mode. - The instrument should have an option of heating and cooling, for sample from +10°C to +75°C in RT and for elution strip from +4°C to +75°C in RT. - Decontamination feature with UV lamp option with UV exposure time maximum up to 10–16 hours. - Easy protocol import / export option using USB stick. - The software and computer should be supplied with the instrument and the software should not have licenses key for unlimited users' access.

12. Uninterrupted power source (3 KVA/1 KVA/5 KVA) with battery backup

S. No.	Technical specifications
1.	**Description of function and use** The UPS must be used in any settings that have frequent problems in the electricity network (e.g. surges, sags, spikes and blackouts) to assure and back-up the function of the BSC or other equipment, so that any current work on hand can be finished and all potentially infectious sources closed. If the BSC or the equipment is connected to a generator, the UPS will maintain the function of the BSC, RT-PCR, automated extractor machine or any other critical the equipment connected during the time needed for the generator to start and to provide full power. Capacity of UPS required to be suited for the equipment connected and battery back up to provided based on local scenario of electrical outage and power back up availability.
2.	**Main specifications:** - UPS: microprocessor controlled, online continuous transducer, 30 minutes (depends on requirements).

	- Booster function to regulate up voltage breakdown to 170 V. - Buck function to regulate down voltage increase up to 280 V. - Filter to protect against voltage spikes. - Protection against overload and short circuit. - Advanced battery check for automated periodic battery inspection. - Indicators for status (e.g. normal function, net down, working on battery, loading battery, battery capacity). - Sleep mode if item consuming power is shut off. - Power: 230 V ± 25%, 50 Hz or 60 Hz (± 10%) with automatic recognition. - Battery: maintenance-free, automatic shut-off before reaching the level of discharge from which recharging to the original capacity will no longer be possible. - Time for recharging: approximately 8 hours to reach at least 90% of total capacity. - Outlet voltage: 230 V ± 3%, 50 or 60 Hz ± 0.5% (if the country's standard voltage is 110 V AC, adjustment will be needed). - Efficiency coefficient: approximately 90%, on battery >85%. - Noise at 1 m distance <48 dBA. - Permissible ambient temperature and relative humidity: 0–40 °C and 95% (not condensing).
3.	**Electricity requirements** - Supply voltage: 230 ± 10 V, AC, 50/60 Hz. - Voltage and plugs to be adapted to meet the country requirements. The line cord/power cord supplied with the equipment shall be of - acceptable durability, length, and current carrying capacity complying with Indian Standards. - Power consumption: Approximately 1500 W (depending on the model chosen). - Protection class (in accordance with EN 60529). - Designed not to interfere with circuit radio (in accordance with EN 55014).
4.	**Documentation** Manufacturer's certificate The manufacturer must have a management system certified to ISO 9001. Quality and safety standards met by the product to be listed.
5.	**Accessories** - Battery pack. - Connection (cable and fittings) for battery pack. - Stand
6.	**Operation, maintenance, and installation** Operation and maintenance manual At least one set of operation, maintenance, and service manuals, written in United Nations languages (or at least in English) and preferably also

	in the official national language of the country requesting the UPS.
7.	**Installation and maintenance** The bidder must arrange for the equipment to be installed by certified or qualified personnel; any prerequisites for installation to be communicated to the purchaser in advance, in detail. The bidder to provide user training (including how to use and maintain the equipment) and a comprehensive maintenance plan. The cost of the maintenance plan to be defined and guaranteed over the period of warranty.
8.	**Standard maintenance tools** All standard accessories, consumables and parts required to operate the equipment, including all standard tools and cleaning material, to be included in the offer. Bidders to specify the quantity of every item included in their offer (including items not specified above).

13. Vortex mixer

S. No.	Technical specifications
1.	- Adjustable speed: 100 to 2,500 rpm, continuous and intermittent "touch-control" modes, - 220-230 V, AC, 50HZ; The line cord/power cord supplied with the equipment shall be of acceptable durability, length, and current carrying capacity complying with Indian Standards. - Cup heads size: suitable for various range of test tubes - Heavy cast-metal base and suction cup to assure stability, prevent "walking" - Disinfectable - Remarks: Equipment quoted should comply with Indian Standards Institutions Guidelines or any other National or International Guidelines.

14. Air conditioner (split AC with voltage stabilizer; capacity 1, 1.5 or 2 tons)

S. No.	Technical specifications
1.	- Air conditioners suitable for 230 V, 50 Hz single phase, AC supply shall be capable of performing the functions of cooling, dehumidifying, air circulating and filtering. The air conditioners shall be complete with automatic temperature control and cut-in and cutout etc. for temperature range 16–30°C. The differential of the thermostat for cut-in and cut-out shall not be greater than +/-1.75°C. - The air conditioners may either be provided with adjustable step less type mechanical thermostat or electronic thermostat as per IS: 11338:1985. - The eco-friendly air conditioners shall have ECO MARK from Bureau of Indian Standards.

	- Outdoor units of the air conditioners shall be fitted discharge cooled type rotary compressor operating on Refrigerant R-22 (or non-CFC refrigerant R-410 in case of Eco friendly Split Air Conditioner) with suitably rated capacitor start electric motor. It shall be equipped with overload protection and shall be mounted on resilient mountings for quiet operation. The rotary compressor shall be of Matsushita/Hitachi/Toshiba/Carrier/Emerson/ LG /Tecumseh make and shall be covered by manufacturers test certificate and TTC to JISS or ASHRAE. - The minimum thickness of the base in outdoor unit shall be 1.20 mm & sheet thickness for rest of the body shall be 0.70 mm (minimum) with galvanized coating thickness of 120 g/ sq. m and shall be provided with stiffeners for robust construction and shall have rounded corners. - Galvanized sheet shall conform to IS: 277/2003. Steel parts/front panel etc. shall have stove-enamelled finish preceded by thorough cleaning of the surface, phosphating and undercoat of anti-corrosive primer paint. Alternate methods of corrosion protection like plastic powder coating, electrostatic paintings shall also be acceptable in lieu of stove-enamelled finish. - The casing of the indoor units shall be made of ABS/HIPS/GS and shall be impact resistant. The control box of indoor unit shall withstand flame retardant test to Grade V-O as per UL-94. For impact resistance the unit duly packed and dropped from a height of 1 m shall show no damage. The filter pads provided shall be washable. - Remote cordless control with LCD/LED Display shall be provided with one On/Off timer, selecting fan speed (three speed) and setting up of temperature. Display shall be provided on indoor unit or on handset or on both. - Maximum power consumption of the split air conditioners shall be measured at capacity rating test conditions. Overall power factor of the unit shall be at least 0.85 at capacity rating test conditions. - The units shall have minimum 3-star rating certified by BEE, for energy efficiency. - Servicing: free servicing shall be provided for 15 months from the date of dispatch or 12 months from the date of installation of air conditioner whichever is earlier. Firm is also required to send service engineer at least three times during the warranty period.
2.	**Installation**: The installation charges consignee's site shall include the following work: - Mounting/fitting indoor and outdoor units at the respective locations. - Laying refrigerant piping of 4-m length and connecting both the units after drilling hole/holes in the wall, if required. The thickness of the copper tubing shall not be less than 0.70 mm. Connecting copper tubing shall have dimensions suitable for the compressors offered with model.

	- Insulating the suction pipe with expanded polyethylene foam with 9-mm thick tubing. - Laying 15-mm drainpipe up to 10-m length to drain the condensate water formed in the indoor unit. - Leak testing of the entire system. - Charging refrigerant gas in the unit. - Suitable electric wiring between indoors and outdoors units up to 10-m length up to switch within 3 m of location of indoor unit. - Good quality 15 A plug and 32 A MCB (appropriately reduced capacity for 1 ton AC) with box for electrical connection of the stabilizer. Stabilizer for Split AC of capacity 1 ton, 1.5 ton and 2.0 ton a) Minimum Input Power: 170 V b) Maximum Input Power: 270 V c) Over volt protection d) Under volt protection e) Built-in thermal overload protection: protects the stabilizer and compressors during high-temperature burnout. f) Time delay system for the compressor proper balancing time in power cuts. Wall-mountable cooling capacity calculation: For each site location, the actual cooling capacity requirement shall be calculated as per ASHRAE guidelines considering factors like the ambient conditions, room size, ceiling height, floor level, windows and glass, lighting load, occupancy factor, equipment load etc., before supplying and installing the split AC units.

Proprietary Equipment:

1. **GENEXPERT/CBNAAT ® SYSTEMS (CBNAAT):**
   GX-VI - 4 module instrument with desktop computer
   Catalog #: GXIV-4-D
   GX-VI - 4 module instrument with laptop computer
   Catalog #: GXIV-4-L

2. **Truelab® Quattro Real Time Quantitative micro–PCR Analyzer (TrueNAT):**

Principle	Patented real-time micro-PCR
Optics	Fluorescence, three wave-length
Speed	40 cycles of PCR/35 minutes
Throughput	4 chip-random access
Interface	Wi-Fi, 3G, Bluetooth
Calibration	Auto-calibration
Memory	20,000 test results
Operating environment	Temperature: 15-40ºC, RH: 10 -80%

Display	5” Capacitive Touch Screen TFT-LCD
Printer	External 2” Bluetooth thermal printer
Power	Rechargeable lithium-ion battery pack: 7.4 V; 8.7 Ah. Input to AC/DC adaptor: Single Phase 100-240 V; 47-63 Hz; 1.35-0.53 A Output from AC/DC adaptor: 10 V; 4.5 A; 45 VA. If the input specifications in your country do not meet the above requirements, please contact your local Molbio representative.
Weight	5.2 kg
Size	400 mm x 242 mm x 159 mm

Annexure 2: Total estimated costs of equipment for the various types of COVID-19 molecular testing laboratories

Total estimated cost of equipment for a COVID-19 laboratory with one Truenat system

Equipment	Unit cost (INR)	Number of units	Total cost (INR)
*Class II Type A2 biosafety cabinet (2'x 2'x7')	150,000–450,000	1	150,000–450,000
Refrigerator with freezer(300L)	65,000–150,000	1	65,000–150,000
Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–250,000	1	65,000–250,000
TrueNat TM Quatro	~6,00,000	1	~ 6,00,000
True Prep Autoextractor (two units) (7"x7"x6")	~ 4,00,000	2	~ 8,00,000
Personal computer (2'3"x2'9") with printer	80,000–100,000	1	80,000–100,000
Complete sets of single-channel pipettes	48,000–60,000	1	48,000–60,000

*A class II Type A2 BSC (2'x 2 x 7') is optional

Total estimated cost of equipment for a COVID-19 laboratory with CBNAAT (with one GeneXpert/CBNAAT system)

Equipment	Unit cost (INR)	Quantity	Total Cost (INR)
Class II Type A2 biosafety cabinet (2'x2'x7')	150,000–450,000	1	150,000–450,000
Refrigerator with freezer (300 L)	65,000–150,000	1	65,000–150,000
-86˚C Freezer (2'6"x2'6"x6')	250,000–500,000	1	250,000–500,000
Vertical autoclave (70) (2'6"x2'2"x4'7")	65,000–250,000	1	65,000–250,000
GeneXpert®/CBNAAT (four modules)	~19,00,000	1	~19,00,000

Personal computer (2'3"x2'9") with printer	80,000–100,000	1	80,000–100,000
Complete sets of single-channel pipettes	48,000–60,000	1	48,000–60,000

Total estimated cost of equipment for a Type 1a COVID-19 laboratory (up to 200 samples/day in 8-hour shifts)

Equipment	Unit cost (INR)	Quantity	Total cost (INR)
Class II Type A2 biosafety cabinet (2'x2'x7')	150,000–450,000	1	150,000–450,000
Refrigerator (300 L)	50,000–100,000	1	50,000–100,000
-86˚C freezer (2'6"x2'6"x6')	250,000–500,000	1	250,000–500,000
-20˚C freezer (2'x2'x5')	30,000–135,000	1	30,000–135,000
Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–250,000	1	65,000–250,000
PCR workstation/hood (2'x1'6"x2'3")	45,000–275,000	2	90,000–550,000
Microlitre refrigerated centrifuge (1' x1'6"x 1')	200,000–350,000	1	200,000–350,000
RT-PCR (2'x1'6"x2') 96 well	1,200,000–,2100,000	1	1,200,000–2,100,000
Personal computer (2'3"x2'9") with printer	80,000–100,000	1	80,000–100,000
Complete sets of single-channel pipettes	48,000–60,000	3	144,000–180,000

Total estimated cost of equipment for a Type 1b COVID-19 laboratory (200–500 samples/day in 8-hour shifts)

Equipment	Unit cost (INR)	Quantity	Total cost (INR)
Class II Type A2 biosafety cabinet (2'x2'x7')	150,000–450,000	2	300,000–900,000
Refrigerator (300 L)	50,000–100,000	1	50,000–100,000
-86˚C freezer (2'6"x2'6"x6')	250,000–500,000	1	250,000–500,000
-20˚C freezer (2'x2'x5')	30,000–135,000	1	30,000–135,000

Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–250,000	1	65,000–250,000
PCR workstation/hood (2'x1'6"x2'3")	45,000–275,000	2	90,000–550,000
Microlitre refrigerated centrifuge (1' x1'6"x 1')	200,000–350,000	1	200,000–350,000
*Automatic RNA extraction machine (2'6"x2'6"x3')	~10,00,000	1	~10,00,000
Real Time PCR (2'x1'6"x2') -96 well	1,200,000–2,100,000	1	1,200,000–2,100,000
Personal computer (2'3"x2'9") with printer	80,000–100,000	1	80,000–100,000
Complete sets of single-channel pipettes	48,000–60,000	3	144,000–180,000
Complete set of multichannel pipettes	75,000–180,000	1	75,000–180,000

* Automatic RNA extraction machine (2'6"x2'6"x3') is optional

Total estimated cost of equipment for a Type 2a COVID-19 laboratory (500–1000 samples/day in 8-hour shifts)

Equipment	**Unit cost (INR)**	**Quantity**	**Total cost (INR)**
Class II Type A2 biosafety cabinet (4'x2'6"x7')	250,000–650,000	1	250,000–650,000
Refrigerator (300 L)	50,000–100,000	2	100,000–200,000
-86˚C freezer (2'6"x2'6"x6')	250,000–500,000	2	500,000–1,000,000
-20˚C freezer (2'x2'x5')	30,000–135,000	1	30,000–135,000
Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–250,000	2	130,000–500,000
PCR workstation/hood (2'x1'6"x2'3")	45,000–275,000	2	90,000–550,000

Automatic RNA extraction machine (2'6"x2'6"x3')	700,000–1,400,000	1	700,000–1,400,000
Real Time PCR(2'x1'6"x2') -96 well	1,200,000–2,100,000	2	2,400,000–4,200,000
Personal computer (2'3"x2'9") with printer	80,000–100,000	2	160,000–200,000
Complete sets of single-channel pipettes	48,000–60,000	3	144,000–180,000
Complete set of multichannel pipettes	75,000–180,000	1	75,000–180,000

Total estimated cost of equipment for a Type 2b COVID-19 laboratory (1000–2000 samples/day in 8-hour shifts)

Equipment	Unit cost (INR)	Quantity	Total cost (INR)
Class II A2 biosafety cabinet (4'x2'6"x7')	250,000–650,000	2	500,000–1,300,000
Refrigerator (300 L)	50,000–100,000	2	100,000–200,000
-86˚C freezer (2'6"x2'6"x6')	250,000–500,000	2	500,000–1,000,000
-20˚C freezer (2'x2'x5')	30,000–135,000	1	30,000–135,000
Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–250,000	2	130,000–500,000
PCR workstation/hood (2'x1'6"x2'3")	45,000–275,000	2	90,000–550,000
Automatic RNA extraction machine (2'6"x2'6"x3')	~10,00,000	2	~20,00,000
Real Time PCR(2'x1'6"x2') -96 well	~15,00,000	3	~45,00,000

Personal computer(2'3"x2'9") with printer	80,000–100,000	3	240,000–300,000
Complete sets of single-channel pipettes	48,000–60,000	4	192,000–240,000
Complete set of multichannel pipettes	75,000–180,000	2	150,000–360,000

Total estimated cost of equipment for a Type 4 COVID-19 laboratory (5000–10,000 samples/day in 8-hour shifts)

Equipment	**Unit cost (INR)**	**Quantity**	**Total cost (INR)**
Class II Type A2 biosafety cabinet (4'x2'6"x7')	2,50,000, –650,000	3	750,000–1,950,000
Refrigerator (300 L)	50,000–100,000	4	200,000–400,000
-86˚C freezer (2'6"x2'6"x6')	250,000–500,000	4	1,000,000–2,000,000
-20˚C freezer (2'x2'x5')	30,000–135,000	1	30,000–1,35,000
Vertical autoclave (70 L) (2'6"x2'2"x4'7")	65,000–2,50,000	2	130,000–500,000
PCR workstation/hood (2'x1'6"x2'3")	45,000–275,000	2	90,000–550,000
Automatic RNA extraction machine (2'6"x2'6"x3')	700,000–1,400,000	4	2,800,000–5,600,000
Real Time PCR(2'x1'6"x2') - 96 well	1,200,000–2,100,000	3	3,600,000–6,300,000
Real Time PCR(2'x1'6"x2') - 384well	2,200,000–3,200,000	3	6,600,000–6,900,000

Personal computer (2'3"x2'9") with printer	80,000–100,000	6	480,000–600,000
Complete sets of single-channel pipettes	48,000–60,000	7	336,000–420,000
Complete set of multichannel pipettes	75,000–180,000	4	300,000–720,000

Costing of equipment for a mobile laboratory for COVID-19 testing** **(with three Truenat systems)**

Equipment	**Unit cost (INR)**	**Quantity**	**Total cost (INR)**
*Class II Type A2 biosafety cabinet (2'x2'x7')	150,000–450,000	1	150,000–450,000
Refrigerator with freezer (300 L)	65,000–150,000	1	65,000–150,000
Truenat TM Quatro	500,000–700,000	3	1,500,000–2,100,000
True Prep Auto extractor (two units) (7"x7"x6")	300,000–400,000	5	1,500,000–2,000,000
Personal computer (2'3"x2'9") with printer	80,000–100,000	1	80,000–100,000
Complete sets of single-channel pipettes	48,000–60,000	1	48,000–60,000

Note: These are only estimated costs and prices may be subject to variation for various reasons.

Annexure 3: Specification of vehicles that can be used for a mobile COVID-19 testing laboratory

Property	Specifications
Dimensions and materials	Overall dimensions (L × W × H): 8000–9000 mm × 2200–2500 mm × 2400–2700 mm
	Wheelbase of approximately 4200–5000 mm
	Ground clearance: 228 mm or more
	Number of tyres: 6+1
	Chassis size to fix a container of 20 ft × 8 ft × 8 ft
	Loading span (L × W × H): 20 ft × 8ft × 8 ft
	Payload of the vehicle: minimum 6 ton
Engine	Engine should be BSVI-compliant to the latest pollution standards
	Engine type: 4 cylinder
	The vehicle should have power steering
Fuel type and tank	Diesel; capacity 150 litres or more
Brakes and suspension	Safety brake features: full air dual line with parking brakes on rear wheels only
	Front suspension: semi-elliptical leaf spring type/parabolic leaf spring type
	Rear suspension: Semi-elliptical leaf spring type
Maintenance	Servicing facility

Annexure 4: Specifications of the container, interior fabrication/fittings and equipment required for a mobile COVID-19 testing laboratory

Specifications	
1	**Container: detailed specifications** **Operational environment:** the container should be designed and constructed so that all materials used in its construction are able to withstand the extreme temperature conditions in India without affecting the container's strength and water tightness. **Standards:** The container should be a standard container of around 2.5 ton and must comply to required ISO standards with and have CSC (Container Safety Certificate) certification **Dimensions:** external length: 20 ft × external width: 8 ft × external height: 8 ft **Door opening:** the door should be a full-length, iron-rod type door of width 3 ft with a lock and key mechanism. The rear door should be a double door, and the side door (entry to the container) should be a single door. **Construction:** The container should be constructed out of mild steel frames; fully vertical mild steel for the side and end walls, with a stamped mild steel roof. This should be customized as per requirement and the layout submitted. **Coating/painting of the container:** All steel surface and welding joints must be coated with primer paint immediately after shot blasting. The exterior surface should be painted white using epoxy paint primer. Paints, oils, varnishes etc. should be of approved brands and manufacturers. **Attachments:** The container must be fitted with external hooks for easy lifting and shifting. **Fitting the container to the vehicle chassis:** The container should be firmly fixed to the chassis of a compatible vehicle using nuts, bolts and welding, as necessary; all fixings should be smooth and as per industrial quality requirements. For improved ground clearance plus maximum stability and driver comfort with any load, the appropriate suspension assembly must be fitted with the vehicle chassis and the container. Three foldable ladders should be fixed to the container.

2	**Fabrication/interiors** **Internal partitions:** The internal partitions should be created inside the container as per the layout and as per the details below. **Internal dimensions:** ● Changing room: length 4 feet × width 7 feet ● BSL-2 laboratory: length 10 feet 6 inches × width 7 feet ● Sanitization and wash basin section: length 4 feet ×width ● 3 feet 4 inches ● Utility section (for the generator set (genset) and UPS with batteries): length 4 feet × width ● 3 feet 4 inches ● Overall height of all sections: 8 feet **Modular walls:** made up pre-engineered 60-mm thick PUF panels with GPSP sheets with PUF insulation of minimum 38 to –40 kg/m^3. Each GPSP sheet should be 0.8-mm thick. The panels must be installed along outer walls, partitions and false ceilings to create an impervious shell that is fully sealed. The panels on each side should be coated with epoxy/powder paint. The panels should be easy to maintain and be aesthetically appealing. **False roofing/ceiling:** The internal ceiling must be monolithic, impervious, non-particle shredding, chemical resistant (especially to hypochlorite cleaning) and able to withstand chemical use during decontamination/fumigation. Modular false ceiling panels suitable for clean room application should be pre-engineered using 60-mm thick PUF panels covered with GPSP sheets; the PUF insulation should be a minimum of 38 to 40 kg/m^3. Both surfaces should be 0.8 mm thick GPSP sheet and installed on the ceiling, to create an impervious shell that is fully sealed. The panels on inside will be painted with epoxy paint and powder coated on the outside. The panels should be easy to maintain and be aesthetically appealing. **Flooring:** The base surface should be made of hardwood; any woodwork to be painted must be dry and free from moisture. Uniform seamless industrial vinyl flooring should be laid on the top of the wood. Welding between corner posts and rails must be continuous single welding. Coving.: covings for entire wall to floor, wall to wall & wall to ceiling joints. Extruded aluminium double cove integrated with top track of the partition panels. Internal and external corner cove joining pieces should be made of aluminium with an anodized finish. This should be of similar construction and finish as the walls and be properly sealed with silicon sealant between the wall and the ceiling.

Any penetrations through the walls, ceiling or floors must be sealed using suitable caulking. Caulking must be applied around pipes and conduits. The interior of electrical and cable conduits should also be caulked.

Internal doors:

Flush door finishes should be 45-mm thick, be resistant to chemicals, and have antifungal and antibacterial properties. 1.2 mm thick GPSP sheet suitable to fix on 60-mm thick wall panel with provisions for double-glazing for all doors, with push plates and handles on both sides. PUF panels should comprise GPSP sheets, epoxy-painted on both sides, and have PUF insulation of minimum 38 to 40 kg/m^3. Concealed hardware should be used to fix door frames, e.g., TS-71 door closures, stainless steel hinges, door handles, ball-bearing butt hinges, concealed tower bolts for double doors, with a lock and key arrangement on both sides. Suitable neoprene "Y-seal"-type gaskets should be used between the door jam and door stop.

Access control system: door interlocking systems should include a controller module, push button stations with LED indicators, and electromagnetic locks. An alternative electrical switch should be provided so that the doors can be opened manually in case of a malfunction.

Vision glass: vision glass for doors should be fixed, vacuumized and insulated with 6-mm-thick toughened glass and should be installed for natural lighting, flush with the surface of the door. All fixings should be flush for ease of cleaning and maintenance. No crevices, joints or sloped profiles are to be used for fixing the glass. This will avoid contamination and dust accumulation.

Engine-driven AC system: this should comprise a suitable roof-top model with a blower. It should have high cooling capacity, including an adequate condensing and evaporating system for both the driver's cabin and the changing room section. The refrigerant must be HFC/CFC-free.

Interiors: The interiors of the van should be suitable for cleaning, scientific-grade fumigation and treatment with disinfectants. Joints should be flush, seamless, hermetically sealed, waterproof and easy to disinfect. All interior materials must comply with fire safety requirements

3	**Power requirements** An autonomous power supply for laboratory components, laboratory equipment and energy efficient internal lighting (LEDs) should be provided. All power supply points, conduits, junction boxes and lighting fixtures inside the laboratory should be sealed to prevent air from leaving or entering the main laboratory. All sockets must be waterproof and airtight. One suitable diesel/petrol acoustic genset (8.5 kVA capacity or higher) should be provided, with very low noise and vibration and a UPS for smooth and safe operation. The genset should conform to Central Pollution Control Board standards. The UPS (of 5 kVA capacity with a 2-hour back-up time) should be able to provide sufficient power to operate the laboratory equipment, lights and the door interlock systems. The genset should be capable of handling the power requirements to operate the laboratory equipment (BSC, refrigerator, 4 True NAT (Quattro equipment), two split AC units, plus internal sockets, lights etc.). The genset should be stationed in a safe location in the utility area that is easy to access and handle. Thus, the genset should be fitted towards the back of vehicle on a rail, have collapsible legs and be behind a closable door. This will allow it to be removed when the van is being cleaned. The collapsible legs support the genset in this position. The genset should be situated on vibration pads to reduce vibrations in the vehicle, so that it can be running while producing minimal vibrations when the laboratory is in operation. The genset manufacturer/authorized dealer must have a pan-Maharashtra servicing facility. There should be provision for a 20-liter canister to be secured outside the vehicle, for the genset to run on. Provision for emergency lighting. All fixtures must be able to withstand laboratory fumigation. Provision to draw power for the entire unit from DG Set or any external source. Provision to run critical equipment using the UPS. Provision to integrate the DG and UPS when required. All equipment and AC should be powered by the genset when stationary or via an external line to the genset, if available at the location. One split AC installed in the BSL-2 laboratory must be able to run on the vehicle's power in case of any emergency situation.

4	**Electrical fixtures and fittings** **Electrical circuits:** there must be two, independent, forward electrical circuits in the van, one receptacles for 12 V DC, of reputable make and meeting IS-1293 standards. There must be both short-circuit and overload protection through the use of fuses or mini circuit breakers (MCBs) for the various segmented electrical installations. Fuse ratings should be clearly marked on each fuse, with three of each fuse housed in a covered fuse box or other appropriate place. The electrical equipment and material indicated for connection to a wiring system rated 220 volts nominal 2 wire with ground shall incorporate a minimum 15 ampere circuit breaker or adequate rating which can be used as a master AC disconnect switch. **Interior illumination**: LED lights must be installed in the BSL-2 section, changing room, sanitization section and utility section (ten in each). All interior lighting should be fixed to prevent movement during vehicle movement or vibration. **Electrical fixtures:** these must be flush-mounted and should not protrude more than 50 mm. All switches, indicators, and controls must be located and installed in a manner that facilitates easy removal and servicing. All exterior housings of lamps, switches, electronic devices, connectors, and fixtures must be corrosion resistant and weather-proof. AC wiring should use standard wire. **Power supply points:** a total of 13 power supply points must be provided inside the BSL-2 laboratory and the changing room. ● All switches, connectors and end-wiring should be rated to carry a minimum of 125% of their maximum ampere load. All wiring should confirm to IS-12645 specifications. ● All wiring must be permanently colour-coded or marked the entire length of the wire for identification, with easily read numbers, letters, or both, and routed through conduits. ● When cables are supplied by a component manufacturer to interconnect system components, these cables need not be continuously colour-coded/identified. However, they must be coded/identified at termination and interconnection points. ● All wiring should be located in enclosed, protected but accessible locations and kept at least 150 mm (6”) away from exhaust system components. **Public address system:** a public address system must be provided, with the following specifications: ● Input voltage: 13.2 V DC ● Base: aluminium dome ● Speaker grill: stainless steel ● Speaker: two 50 Watt unit horns

	● The speaker must be protected by the speaker grill, which must be completely waterproof ● There should be a selector switch for radio/wail/yelp/hi-lo of 100 W.
5	**Equipment to be supplied and installed in the BSL-2 laboratory section** **Laptop, one unit** ● i5 7th Gen - (8 GB/1 TB HDD/Windows 10 Pro) 348 G4 Business Laptop (14 inch, black, 2 kg) ● OS: Windows 10 PRO ● Processor: i5 or above ● RAM: 8 GB or above ● Storage: 1 TB HDD or 512 SDD ● Screen size: 13 to 14inch **Printer, one unit** ● Black/white (A4 normal), up to 8 ppm ● Duty cycle (monthly, A4), up to 2000 pages ● Recommended monthly page volume: 500–1000 ● Laser print technology, connectivity USB 2.0, 2 MB RAM ● Compatible Operating System: Windows 10 (64 bit/32bit) , Windows Vista, and Windows XP **Tablet, two units** ● Screen size: minimum 10 inch ● Camera: minimum 8 MP ● CPU type: minimum octa core or above ● RAM: 4GB, provision of external memory support ● 4G-compatible ● OS: Android ● Connectivity: USB, wi-fi and Bluetooth ● Battery: approx. 7000 mAH ● Video/audio playing formats: MP4, M4V, 3GP, 3G2, WMV, ASF, AVI, FLV, MKV, WEBM/MP3, M4A, 3GA, AAC, OGG, OGA, WAV, WMA, AMR, AWB, FLAC, MID, MIDI, XMF, MXMF, IMY, RTTTL, RTX, OTA ● Weight: < 500 g **Wi-fi router (plug and play):** to be provided for internet connectivity **GPS** tracker **Online UPS, one unit** ● Minimum 5 kVA with external battery; charging via genset/external source ● The UPS should be microprocessor controlled, with an online continuous transducer ● Backup of 2 hours ● Booster function to regulate up-voltage breakdown to 170 V ● Buck function to regulate down-voltage increase up to 280 V

- Protection against overload and short circuit
- Indicators for status (e.g. normal function, net down, working on battery, loading battery, battery capacity)
- Sleep mode if item consuming power is shut off
- Power: 230 V ± 25%, 50 Hz or 60 Hz (± 10%) with automatic recognition
- Battery: maintenance-free, automatic shut-off before reaching the level of discharge from which recharging to the original capacity will no longer be possible
- Outlet voltage: 230 V ± 3%, 50 or 60 Hz ± 0.5%
- Efficiency coefficient: approximately 98%, on battery >85%
- Noise at 1 m distance <48 dBA
- Supply voltage: 230 ± 10 V, AC, 50/60 Hz
- The line cord/power cord supplied with the equipment must be of acceptable durability, length, and current carrying capacity, in compliance with Indian standards
- Accessories: battery pack, connectors (cables and fittings) for battery pack, stand

Split air conditioner of 1 TR capacity, two units

- These must be inverter ACs (minimum three star) manufactured by Hitachi/Bluestar/Carrier/Lloyd/Godrej or equivalent OEM and have a suitable voltage stabilizer
- They must be roof-mounted, split ACs of 1 tonne capacity, that run on 240 V
- The manufacturer/authorized dealer must have a pan-India servicing facility
- The outdoor units of both split ACs are to be located in the space between the driver's cabin and the container; an appropriate frame and fixings must be provided
- Drainage pipes must be sufficiently long and connected to the drainage system of the vehicle

Biosafety safety cabinet (Class II type A2, 3 feet), one unit

A biological safety cabinet (BSC) must be commissioned, installed and validated inside the mobile laboratory at the required location, as per the plan. The BSC should be placed away from doors, air supply vents or anything else that may disrupt the cabinet airflow. The exhaust from the BCS must be thimble-connected and individually ducted out. (The ducting material and an external blower of sufficient capacity for the BSC ducting should be provided by an Identified Agency.)

Laboratory refrigerator, one unit

- Temperature range: 2–8˚C
- Capacity: 300–350 L
- Features: vertical (floor-standing), frost-free, CFC-free, single door

	• Supply voltage: 230 ± 10 V, AC, 50/60 Hz; voltage and plugs must be adapted to meet the country's requirements. • Must be fixed to the floor to avoid sliding or dislocation of equipment when the vehicle is moving • The equipment must comply with Indian Standards Institutions Guidelines and any other national or international guidelines • The line cord/power cord supplied with the equipment must be of acceptable durability, length and current carrying capacity, in compliance with Indian standards. • The refrigerator must be able to run on vehicle-driven mode, an external power source and a genset source, as the reagents and kits will be stored in it and the refrigerator therefore needs a continuous power supply, 24/7 **All equipment must be securely fastened, as per the details in section 5.**
6	**Interior fitting requirements** The following interior fittings should be provided: A work bench made of SS304-grade stainless steel. Each work bench should have two storage shelves, with a lock and key arrangement for the secure storage of laptops. The work benches should have the following dimensions: • L- shaped work bench: 7 ft 6 in × 2 ft × 2ft 6 in (L × W × H), one unit • Straight work bench: 5 ft 6 in × 2 ft × 2 ft 6 in (L × W × H), one unit Two lockable (lock and key arrangement) wall-mounted cabinets made of SS304-grade stainless steel, of the following dimensions: • BSL-2 laboratory section: 5 ft x 1 ft x 1ft (L × W × H) • Changing room section: 3 ft X 1 ft x 1 ft (L × W × H) • Two elbow or foot operated wash-basins made of SS304-grade stainless steel and of appropriate size to fit in the changing room and the sanitization section. • Freshwater tank wash basin with a minimum capacity of 20 L- 2 units freshwater tank (wall mounted) capacity. The tap should be foot- or elbow-operated. The tap must be positioned such that when washing hands, no water should fall outside of the sink area. A dispenser for liquid hand-soap should be installed near the wash basins. • Wastewater storage tank: Wash basin and drain water tank (20 L- 2 units). All hose connection joints must be firmly fixed with clamps and sealant to prevent water overflowing into the BSL-2 section. • Water disposal: a 5-cm diameter galvanized-iron pipe must be fixed under the sink to drain the water. All liquid draining out must be connected to a single drain with back-flow prevention and a disinfectant portion in the holding tank close to the outlet. The drains must be equipped with P-traps. Any penetrations made in walls

and floors must be properly sealed. Another plastic pipe of appropriate diameter and length must be provided to drain the water away to a safe area.

- Sanitization section: a sanitization tank (approximate capacity 10 L) with a handheld spray-head must be provided. The sanitization tank must be refilled as and when required by the laboratory.

Truenat Quattro or GeneXpert/CBNAAT equipment and shock-absorber pads: The Truenat GeneXpert/CBNAAT equipment must be fixed on a fabricated base (as per the manufacturer's recommendations) and further secured with Velcro straps. The base absorbs shocks when the vehicle is moving and prevents vibration and movement of the machine during transportation (this is critical for the smooth running of the operation).
UPS and other ancillary equipment: This must also be securely fastened, using bolts, to a base-plate to prevent movement while the vehicle is mobile.
Adjustable-height, stainless-steel stools (four units) must be provided. These must also be securely fastened, using bolts, to a base-plate to prevent movement while the vehicle is mobile.

Two folding-type, single seats should be installed in the changing room for the safe and secure travel of personnel when the vehicle is moving.
A shoe rack (one unit), made from SS304-grade stainless steel and of dimensions 1 ft × 1 ft (W × H) must be provided in the changing room section.
Dustbins (three units, 10-L-capacity each): these should be foot-operated dustbins, made of plastic or stainless steel, and installed in the BSL-2 laboratory section, the changing room and the sanitization section.

Any tools necessary for the repair of any fabrications must be provided in the vehicle.
The following items are required for the patient sample collection area and sitting area:

- Canopy, two units:
  - Dimensions: 8 ft (H) × 6 ft (L) × 6 ft (W)
  - Material: PVC (white in colour); it must be waterproof, washable and resistant to fumigation
  - It should be foldable, so it is portable and easily dismantled and installed
  - The frame should be durable, sturdy, metallic and MS powder-coated
- One fogging machine, SS304 stainless steel, 10 L capacity tank, equipped with a spray-head unit; it should run on an AC line supply/genset and have a timer. It should be filled as and when required by the laboratory.
- Four sturdy, foldable chairs that are resistant to chemicals
- Two sturdy, foldable tables, 2 ft (L) × 1 ft 6 in (W), that are resistant to chemicals

Storage space should be provided below the container to store the foldable canopy, two chairs, table, fogging machine and sanitization tank after use. A lock and key facility must be provided. If space permits, some of the items may be stored in the utility area if properly

	arranged. Under no circumstances should any of these items be stored inside the laboratory.
7	**Fire safety** Three multipurpose fire extinguishers (4kg) must be provided. These should be of ABC type (ISI marked and conforming to BIS: 15683-2006), duly filled, of capacity and quantity as per the provisions of the Central Motor Vehicle Rules 1989. One extinguisher should be wall-mounted in each of the following: the changing room, the utility room and the driver's cabin.
8	**Driver's cabin** The driver's cabin should have provision for external charging.
9	**Warranty details** The period of warranty for the following equipment should be a minimum of 1 year: vehicle, genset, AC units, BSC, refrigerator, laptop and printer.
10	**Other requirements: partners' logos; messaging** All external markings should be retro-reflective in nature and materials used for the same should meet or exceed requirements of ASTM D 4956. Other points to note with regards to labelling include: ● Standard specifications required for retro-reflective sheeting ● Biohazard markings/labels as per standard Electrical points for specific equipment should be labelled
11	**Operating manual and instruction sheets** ● A comprehensive User Manual(s), written in English, must be provided. This should include detailed descriptions of parts and equipment, operating instructions, service contact numbers, etc. for the base vehicle, compartment, equipment, fittings, etc. The manuals should be printed on high-quality paper and housed in water-resistant pouches. ● Laminated sheets must be produced, clearly showing the layout of the BSL-2 laboratory section, changing room, sanitization section, utility section and the driver's cabin, with the location of equipment, fittings, switches, consumables, etc. suitably depicted. These should be fixed in the patient and driver cabins at suitable locations.

	Laminated sheets must be produced showing the OEM and non-OEM electrical wiring diagrams, complete with the locations of various fuses and circuit breakers. These should be displayed in the vehicle at appropriate locations.

Annexure 5: Specifications of the biological safety cabinet required for a mobile COVID-19 testing laboratory

SR No	Technical specifications
1	Biological Safety Cabinet Class II Type A2 with thimble canopy.
I	Application: In a microbiology laboratory, a BSC Class II Type A2 is used to provide protection to the operator, specimens and the environment during the handling of potentially infectious specimens, cultures and microbial strains.
i	Technical specifications: the BSC must meet the requirements of Class II Type A2 NSF 49 or Class II EN 12469; specifically, the inward airflow should be ≥0.40 m/s according to EN 12469:2000 or ≥0.50 m/s according to NSF 49:2004.
ii	Class II, Type A2 BSC internal working area (approximate) ● Vertically adjustable sliding window: aerosol-tight safety glass (laminated multilayer safety glass only), thickness ≥6.7 mm, counterbalanced ● Cabinet made of high-quality stainless steel (e.g. grade 304) ● High optical transmission, but absorption of UV light; minimal reflection ● Working aperture: ≥170 mm, measured from the work surface to the bottom of the sash window ● Maximal lifting height of front window: 500 mm ● Single-piece working surface ● Noise level: ≤60 dBA ● External housing: made of stainless steel or equivalent resistant galvanized (zinc-coated) sheet steel, subsequently powder-coated and thermally hardened; minimum 80 µm thick GI sheet.

	● ULPA/HEPA-filtered, re-circulated mass airflow within the workspace ● Exhaust air from the cabinet to be filtered by ULPA/HEPA filters of classification at least H14; conforming with EN 1822 ● Metal-framed
iii	Size (modify according to the BSC dimensions): Approx. 3 ft. (90 cm) 900–1000 mm (W) × 620–630 mm (D) × 650–750 mm (H)
	Air downflow velocity: ● NSF 49–2002: requires compliance with the manufacturer's set points or downflow velocity with a deviation of <0.025 m/s from a nominal set point. ● EN 12469: Airflow velocity should be between 0.25 and 0.50 m/s and is defined by the manufacturer according to the construction. Additionally, no individual measurement should differ by more than 20% of the value requested by the manufacturer within the limits given. Inflow air velocity: ● According to NSF 49, the average airflow velocity at the front aperture should be 0.51 m/s for class A2 ● EN 12469 does not sub-classify within class II BSCs. The average airflow velocity at the front aperture should be at least 0.4 m/s, according to the manufacturer's specifications ● Blower system must be able to maintain the airflow within a minimum window (narrow limits) on voltage fluctuations
	● Microprocessor-controlled functions with LCD display on the front of the BSC ● Electrical control or indicators ● Electronic fan control ● Flow meter for air inflow velocity and air down flow velocity ● Operating hours indicator (counter) ● UV-light timer ● Filter and flow conditions
	Alarms, visible and/or audible, for any unsafe conditions of the BSC (e.g. airflow, window position, hardware or software errors)

	Flicker-free, low-glare, warm-coloured light, >1000 lux
	Ultraviolet C (UVC) light (253.7 nm wavelength); 30 W with hour counter; with interlock with white light so that the UVC light can be switched on only when the white light source is switched off.
iv	Electrical requirements: As per country electrical standards, lead fuse T16A (slow blow) or circuit breaker B16 Internal fittings: Two plugs as per country electrical standards, protected with separate T5A (slow blow) fuses Voltage and plugs to be adapted to meet the country requirements. The line cord/power cord supplied with the equipment must be of acceptable durability, length, current carrying capacity, and comply with country electrical standards.
V	Power consumption (approximate; modify according to BSC dimensions): ● For a BSC of 90 cm (3 ft): 500 W or less ● Power consumption for plugs inside: approximately 1000 W ● Conform to electrical safety standards IEC 60601–1, UL 61010–1 and EN 61010–
vi	Should have an appropriate support stand on castor wheels
vii	Ergonomic laboratory chair, designed for use in potentially infectious laboratory areas: ● Adjustable height to suit different users, seat range approximately 400–490 mm ● Adjustable-angle back-rest (no arm-rest) ● Castor wheels (five) ● All metal parts must be chrome-plated, so they can be disinfected with alcohol-containing disinfectants
viii	External height ≤2200 mm including support stand, allowing an available space of at least 400 mm from the top of the BSC to the ceiling. Taller versions may be acceptable, provided the 400 mm above the BSC is available to measure air velocity above the exhaust filter, and so there is sufficient space for changing the filter, for ducting and/or a thimble connection to the outlet.

xi	**Thimble ducting:** ● Air duct construction with thimble to vent air from the BSC ● The power of the external extraction fan installed at the end of the ducting should exceed the volumetric flow rate of the BSC by 30–50% and should be controllable with an uninterrupted power supply ● The air from the BSC should be ducted with ventilation pipes (diameter > 20 cm) ● The extractor fan assembly must be easily accessible and preferably located at the end of ducting with a stable fitting ● Ducting design should be straight with minimum bends; any bends should be rounded ● Ducting should have adjustable balancing dampers that are easily accessible so that flow can be controlled as and when required ● Ducting of suitable length must be provided ● The unit rate of ducting must be quoted in the proposal separately ● At the end of the ducting a wire mesh arrangement must be provided such that no external materials/rainwater can enter the BSC, which could result in damage to the BSC
X	All standard accessories, consumables and parts required for the proper installation, operation and maintenance of the BSC should be included in the offer by the supplier and be specified and quantified One set of operation, maintenance and service manuals in English should be provided with each BSC
xi	A certificate to state that the BSC has been calibrated at the factory must be obtained. A certificate should be provided for each unit supplied.
xii	**Warranty:** One year, with the warranty to start from the date of successful installation and validation on-site
xiii	**Validation of BSC during installation and warranty period (annually):** ● Particle count test ● PAO filter integrity test for HEPA filters ● Air inflow velocity and downflow velocity tests as per NSF 49 and EN 12469 standards, with devices acceptable by national/international standards

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Chapter 3

Biosafety and waste management protocols for the COVID-19 laboratory

Chapter 3: Biosafety and Waste Management Protocols for the COVID-19 laboratory

3.1 Introduction to biosafety

A laboratory safety programme is essential, to protect the laboratory staff; equipment and facilities, and to protect the environment. Everyone in the laboratory is responsible for quality and safety.

The predominant routes of laboratory-associated infection (LAIs) are:

- Inhalation of infectious aerosols
- Parenteral inoculations with syringes or other contaminated sharps
- Spills and splashes onto skin or mucous membranes
- Ingestion or exposure by touching the mouth or eyes with fingers or a contaminated object

3.2 Recommendations for biosafety in a COVID-19 laboratory[1]

Risk assessment: No procedures must be performed until a risk assessment has been carried out, involving the following aspects.

- Risk group classification
- Risk identification in the laboratory
    - Exposures
    - Sharps: elimination or precautions
    - Chemical safety
    - Electrical hazards
    - Fire safety
- Biosafety level requirements
- Personal protective equipment (PPE)
- Biosafety cabinet: Specimen processing should be carried out inside a certified biosafety cabinet
- Safe handling and containment
    - Training of laboratory personnel
    - Safety equipment
    - Good laboratory practice
    - Facility design
    - Laboratory procedures
    - Decontamination and disinfection
    - Biomedical waste management
    - Decontamination by autoclaving
    - Spills
    - Medical surveillance/occupational health and safety

Appropriate biosafety practices must be always followed by COVID-19 molecular testing laboratories.

3.3 Risk assessment

Each laboratory should conduct a local/institutional risk assessment, from sample collection to performing a PCR test to ensure the laboratory is competent to safely perform the intended testing, with appropriate risk-control measures in place.

3.3.1 Risk assessment team

A risk assessment team should be established to perform risk assessments, to ensure a variety of perspectives are considered and to reduce bias.[1] This team could be comprised as shown in Figure 1.

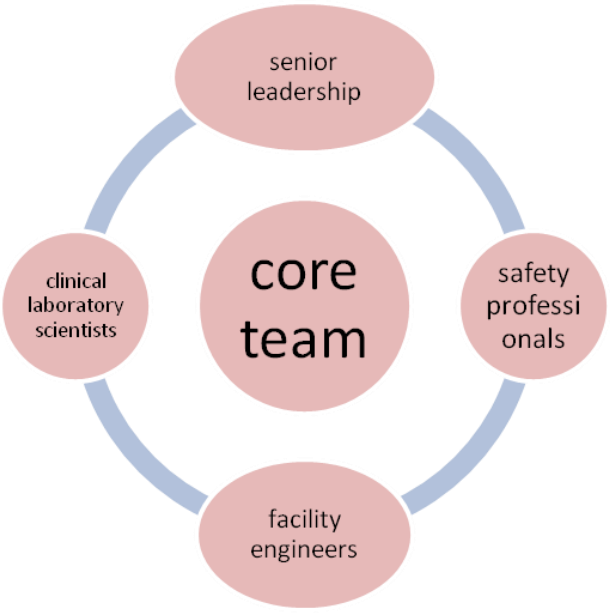


Figure 1: Elements of the risk assessment team

Specific hazards will be identified for each step of the processes involved. For each identified risk, appropriate risk control measures, including the following recommendations, should be selected and implemented, to mitigate any risks and reduce them to an acceptable level, as shown in Figure 2. An example of a risk assessment template is provided in Annexure 6.

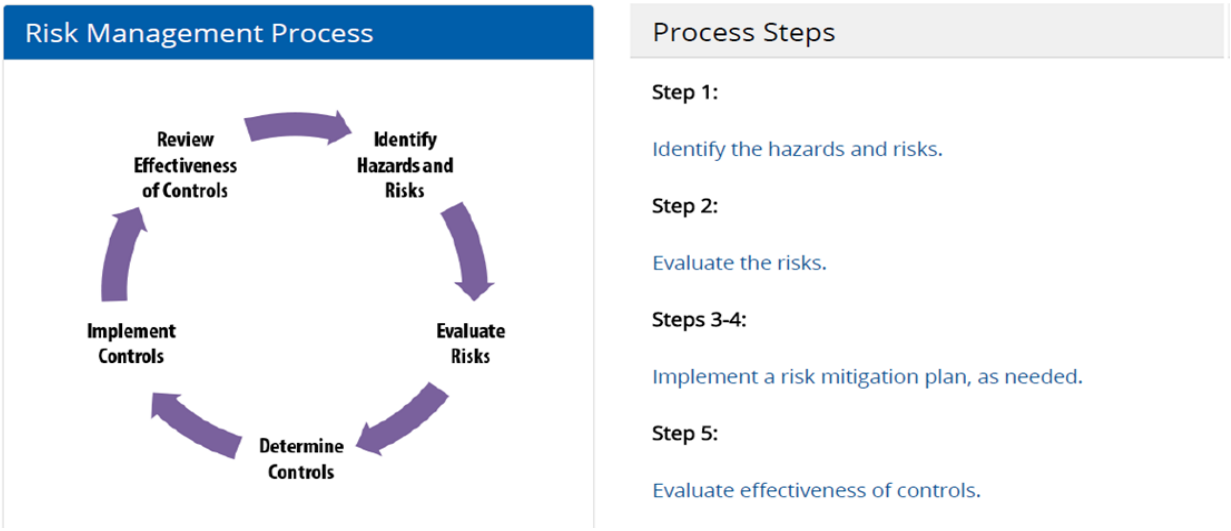


Figure 2: Steps in the risk management process

3.3.2 Risk group classification

Risk groups are classifications that describe the relative hazard posed by infectious agents or toxins in the laboratory[2] (Table 1).

Table 1: Risk group classification

Risk group 1	No or low individual and community risk: a microorganism that is unlikely to cause disease in humans or animals
Risk group 2	Moderate individual risk, low community risk: a pathogen that can cause disease in humans or animals but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposure 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: These pathogens present a serious risk to laboratory staff. The organisms could present a significant community risk if spread in the environment, but there are usually effective measures for treatment and/or prevention. A pathogen that usually causes serious disease in humans or animals. Effective treatment and preventive measures are available.
Risk group 4	High individual and community risk: a pathogen that usually causes serious disease in humans or animals and 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 in risk group 3.

Risk assessment is essential so that a laboratory director can manage and reduce the risks posed to laboratory staff.

3.3.3 Exposure

An immediate response to any exposure is required; such exposures include cuts, lacerations, or splashes to the eyes, nose or mouth. Responses should include the following, as appropriate.[2]

- Immediately wash the affected area
- Punctures/cuts from contaminated sharp objects, or splashes, should be washed with soap and water for 15 minutes
- Any contamination of the eyes, nose or mouth should be washed with water for 15 minutes
- The supervisor should be notified if they are available

3.3.4 Sharps: elimination or precautions

- Avoid the use of glass Pasteur pipettes and syringes. Substitute plastic for glass whenever feasible.
- Alternatives to glass Pasteur pipettes include plastic pipettes, plastic transfer pipettes, plastic gel-loading pipette tips and pipette tip extenders, aspirators, and flexible plastic aspiration pipettes.
- If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use and discard intact needles and syringes immediately after use. Use a one-handed disposal method (keep a hand behind your back or by your side, and do not place your other hand on or near the opening of the sharps container).
- Never re-cap, bend, break or otherwise manipulate sharps by hand.

3.3.5 Chemical safety

Guidelines for the storage of chemicals

Lab should have lab safety policy / safety manual that includes section on chemical safety, in accordance with good chemical laboratory practice standards

Look for any unusual conditions in the chemical storage areas, such as:

- Improper storage of chemicals
- Leaking or deteriorating containers
- Spilled chemicals
- Temperature extremes (too hot or cold in the storage area)
- Lack of light
- Doors propped open or other lack of security

- Accumulation of trash
- Open lights or matches
- Fire equipment blocked, broken or missing
- Lack of information or warning signs for e.g. flammable liquids, acids, corrosives, poisons, etc.

Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) should be maintained for the identity of chemicals, hazardous ingredients, health hazards, first-aid measures, fire and explosion hazards, accidental release measures, precautions for safe handling and use, and control measures.

3.3.6 Electrical hazards

Types of electrical hazards:

- Shock
- Burns
- Arc-burns
- Fire

General electrical safety measures:

- All electrical equipment should be inspected annually
- Periodic checks should be made on all electrical wires
- There should be no joints in any electrical wires
- Power wiring must be kept separate or distinct from control wiring
- If frayed wires are found, the equipment should immediately be removed from use and repaired
- If you receive any shock or tingling from electrical equipment, report it to your supervisor

Electrical safety devices

UPS	Fuses
● An uninterruptible power supply (UPS), also known as an uninterruptible power source, is an electrical device that provides emergency power when an input power source fails ● A UPS also plays a major role in surge protection of equipment that is very costly, playing a critical role in cases of major electrical fluctuations/electrical short-circuits and during thunderstorms ● Equipment that must be connected to a UPS includes: 1) biosafety cabinet 2) thermocycler 3) centrifuge 4) GeneXpert/CBNAAT /Truenat machine and monitor	● Protect electrical wiring from carrying too much current ● Protect malfunctioning equipment from drawing too much current ● The conductor melts if too much current flows through the fuse

Maintenance of a UPS

- Visually inspect equipment for loose connections, burned insulation or any other signs of wear
- Check equipment and performance related to load
- Provide a complete operational test of the system, including a monitored battery-rundown test (at least once a week, discharge the batteries completely and then recharge)
- Make sure the UPS always remains in contact with its electrical ground
- Do not overload the UPS
- To extend the battery life, avoid deep discharges, and do not expose the battery to extremes of heat, cold or humidity.

Good electrical practice

- Grounding: all electrical equipment must be grounded by using a three-pronged plug; three-to-two plus conversion adapters are prohibited
- Unplugging: electrical cords should be unplugged by holding the cap and not by pulling on the cord; unplug all equipment during servicing

- Junction boxes: pull boxes and fittings must have approved covers
- Unused openings in cabinets, boxes and fittings must be closed.

Hazards of overloading

- Too many devices plugged into circuit can heat the wires to a very high temperature, which could lead to a fire
- If wire insulation melts, arcing may occur and could cause a fire in the area where the overload exists (even inside a wall).

Bad electrical practice

- Light fixtures, lamps and light bulbs are another common cause of electrical fires
- Installing a bulb with a wattage that is too high for the lamp or light fixture is a major cause of electrical fires
- Always check the maximum recommended bulb wattage on any lighting fixture or lamp and never exceed the recommended wattage.

Electrical evaluation

- The total electrical load of the laboratory should be calculated as per available equipment/ machineries
- An electrical evaluation should be performed on at least an annual basis
- The electrical evaluation should be carried out with a local electrical engineer
- The evaluation must include the incoming supply voltage, current load, grounding, wiring quality, generator and UPS status
- An evaluation can be completed within a day or two if everything is fine; however, in case of incidents or issues in past, the evaluation could extend to a week or even a month

Electrical audit

- An electrical audit should be carried out regularly to cover the following:
- Confirm whether the electrical load requirement of the laboratory is appropriate
- Confirm that back-up power is available for critical equipment and is functional
- Confirm that any UPSs linked to equipment are functional
- Confirm the distribution to the laboratory/zone is acceptable
- Check for voltage variations (input and output) across various sections of the laboratory
- Check the quality of the wiring and whether the wiring is adequate to handle the power load being carried
- Check plug points are functional and not getting short
- Check fire safety measures (due to electrical sparks etc.) are in place and working

Staff training

- Employees should continually educate themselves and their colleagues on the proper use of electrical equipment
- Operating instructions should be attached to electrical equipment when feasible, or a label should be attached stating where the operating instructions are conveniently located
- Know the location of emergency power sources in the event of a power outage.

Steps to prevent electrical accidents

- Unplug equipment first
- Use a dry chemical fire extinguisher
- If the fire is large, unplug equipment and call the fire department
- Never use water on an electrical fire!!

3.3.7 Fire safety

Common causes of fires in laboratories include:

- Electrical circuit overloading
- Poor electrical maintenance, e.g. poor and perished cable insulation
- Excessively long gas tubing or long electrical leads
- Equipment left switched on unnecessarily
- Equipment that was not designed for a laboratory environment
- Open flames
- Deteriorated gas tubing
- Improper handling and storage of flammable or explosive materials
- Improper segregation of incompatible chemicals
- Sparking equipment near flammable substances and vapors, and improper or inadequate ventilation.

Considerations to ensure fire safety

- Close cooperation between safety officers and local fire prevention officers is essential
- The effect of fire on the possible dissemination of infectious material must be considered, as this may determine whether it is best to extinguish or contain a fire
- Fire warnings, instructions and escape routes should be prominently displayed in all rooms, corridors and hallways
- Fire-fighting equipment should be placed near room entrances and at strategic points in corridors and hallways (RT-PCR lab will require Class A: Ordinary materials, such as paper, wood, plastics, and cardboard; Class B: Flammable and combustible liquids, as

well as organic solvents; Class C: Energized electrical equipment, such as appliances, burners, hot plates, power tools, and panel boxes)

- Fire extinguishers should be regularly inspected and maintained, and their shelf-life kept up-to-date

General measures to handle a fire in the laboratory

- All laboratory personnel must learn how to operate a fire extinguisher
- Fire extinguishers must be inspected annually and replaced as needed
- The laboratory should have appropriate class of fire extinguisher(s)
- In general, fire extinguishers of type ABC (2 kg) inert gas system are appropriate
- Contact information for the fire department should be posted at various locations throughout the laboratory

3.4 Biosafety level requirement for a molecular testing laboratory

DNA polymerase and PCR assays are highly susceptible to amplicon contamination. If a contaminant is introduced early in the reaction, then the error is amplified in an exponential fashion, which can lead to false or inaccurate results[3]. The most common causes of PCR contamination include:

- Incorrect laboratory set-up and improper workflow
- Untrained lab staff
- Improper sterilization of laboratory equipment

3.4.1 Personal protective equipment (PPE)

A range of PPE is required for staff working in a molecular testing laboratory for COVID-19, as outlined below.[1 4]

- Full sleeves laboratory coats must be worn at all times in a laboratory, to prevent personal clothing from getting splashed or contaminated by biological agents. Laboratory coats must have long sleeves, preferably with elasticated or fitted cuffs, and must be fastened when worn in the laboratory. The 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 properly stored; they should not be hung on top of other laboratory coats or kept in lockers or on hooks with personal items.
- Powder free disposable gloves must be worn for all procedures that may involve planned or inadvertent contact with blood, other body fluids or other potentially infectious materials. Disposable gloves 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.
- Footwear must be worn in the laboratory and must be of a design that minimizes slips and trips and reduces the likelihood of injury from falling objects and exposure to biological agents.
- Respiratory protection is generally not among the core requirements. In the present COVID-19 context, this is required during the process that involves handling the infectious specimens like sample collection, aliquoting and RNA extraction protocols.

The specific PPE required must be determined by conducting a risk assessment, as shown in Table 2.

Table 2: Recommended PPE for various biosafety levels

S no.	Setting	Activity	Risk/BSL	Recommended PPE
1.	Dedicated sample collection centre/laboratory	Sample collection, transportation	High risk/NA	Full complement of PPE, including: ● Non-sterile gloves (single use only) ● Gown[a] ● Eye protection ● N95 respirator
2.	Laboratory	Sample processing (aliquoting and testing)	High risk/BSL-2	● Non-sterile gloves (single use only) ● Gown ● N95[b] respirator mask

BSL, biosafety level; NA, not applicable

[a] Solid front or wrap-around gowns, scrub suit, or coveralls with sleeves that fully cover the forearms; head coverings shoe covers or dedicated shoes
[b] Applicable for aerosol-generating procedures performed outside the BSC and for cleaning up spills outside of the BSC

Steps for donning full PPE

- Remove all personal belongings: mobile phone, watch, bangles, jewellery, pen, etc. and store them safely in a locker
- Drink water and use the bathroom 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
- Put on PPE (see Figure 3)
- See Figure 4 for steps for doffing PPE.

Please also click the following link to play the donning and doffing video: https://www.youtube.com/watch?reload=9&v=8PSBOZUelTc.

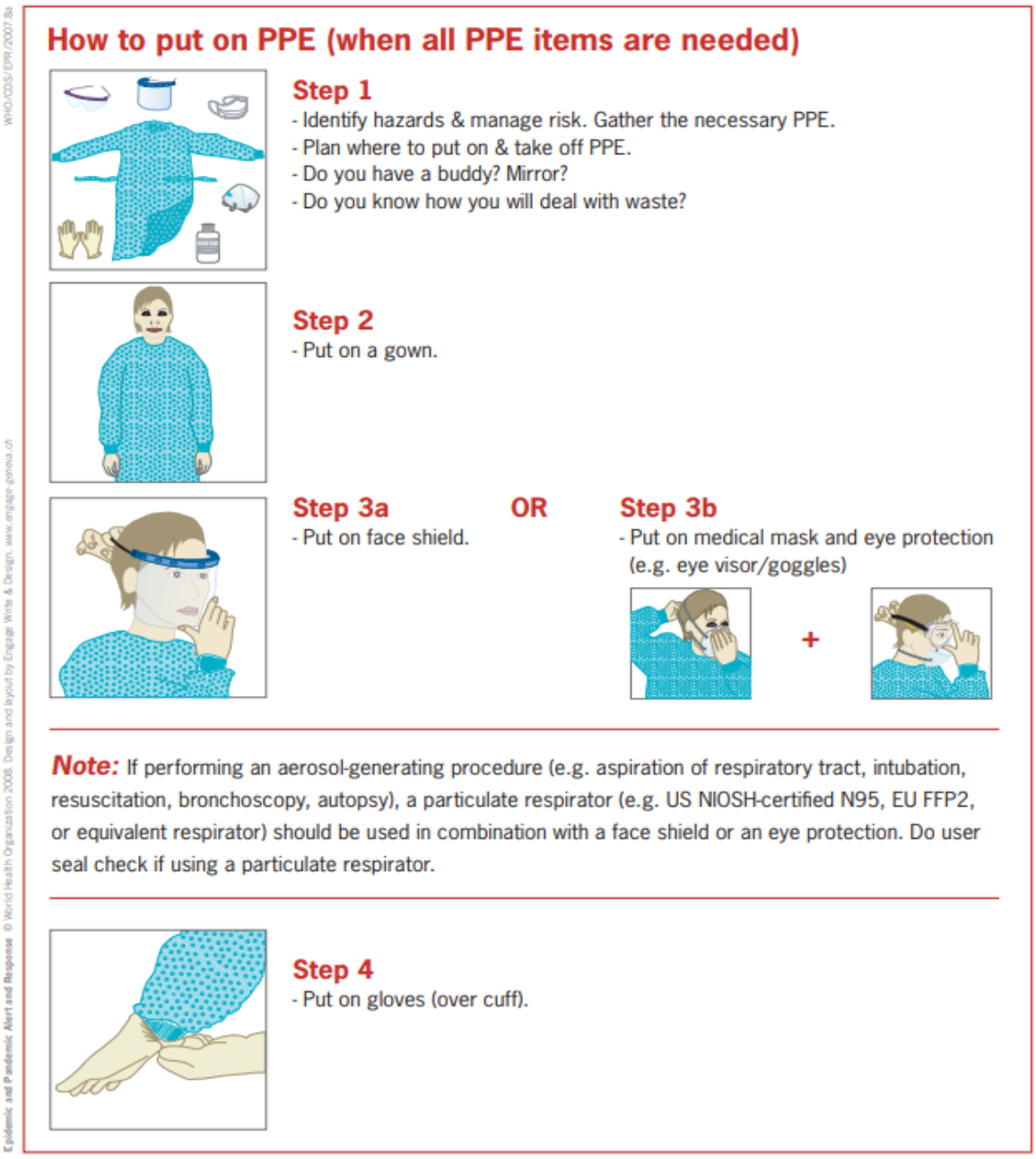


Figure 3: Steps for donning PPE

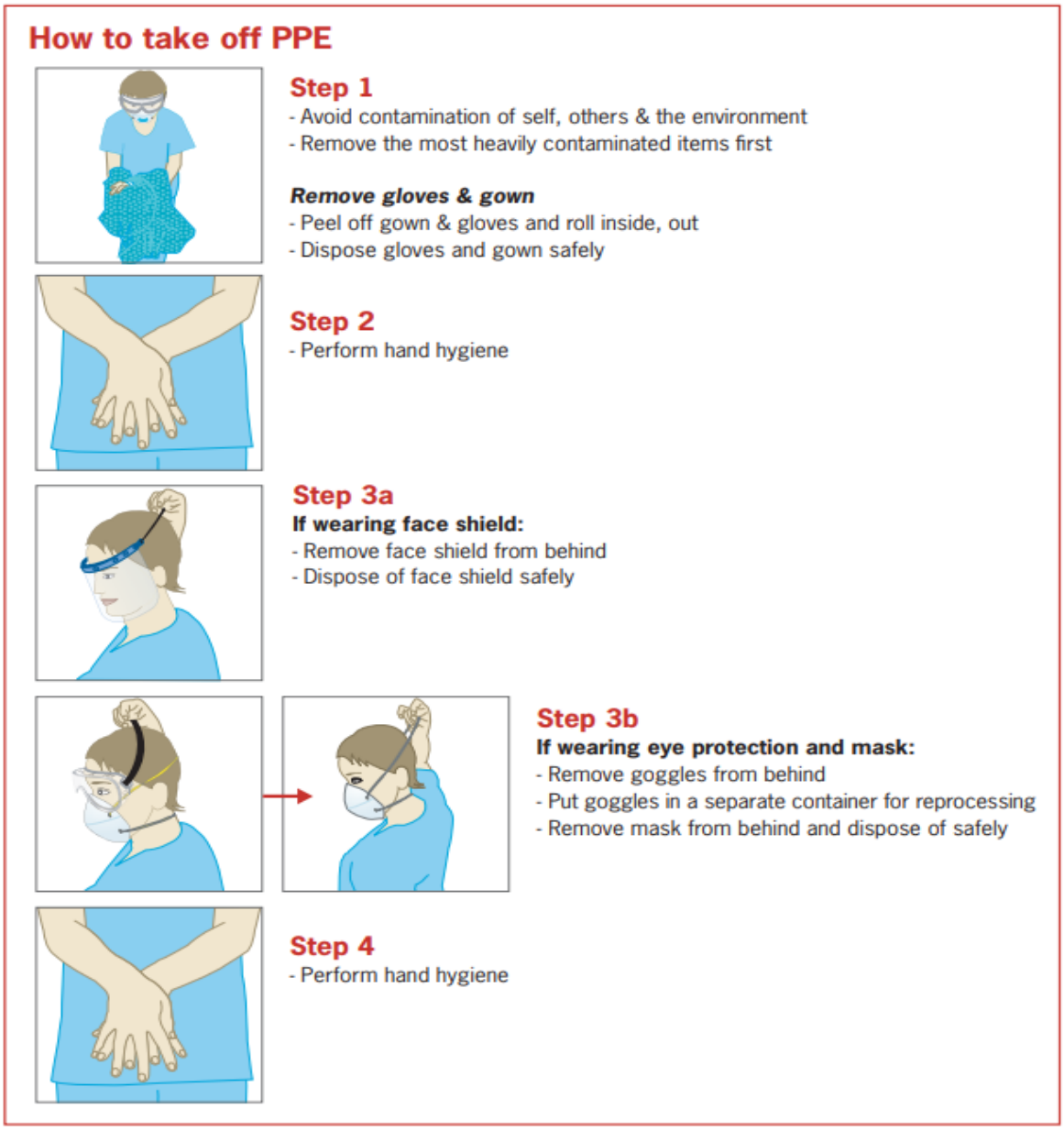
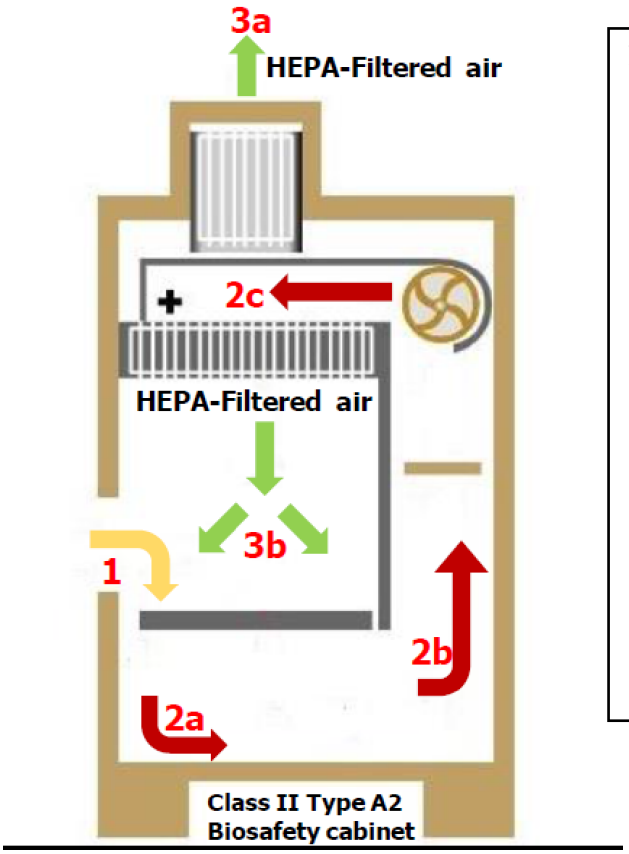


Figure 4: Steps for doffing PPE

3.4.2 Biosafety cabinets (BSC)

As per WHO SARS-CoV-2 biosafety guidelines,[4] non-propagative diagnostic laboratory work (e.g. sequencing; nucleic acid amplification tests, NAATs) should be conducted at a facility using procedures equivalent to Biosafety Level 2 (BSL-2), while propagative work (e.g. virus culture, isolation or neutralization assays) should be conducted at a containment laboratory with inward directional airflow and that meets Biosafety Level 3 (BSL3) requirements. Procedures with a high likelihood of generating aerosols or droplets must be carried out in a certified biosafety cabinet (BSC), Class II Type A2 (Figure 5 & 6).



Features of a Class II biosafety cabinet:

1. Unfiltered room air is drawn into the front of the cabinet
2a. Unfiltered air is pushed below the work surface
2b. Unfiltered air is drawn up through the plenum
2c. Unfiltered air is pushed through HEPA filters by the cabinet blower meter
3a. 30% of filtered air leaves the top of the cabinet and recirculates into the room or is removed by the canopy exhaust
3b. 70% of HEPA-filtered air enters the cabinet from above and flows down on work surfaces under unidirectional flow

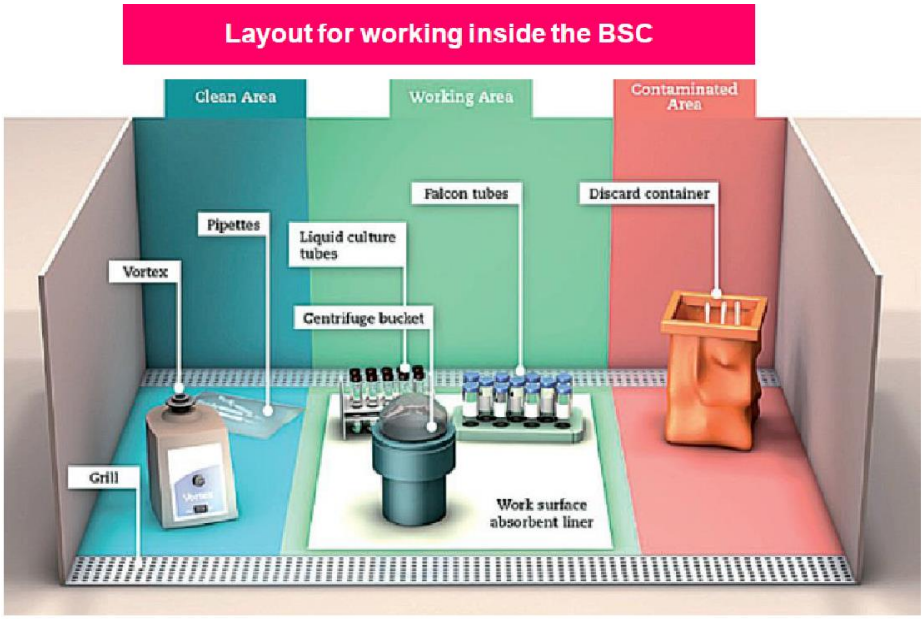
Figure 5: Features of a Class II Type A2 biosafety cabinet

Operating instructions for a biosafety cabinet

All staff who use a BSC should be familiar with the user manual, daily disinfection procedures and how to work safely within the cabinet. Specific steps are outlined below. The principles behind a BSC are shown in Figure 5, while the correct layout of equipment in a BSC is shown in Figure 6.

- The BSC must be turned on 5 minutes before starting work in it
- Any materials going into the BSC must be disinfected with appropriate disinfectant followed by 70% ethanol

- Tasks inside the BSC must always be performed on disinfectant-soaked absorbent towel (to capture any droplets should they occur)
- The front grill must not be blocked with paper, equipment or any other items
- All materials should be placed as far back in the cabinet as possible without blocking the rear grill
- Large items, such as biohazard bags, should be placed to one side of the interior of the cabinet
- To avoid cross-contamination, always work from the clean to the contaminated areas across the work surface of the BSC
- The BSC should be turned off 5 minutes after completion of work



From: https://www.who.int/tb/publications/2012/tb_biosafety/en/

Figure 6: Arrangement for working inside a biosafety cabinet

Location of biosafety cabinets

- Keep BSCs away from foot traffic and from potentially disruptive air currents
- A clearance of 30 cm should be provided behind and on each side of a BSC to allow easy access for maintenance
- A clearance of 30 to 35 cm above the cabinet is required to accurately measure the air velocity across the exhaust filter and to change the exhaust filter

Maintenance of biosafety cabinets

BSCs must be carefully maintained (see Table 3 for details). It is also important to keep the BSC maintenance log sheet up to date (see Annexure 7 for details).

Table 3: Maintenance of a biosafety cabinet

Maintenance to be carried out	Work to be performed
Daily maintenance	● Check and record the vertical air pressure in the BSC log ● Under no circumstances should a BSC be worked in if the vertical air pressure is incorrect ● The interior surfaces of BSCs should be decontaminated with an appropriate disinfectant, before and after each use (a solution of bleach and then 70% alcohol should be used) ● At the end of the workday, a final surface decontamination must be performed that includes a wipe-down of the work surface, the sides and rear of the BSC, and the interior of the BSC glass
Weekly maintenance	● Surface decontamination of the drain pan ● Surface cleaning of fluorescent and UV lamps ● Cleaning of the front sash
Annual maintenance	● Check downflow and inflow velocities ● Particle count test ● Airflow smoke patterns ● PAO (poly alpha olefin) test for HEPA filter integrity
Certification	● At the time of installation ● Whenever a BSC is moved ● Following any repairs or filter changes ● BSCs also require regular (annual) maintenance and validation to ensure proper functioning; delaying maintenance or having under qualified personnel conduct maintenance can put laboratory workers at risk

3.4.3 Training of laboratory personnel

It is essential that all laboratory staff are trained in general laboratory safety as well as in any specific tasks and duties for which they are responsible. All staff should have safety training that includes:

- Reviewing the code of practices and procedures
- Information on safe practices that should be followed to avoid or minimize risks of inhalation, ingestion and inoculation
- Information on proper decontamination and disposal of infectious materials

Additional points to note are as follows.

- Laboratory personnel should be made aware of SARS-CoV-2 infection, transmission and potential risks to health related to COVID-19 disease
- All personnel involved in sample collection, receipt and analysis should be tested for COVID-19 beforehand; only staff who test negative should be involved in testing
- Options for reassignment should be considered for personnel with comorbid conditions that put them at high risk should they contract COVID-19, such as diabetes, chronic respiratory diseases, high blood pressure, or immunosuppression, to keep these individuals away from high-risk areas of a COVID-19 testing laboratory
- If human resources are limited, staff should be made aware of the risk of experiencing severe symptoms of the disease
- It is essential that all laboratory personnel are trained in biosafety and any routine laboratory procedures assigned to them
- The laboratory manager should ensure that all staff are appropriately trained and that their technical competency in performing various procedures is evaluated on regular basis

3.4.4 Safety equipment

The safety officer should be assigned responsibility for ensuring that there is an adequate supply of appropriate equipment for safety and biosafety, including:

- PPE
- Fire extinguishers and fire blankets
- Appropriate storage and cabinets for flammable and toxic chemicals
- Eye washers and emergency shower(s)
- Waste disposal supplies and equipment
- First aid equipment

3.4.5 Good Laboratory Practice

The biosafety of any laboratory depends on Good Laboratory Practice, including the following:

- Laboratory access should be limited to essential staff
- No eating, drinking or smoking in the laboratory
- No placing of pencils or pens in the mouth
- Keep hands away from eyes and face
- Always wash hands before leaving the laboratory
- Remove gloves before handling phones, instruments or computers
- Laboratory coats must be decontaminated and laundered regularly (never take them home for laundering!)

3.4.6 Facility design

The correct design and construction of laboratory facilities with unidirectional workflow contributes to the protection of all laboratory workers and provides a barrier that protects the community from SARS-CoV-2 aerosols that may be created within the laboratory. (Please also refer to the section above regarding workflow processes.)

3.4.7 Laboratory procedures before starting testing

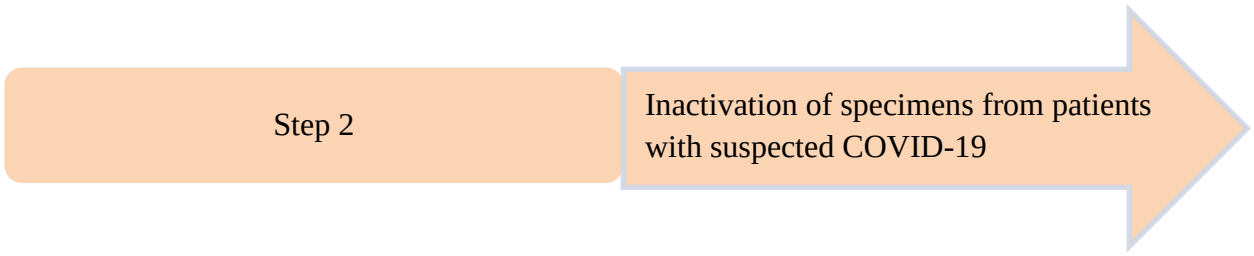
- Adequate PPE must be readily available to all laboratory staff, including support staff
- All staff should receive training on the appropriate use of PPE, including the recommended sequence for safely putting on and taking off PPE, as well as safe disposal or decontamination before re-use
- Always remember to:
    - Change gowns and gloves if they become soiled or contaminated
    - Remove gowns and gloves before leaving the laboratory
    - Discard disposable gowns after single use; cloth gowns should only be re-used following proper cleaning and decontamination
    - Always perform hand hygiene after working with specimens

Below, examples are given of the biosafety considerations required for each step of the molecular diagnosis of SARS-CoV-2:

Step 1 (collection to receiving of samples in the laboratory)	• Appropriate container (sterile, leak proof) • PPE worn during sample collection (respiratory and barrier precautions) • Transport (properly labelled, no spillage, triple packaged) • Safe practices to minimize the risk of inadvertent exposure

- Following the arrival of a sample in the laboratory, the sealed transport container must be disinfected and wiped prior to opening the container within a certified biological safety cabinet.
- Wear dedicated PPE within the laboratory for all procedures. Do not wear PPE to non-laboratory areas and remove all PPE prior to leaving the laboratory. Depending on the number of laboratories and the nature of the work, staff may need multiple laboratory coats for each area, to avoid cross-contamination.

3.4.8 Laboratory procedures for inactivation of specimens



When inactivating specimens:

- Wear safety glasses or a full face-shield, a laboratory coat and examination gloves (consider double gloving)
- Make sure that wrists are not exposed and are sufficiently covered (e.g. long gloves over a laboratory coat with banded cuffs)
- Disinfect the exterior of the primary and secondary transport containers within a certified biosafety cabinet (BSL-2 facility, BSC Class II)
- Follow any inactivation procedures and allow the required contact time prior to considering the specimens to be "inactivated"
- Limit aerosol-generating procedures (e.g. vortexing, shaking of specimen)
- Follow the PPE removal (doffing) and hand-washing guidance as noted in the sample collection document

3.4.9 Laboratory procedures for RNA extraction

During RNA extraction:

- PPE should be worn during RNA extraction (properly fitting, tested N95 respirator, double gloves, face shield, sufficiently covered (e.g. long gloves over a laboratory coat with banded cuffs)
- Work should be performed inside a biosafety cabinet (BSL-2 facility, BSC Class II)

- Limit aerosol-generating procedures (e.g. vortexing, shaking of specimen)

3.5 Decontamination and disinfection

The "5 Cs" of decontamination and disinfection (Figure 7) 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 (Table 4), including:

- Sodium hypochlorite (bleach); for example, 1000 parts per million (ppm, 0.1%) for general surface disinfection and 10 000 ppm (1%) for disinfection of sample spills
- 70% ethanol
- Povidone-iodine (7.5%), chloroxylenol (0.05%), chlorhexidine (0.05%), benzalkonium chloride (0.1%), if used according to the manufacturer's recommendations[5]

Note that SARS-CoV-2 and human coronaviruses in general are known to persist on inanimate surfaces such as metal, glass and plastic for up to 7 to 9 days.

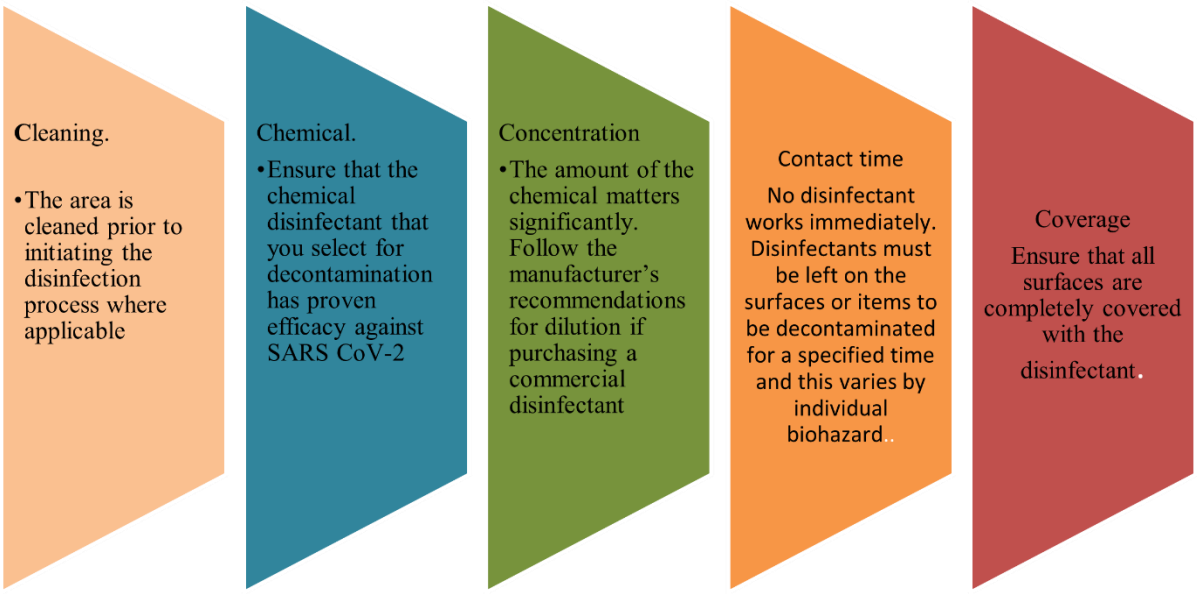


Figure 7: The 5 Cs of decontamination and disinfection, which should be practiced at all times

Table 4: Disinfectants suitable for SARS-CoV-2

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)

3.6 Biomedical waste management

The following section outlines relevant biomedical waste management considerations.[4] Please note that:

- PPE to be used: laboratory coat, N95 mask, gloves and goggles
- The availability of PPE and materials for biomedical waste procedures must be ensured at all times
- Laboratory personnel must supervise the activities of housekeeping staff throughout the entire protocol

Biomedical waste segregation[6 7]

- Keep separate colour-coded bins/bags/containers in wards and maintain proper segregation of waste as per biomedical waste management (BMWM) Rules, 2016, as amended and central pollution control board (CPCB) guidelines for the implementation of BMW Management Rules.
- Colour-coded bags should be used for the disposal of different categories of biomedical waste generated as detailed in Table 5.
- Report opening or operation of COVID-19 lab to state pollution control board (SPCB) and respective common biomedical waste treatment facility (CBWTF) located in the area.
- As a precaution, double-layered bags (using two bags) should be used for collection of waste from COVID-19 isolation wards to ensure adequate strength and prevent leaks.
- It is mandatory that bags/containers used to contain biomedical waste from COVID-19 laboratories should be labelled as “COVID-19 waste”.
- Use a dedicated collection bin and trolleys labelled as “COVID-19” to store COVID-19 waste and keep them separately in a temporary storage room prior to handing waste containers over to authorized staff of a biomedical waste-handling agency.
- The inner and outer surfaces of containers/bins/trolleys used for storage of COVID-19 waste should be disinfected with 1% sodium hypochlorite solution daily.
- Collect used PPE, such as goggles, face-shields, splash-proof aprons, plastic coveralls, hazmat suits, and nitrile gloves, in RED bags.

- Collect used masks (including triple-layer masks, N95 masks, etc.), head covers/caps, shoe-covers, disposable linen gowns, non-plastic or semi-plastic coveralls in YELLOW bags.
- In a sink connected to the effluent system, pre-treat viral transport media, plastic vials, vacutainers, Eppendorf tubes, plastic cryovials, and pipette tips with 1% hypochlorite solution. Drain the liquid from the sink and then dispose of the waste in RED bags.
- Used PPE such as goggles, face-shield, splash proof apron, plastic coverall, hazmat suit, nitrile gloves into a RED bag. Used masks (including triple-layer masks, N95 masks 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 as per BMWM Rules, 2016 and collect in RED bags and specifically label as "COVID-19 waste". Biomedical Waste Agency signed in the MOU.

The following COVID-19-specific guidelines should be used in addition to existing practices under Biomedical Waste (BMW) Management Rules, 2016, and Amendments 2018 and 2019, issued by the Ministry of Environment Forest and Climate Change with regards to the segregation of biomedical waste at source, collection, transportation, storage and disposal.

Biomedical waste disposal for COVID-19 samples

- COVID-19 samples should only be opened within a biosafety class II type A2 cabinet
- At the time of sample disposal, the VTM with swabs should be discarded in a yellow biohazard bag containing freshly prepared 1% hypochlorite solution and must be incubated for at least 20 minutes
- All dry waste (PPE) should be discarded in a biohazard bag and should be autoclaved prior to disposal
- All wet waste generated from viral RNA extraction and PCR testing should first be treated with detergent and water and then treated with 1% sodium hypochlorite solution
- Any spillage should be decontaminated with 1% bleach followed by wiping with 70% ethanol; spillages should also be reported to the facility manager
- Any biomedical waste should be labelled as "COVID-19 waste" before being handed over to a local, licensed external agency for biomedical waste management
- Effluent treatment: all liquid biological and chemical waste should be disinfected with 1% sodium hypochlorite then discarded into a sink within the laboratory that is connected to an effluent collection tank; this waste should then be handed over to a local, licensed biomedical waste collection agency

Table 5: Types of liners and containers used for the disposal of biomedical waste[8]

Liner/container colour	Type of liner/container	Type of waste	Treatment disposal options	Applicable/not applicable
Yellow	Autoclave-safe plastic bags/containers	Disposable gowns, masks	Discard COVID-19 samples in 5% hypochlorite solution and double cover, followed by autoclaving on-site	Applicable
Red	Non-chlorinated plastic bags or containers	Contaminated waste, including VTM swabs and media ● Body fluids, tissue samples, swabs, stool samples in screw cap containers ● Vacutainers ● Tips ● Tip boxes ● Microfuge tubes ● ELISA plates ● Vials ● Disposable forceps ● Gloves ● Shoe covers ● Falcons	Discard in 5% hypochlorite and autoclave on-site. Discard in a double bag, pre-treat by autoclaving on-site. Give to registered or authorized recyclers for autoclaving	Applicable

White	Puncture-proof, tamper-proof, leak-proof containers	Waste sharps including metals: Syringes with needles Scalpels Blades Any other contaminated sharp objects	Autoclaving/dry heat sterilization followed by shredding/mutilation/encapsulation; given to registered or authorized recyclers	Not applicable
Blue	Waterproof cardboard box	Glassware waste, including broken or discarded contaminated glass, such as medicine vials and ampoules	Given to registered or authorized recyclers	Applicable

3.7 Decontamination by autoclaving

Collection of waste for autoclaving

Biomedical waste bags and sharps containers should be filled no more than three-quarters full. Once this level is reached, they should be sealed ready for collection. Plastic bags must never be stapled but should 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.

Standards for autoclaving biomedical waste

Two factors are essential for the optimum function of an autoclave: (1) all the air in the chamber should be replaced by steam, and (2) the temperature must reach 121°C, as shown in Figure 8.

When operating a 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 min	When operating a vacuum autoclave: ● Use a temperature of not less than 121°C and pressure of 15 psi for an autoclave residence time of not less than 45 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 30 minutes

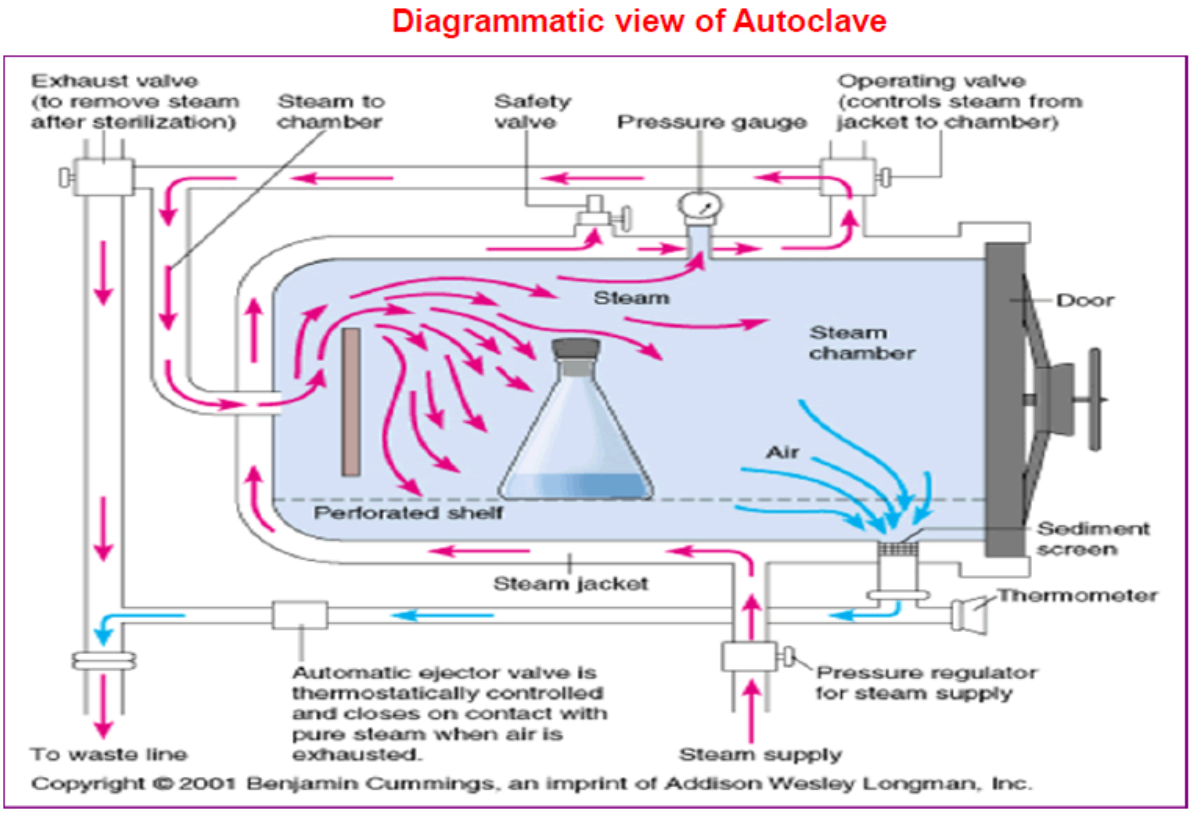


Figure 8: Diagrammatic view of an autoclave

Validation test for an autoclave

The procedure for validating an autoclave is as follows:

- Four biological indicator strips are required, one to be used as a control and left at room temperature and three to be placed in the approximate centre of three containers of waste.
- At least one of the containers with a biological indicator should be placed in the most difficult location, generally at the bottom centre of the waste pile.
- This test must be performed three consecutive times to define the minimum operating conditions. After determining the minimum temperature, pressure and residence time, the operator must repeat this test every 3 months and maintain accurate records of the results.

Disposal of autoclaved waste

Colour-coded waste bags and containers should be printed with the biohazard symbol, labelled with details such as the date, type of waste, quantity of waste, sender's name and recipient's details, as well as a barcoded label to allow the containers to be tracked until final disposal. Microbiological waste and all other clinical laboratory waste must be pretreated by sterilization

prior to packing and being sent to the common biomedical waste treatment facility (see Annexure 8 for proformas used).

Maintenance of an autoclave

It is essential that autoclaves are well-maintained to ensure their efficient functioning. Routine maintenance details are shown in Table 6, while Table 7 outlines common problems that can occur with autoclaves and how to troubleshoot these problems.

Table 6: Routine maintenance of an autoclave

Maintenance to be carried out	**Work to be performed**
Daily maintenance	● Check that the lid/door gasket is clean ● Check for cracks in the gasket ● Check for leaks while the autoclave is running, e.g. bubbles ● Clean the autoclave and work area after every use ● Check for any damage to sensors
Weekly maintenance	● Remove and clean the drain strainer if necessary ● Register in the logbook ● Check the operation of the pressure-release safety valve to verify its proper functioning (the operator must keep away from the release valve exhaust during this check to prevent burn injuries
Monthly maintenance	● Inspect autoclave gaskets, lid/door, and internal walls for any residue built ● Register in the logbook ● Notify the laboratory manager if any deterioration is observed
Annual maintenance	● All autoclaves must be inspected and certified annually by a qualified service technician ● At a minimum, pressure gauges and thermometers should be tested ● The service technician must issue a certificate of inspection, indicating compliance with safety and proper operation

Table 7: Common autoclave problems and how to troubleshoot them

Problem	Causes	Solution
▪ Clogged drain ▪ Exhaust takes more time	Drain value may be clogged with dust	Clean chamber drain filter
Scaling & salt deposition	▪ Proper Cleaning ▪ Moisture ▪ Exposure to chemicals like acids & detergents	▪ Improve cleaning ▪ Check sterilizer for drying efficiency. Store in a dry area ▪ Do not expose instruments to these chemicals. If exposure occurs, rinse thoroughly after contact ▪ Use only Distilled water in the boiler
Caps “blow off" of liquid containers	Exhausting sterilizer too rapidly	Use slow exhaust cycle
Solution is boiling when door is opened	Door opened too quickly	Do not open door until temperature gauge is below 85º C and pressure gauge is at "0". Do not touch or move a load of boiling solutions

Common Problem & Troubleshooting

Problem	Causes	Solution
Indicator Shows Sterilization Not Complete	▪ Load may be too large, too dense, or improperly loaded in chamber ▪ Time not sufficient for load ▪ Sterilizer may be malfunctioning	▪ Don’t over load material ▪ Increase time ▪ Strips may need to be changed
Steam Leakage	▪ Worn gasket ▪ Door closed improperly	▪ Gasket needs to be changed ▪ Door needs to close properly
Chamber Door Won't Open	▪ Vacuum in chamber ▪ Door lock clutch may be jammed ▪ Gasket sticking to door frame	Follow manufacturer instructions

3.8 Spills

Any laboratory conducting experiments involving biological hazards, such as microorganisms, human-derived materials, and recombinant/synthetic nucleic acid molecules must have a plan for handling accidental spills.[1 2] Spill response kits should be made available in the sample receiving area and in the specimen processing area of the laboratory. Each spill response kit must contain the following items:

- Spill incident logbook
- 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)
- Shoe covers
- N95 masks
- Gloves

Note: 1% sodium hypochlorite solution must be freshly prepared every morning. Therefore, a pre-marked disinfectant bottle containing indicated volume of water should be stored in the spill kit. Immediately prior to use, add the required amount of sodium hypochlorite stock solution to the bottle to produce 1% sodium hypochlorite (see Annexure 9 for more details).

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

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

Procedures for dealing with spills

The procedures for dealing with spills in specific areas are outlined below, followed by the general procedure to be followed when cleaning up a spill.

Leakage in a sample transport box

- Immediately notify laboratory staff and any other individuals who are nearby.
- Cordon off the area and restrict access.
- Wear proper PPE while handling the sample transport box and opening it.

- 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.
- The ample transport box should always be opened inside BSC whenever a BSC is available.
- Document the spill incident.

A spill of infectious material inside a biosafety cabinet

- Place absorbent tissue papers 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. Do not turn off the BSC.
- 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. If using bleach, follow by wiping again with sterile water and then 70% alcohol to protect metal surfaces from corrosion.
- Any equipment or reusable material that has been splashed should be cleaned with the same disinfectant.
- Remove any contaminated PPE in such a manner as to avoid cross-contamination.
- 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.
- 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.

Spill clean-up 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.

3.9 Medical surveillance and occupational health and safety

When working with COVID-19, staff may be subjected to additional hazards beyond the occupational risk of infection, as outlined below.

- Staff may be required to work extended hours, have an increased workload, and experience psychological stress.
- The health status of laboratory staff may impact their susceptibility to infection or their ability to receive immunizations or prophylactic interventions as they become available.
- Individuals who are immunosuppressed or have medical conditions that might contribute to negative outcomes if infected by SARS-CoV-2 are strongly discouraged from working with material that could potentially contain this virus.
- Laboratory staff should have an acute awareness of the symptoms of COVID-19 and report any COVID-19 illness to their supervisor within 24 hours. Any individual experiencing COVID-19-like symptoms should self-quarantine.
- Laboratory staff should have both the hepatitis B and the COVID-19 vaccine

Annexure 6: Risk assessment template

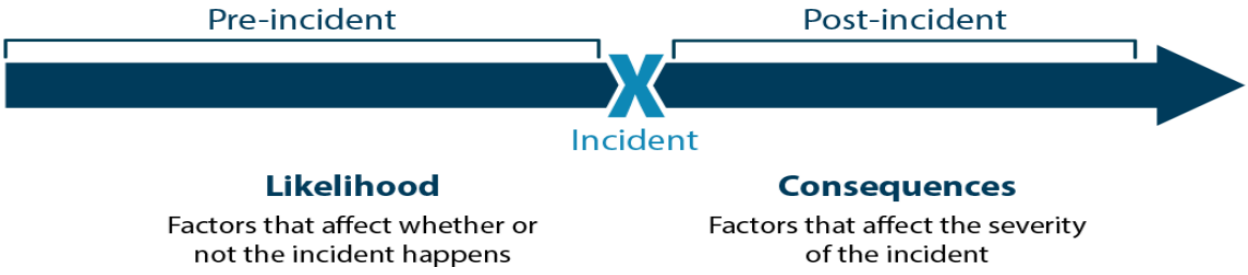
Details are from WHO guidance.[1]

- 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 an 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?	

Step 4 – Evaluate the 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.*

Annexure 7: Maintenance of biosafety cabinets

Date	Time of use	Cumulative duration of use	Cumulative duration of use of UV lamps	Visual alarm	Sound alarm	Smoke test	Airflow, m/s (for class II BSC)	Observations	Operator's Signature

SOP Code COVID # 009	Standard Operating Protocol for for the Qualitative Detection of Nucleic acid from SARS CoV-2 using Real time PCR (CoviPath COVID-19 RT-PCR Kit)
Version-I – (10/2021)	

Annexure 8: Pro forma for biomedical waste to be sent for disposal

Lab Name_____

NOTE	Biochemical indicator acceptance/rejection criteria	Black strip	Accepted	Autoclaving conditions
		White strip	Rejected	Temperature (121*C)
	Biological indicator acceptance/rejection criteria	Purple strip	Accepted	Hold time (60min)
		Yellow strip	Rejected	Pressure (15psi)

BIO MANAGEMENT (PRE-TREATMENT & DISPATCH)

Facility type: [ ]										
DATE (DD/MM/YY)										
Check	Presence of steam and/or water leak through door (Y/N)									
	Chamber door sealed properly (Y/N)									
	Autoclave start									

	Autoclave temperature (*C)									
	Autoclave pressure (psi)									
	Autoclave stop time									
QC	Tape strip colour changed	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)	[ ] (White)
		[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)	[ ] (Black)
	Biological test passed (capture weekly)	[ ] (Purple)				[ ] (Purple)				
		[ ] (Yellow)				[ ] (Yellow)				
	Comment									
	Corrective action									
	Technician's sign									
	Verified by									
	Collection by									

Annexure 9: Sodium hypochlorite change record sheet and preparation details

HYPO CHANGE RECORD MONTH_____YEAR_____

Date	**Hypo To Be Changed In Morning By Fresh 1% Hypo Solution (9:00-10:00am)**	**Signature Of Person**	**Technician Signature**
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
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22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

Details for preparing disinfectant solutions

Formula for preparing a working solution of sodium hypochlorite from the stock solution:

Amount of stock required (mL)	**Concentration of stock**	**Working concentration**	**Working volume required (mL)**
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 stock solution: parts 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 (mL)**
250	62.5	187.5
500	125	375
1000	250	750
2000	500	1500

Preparation of disinfectant 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 a label to the bottle with the following information:

    - Name of reagent
    - Strength
    - Date of preparation
    - Use before date
    - Prepared by

Chapter 3 References

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2. WHO. Laboratory Biosafety Manual: Third Editon
3. Laboratory Equipment.com. Preventing Contamination in PCR Reactions nd [Available from: https://www.laboratory-equipment.com/blog/preventing-contamination-in-pcr-reactions/.
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6. https://cpcb.nic.in/uploads/Projects/Bio-Medical-Waste/BMW-GUIDELINES-COVID_1.pdf
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Chapter 4

Sample collection, registration, transportation and storage, report management, including sample management for whole genome sequencing

Chapter 4: Sample collection, registration, transportation and storage, report management, including sample management for whole genome sequencing

4.1 Sample collection, packaging, transport and storage for COVID-19 testing

4.1.1 Introduction

Proper specimen collection is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to false or inconclusive test results**.** Diagnostic testing can involve detecting the virus itself (viral RNA or antigen) or detecting the human immune response to infection (antibodies or other biomarkers).

Table 1 denotes the minimum materials required at the various steps involved in sample collection, packaging and transportation.

Table 1: The minimum materials required at the various steps involved in sample collection, packaging and transportation

Process	**Materials needed**
Biosafety	Appropriate PPE, waste disposal and disinfectants
Specimen collection	Swabs, transport medium, suitable collection tools and containers
Specimen labelling	Marker pens or barcodes
Triple packaging	Absorbent material, plastic bag, sturdy outer container, racks, cooler box, thermometer
Documentation	COVID-19 test sample request form, transport register form
Transportation	Regular schedule and method to move the samples to the laboratory, with personnel trained in sample handling

Information on the appropriate collection, handling and submission of specimens for testing should include the following:

- Appropriate type and quantity of specimens to be collected
- Collection container or device to be used
- Special timing of specimen collection (if necessary)
- Specimen preparation and handling prior to submission to the laboratory
- Specimen stability information, including the timeframe beyond which the stability and integrity of a specimen or the analytes to be detected in a specimen might be compromised

- Specimen transport conditions (e.g. ambient temperature, refrigeration and immediate delivery requirements)
- Reasons for rejection of specimens

4.1.2 Criteria for specimen acceptance or rejection

Laboratories should have written criteria for the acceptance or rejection of specimens for the tests they perform and should promptly notify the authorized person when a specimen meets the rejection criteria and is determined to be unsuitable for testing. The criteria should include information on determining the existence of and addressing the following situations:

- Improper handling or transport of specimens
- Specimen exposure to temperature extremes that may affect sample stability or integrity
- Insufficient specimen volume received
- Use of inappropriate anticoagulants or media, specimen degradation, or inappropriate specimen types
- Commingled specimens or possible contamination of specimens that might affect results of molecular amplification procedures
- Specimens that are mislabelled or lack unique identifiers
- Lack of unique identifiers on the test request form
- Lack of other information needed to determine whether the specimen or test requested is appropriate for answering the clinical question

4.1.3 Sample collection

The general guidelines for sample collection for COVID-19 testing are as follows. Information on sample collection and specimen types is based on World Health Organization (WHO) guidelines.[2]

- The specimen(s) should be collected as soon as possible once a decision has been made to pursue COVID-19 testing
- Appropriate clinical samples should be collected by laboratory personnel/healthcare workers trained in specimen collection in the presence of a clinician
- It is imperative to wash hands thoroughly, for 40 to 60 seconds, before donning the recommended personal protective equipment (PPE) prior to sample collection; Figure 1 illustrates the WHO-recommended nine steps for correct handwashing
- Caution should be exercised due to the high risk of aerosolization; therefore, strict adherence to infection prevention and control (IPC) procedures during sample collection should be followed
- Label each specimen container with the patient's unique identification (ID) number (e.g. SRF ID), specimen type, and the date and time the specimen was collected
- If required, the specimen must be identified as a priority as per the collection facility's guidelines

- The optimal specimen depends on clinical presentation and time since symptom onset.
- At a minimum, respiratory specimens should be collected

Figure 1: The nine steps of correct handwashing[3] [4]

4.1.4 Types of specimens for COVID-19 testing [2]

Respiratory specimens

- Upper respiratory tract (URT) specimens
    - These specimens are suitable for testing early-stage infections, especially in asymptomatic or mild cases
    - Testing combined nasopharyngeal and oropharyngeal swabs from one individual has been shown to increase sensitivity for the detection of respiratory viruses and improve the reliability of the result
    - Two individual swabs can be combined in one collection tube, or a combined nasopharyngeal and oropharyngeal swab can be taken
    - A nasopharyngeal swab is the most widely used and recommended specimen for conducting molecular tests, such as RT-PCR, for COVID-19 detection
- Lower respiratory tract (LRT) specimens
    - These specimens are advised if collected later in the course of COVID-19 disease or in patients with a negative URT sampling and if there is a strong clinical suspicion of COVID-19
    - LRT specimens can consist of sputum if spontaneously produced (induced sputum is not recommended as this poses an increased risk of aerosol transmission) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease
    - Caution should be exercised due to the high risk of aerosolization; therefore, strict adherence to IPC procedures during sample collection is required; the indication for an invasive procedure should be evaluated by a physician

Figures 2 and 3 show how to collect nasopharyngeal and combined nasopharyngeal and oropharyngeal specimens.

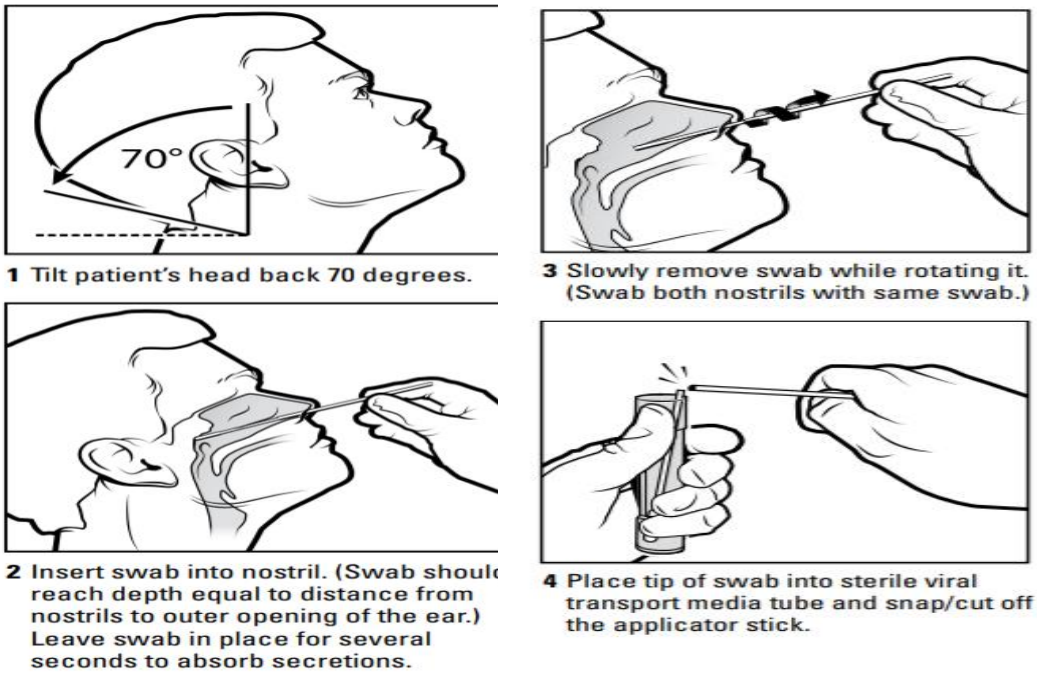


Figure 2: Nasopharyngeal swab collection[5]

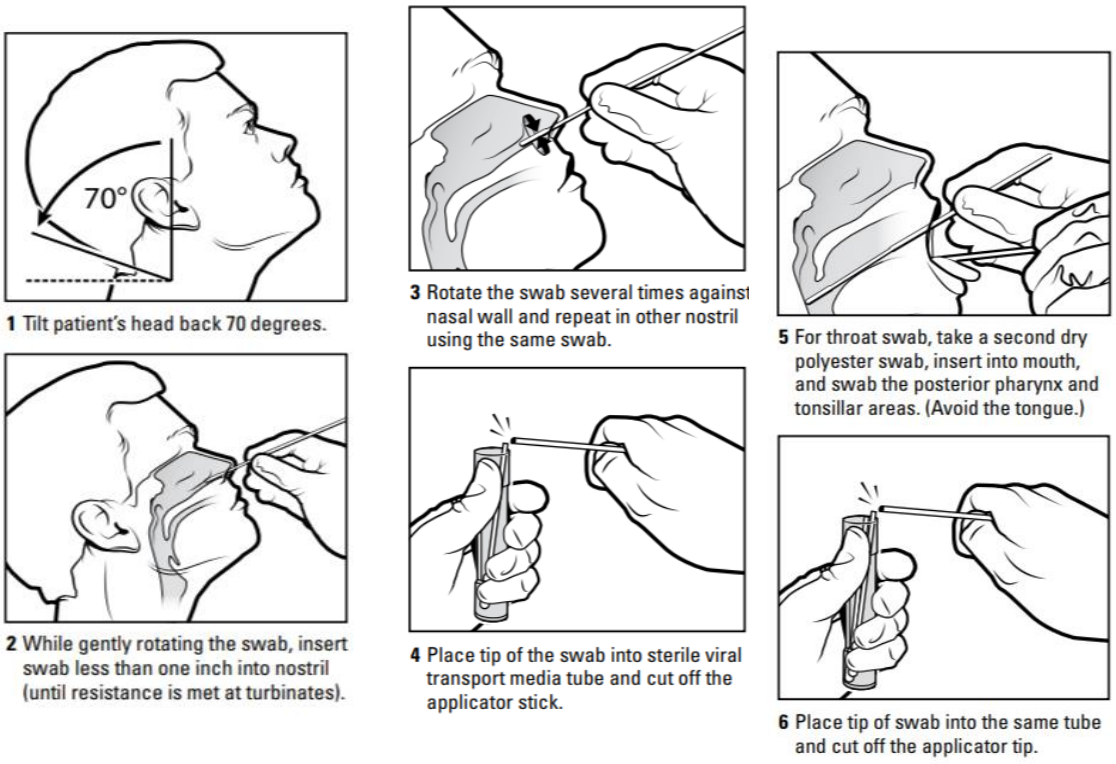


Figure 3: Combined nasal and oral swab collection[5]

The following link from WHO illustrates how to collect oropharyngeal and nasopharyngeal specimens for the diagnosis of COVID-19: https://www.youtube.com/watch?v=rsyFlpe71oQ

Alternate Specimens for COVID-19 testing

- There are specific cases where collecting nasopharyngeal and oropharyngeal swabs can be problematic, such as mass screening in schools or nursing homes, especially when elderly people with dementia or young children are involved. In these scenarios, oral fluids could potentially be a suitable specimen, as the collection methods are less invasive and there is a lower risk of exposure to others upon collection, as compared with the collection of URT specimens.[2]

Serum specimens

- If a negative nucleic acid amplification test (NAAT) result is obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen should be collected
- One specimen taken in the acute phase and one in the convalescent phase 2 to 4 weeks later can be used to look for seroconversion or an increase in antibody titers
- These two samples can be used retrospectively to determine whether an individual has had COVID-19, especially when the infection could not be detected using NAAT

Faecal specimens

- From the second week following symptom onset and onwards, NAAT can be considered for faecal specimens in cases where URT and LRT samples are negative and the clinical suspicion of a COVID-19 infection remains
- When testing faeces, ensure that the intended extraction and testing methods have been validated for this type of sample.

Post-mortem specimens

- If an individual is deceased, consider taking a post-mortem swab, needle biopsy or tissue specimens, including lung tissue, from the autopsy, for further pathological and microbiological testing

Table 2 shows the recommended temperatures for transportation and storage of various types of specimens for SARS-Cov-2 testing.

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.

4.1.5 Sample registration and receiving reports using the ICMR portal[7]

ICMR has designed a specimen referral form (Figure 11) for use with every COVID-19 sample. The National Informatics Center (NIC) has developed the RT-PCR and rapid antibody test for India (RATI) mobile apps for ICMR, for use on Android, iOS and Windows mobiles; NIC has also developed the web portal for registered phlebotomists (sample collectors) to use the platforms as centralized patient sample registration systems

- The RT-PCR app is a handheld tool for use by medical staff at sample collection centres across the country
- All patients must be assigned a Sample Referral ID or SRF ID, by completing the Sample Registration Form either on paper or online, via the ICMR RT-PCR app.
- On saving any sample successfully, the collection centre and the patient can view the collection details from the registration link received as a text message on their registered mobile number
- With a registered SRF ID, patients can receive test results over their registered mobile phone, thereby removing the need to physically visit the collection centre or testing laboratory to collect test results
- Authorized personnel from collection centres/laboratories can access and download registered patients' RT-PCR and RAT results from the GoI portal (https://covid19cc.nic.in/icmr/Login.aspx)
- Visit the following link to view a video on how to use the ICMR RT-PCR app: https://youtu.be/CXTmT6JIXlI

4.1.6 Sample labelling, packaging and transportation: instructions to be followed by collection centres[6]

Labelling

- Ensure all staff wear appropriate PPE (apron, gloves, face shield, N95 masks etc.) and follow all biosafety precautions to protect individuals and the environment
- Correctly label the specimen container with patient details (name/age/gender/specimen SRF ID) and details of the sender (name/address/phone number); also write "To be tested for SARS-CoV-2" on the outer container
- Follow the nine steps of handwashing (as outlined above) before donning and after doffing PPE

Packaging and transportation

- While samples are being transported, there is the possibility of exposure for people involved in the transport of the samples and the environment through which the material passes
- Triple pack all samples for COVID-19 testing and transport them under a strict cold-chain to the reference laboratory, with prior notice of their arrival. Triple packaging consists of three layers, as follows:[8]

    i) Primary receptacle: a labelled, watertight, leak-proof primary receptacle containing the specimen, wrapped in sufficient absorbent material to absorb all fluid in case of breakage
    ii) 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

iii) Outer shipping package. The secondary receptacle must be placed in an outer shipping package, which protects it and its contents from outside influences such as physical damage and water while in transit.

- The following documents are mandatory to be sent with samples:
    - Packaging list and invoice, including Sample Referral Form (SRF) details of patients
    - Airway bill in case of air transport
- Packages containing dry ice should be designed and constructed to prevent pressure build-up and allow gas release that avoid rupture of the packaging[9]
- A shipper's declaration is not required for UN 3373 Biological Substances, Category B shipped specimens[9]
- All work surfaces should be decontaminated once specimens are packaged[9]
- Communicate with the receiving laboratory:
    - Maintain open and efficient lines of communication with the laboratory and provide all requested information
    - Alert the laboratory before sending specimens and providing all essential background information with the diagnostic request to enable the proper and timely processing of specimens and reporting of results

Figure 4 illustrates the procedure for safely packaging and transporting samples as per the recommended guidelines.

Figure 4: Procedure for safe packaging and transportation of COVID-19 samples[6]

Biomedical waste management at a collection centre

- All non-infectious solid waste collected in the sample collection room must be discarded in labelled discard bins only
- Removed PPE should be discarded in marked designated bins lined with biohazard bags
- Proper handwashing should be performed after doffing and discarding PPE
- Biohazard bags containing soiled PPE should be handed over to the autoclave team

For further details on the management of biomedical waste, please refer to the chapter on Biosafety.

Resampling

- Certain scenarios may lead to a sample(s) not being tested, e.g. in cases of damaged sampling tubes, sample leakage, incorrect sample taken, incomplete/incorrect

labelling of a sampling tube, improper storage conditions, or interrupted cold-chain during sample transportation; in such situations, resampling may be necessary
- It then becomes imperative to reach out to the patient(s) concerned and convey the need for resampling
- The patient SRF is a useful reference tool for obtaining the contact details of the patient(s) concerned if repeat sampling is required
- It is thus of utmost importance to note correct and legible patient information in the SRF while registering the patient on the ICMR portal

4.1.7 Spillage/leakage during collection/packaging/transportation

The procedures for dealing with spillage or leakage during collection, packaging and transportation are as follows.[10]

Sample spill or leakage during sample collection/packaging

- Ask everyone to immediately vacate the affected area and follow the personal safety protocol outlined below before cleaning the spill/leak:
    - If the spill has contaminated an individual's gown and/or shoe-covers, spray them with disinfectant before they step out of the room
    - Remove contaminated PPE and place it in a biohazard bag
    - Change contaminated clothing items and place them in an autoclave bag for decontamination later on
    - Disinfect hands and remove N95 mask
    - Wash hands thoroughly and don a fresh set of PPE
- Put up a sign indicating that entry is forbidden during the clean-up procedure
- Inform the collection centre in charge of the incident immediately
- Prevent staff from re-entering the laboratory for at least 30 minutes to an hour to allow aerosols to be removed through the laboratory's ventilation system and allow time for heavier particles to settle
- Document the incident
- Follow the steps shown in Figure 5, illustrating how to manage a spill

Sample leakage in a sample transportation box

- Notify laboratory staff and any other people nearby immediately
- Cordon off and restrict access to the area
- 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 any other contaminated items in a biohazard bag
- Transport these biohazard bags to an autoclave facility

- Document the spill incident

Figure 5: Management of sample spillage

For further details on spill management, refer to chapter 3, section 8 on spill management.

4.1.8 Sample receipt and storage and disposal of biomedical waste: instructions to be followed by testing laboratories

Sample receipt

Any personnel involved in receiving and unpacking specimens must be adequately trained on the hazards involved, how to adopt necessary precautions according to good microbiological practices and procedures (GMPP), how to handle broken or leaking containers, and how to handle spills and use disinfectants to manage any contamination.[2 11]

Instructions for sample receipt:

- Sample containers containing samples must be opened inside a certified biosafety cabinet (BSC Class II Type A2) in a BSL2-equivalent laboratory
- Don appropriate PPE (coverall/laboratory gown, N95 mask, head cover, goggles, outer shoe-covers, and two pairs of gloves) required for the procedure
- After donning the PPE, transfer the specimen box from the cold room/sample reception area on a trolley to the specimen processing area
- Appropriate biosafety instructions should be followed for accessing the biosafety cabinet (refer to chapter 3, section 3.4.2 on biosafety for instructions on the safe use of biosafety cabinets)
- Place the sample box in the biosafety cabinet
- Open the box and remove all sampling tubes/viral transport medium (VTM) containing the specimens and place them on a stand
- Adhering to biosafety instructions, inspect the sampling tubes and apply the specimen acceptance/rejection criteria

Sample storage at a testing laboratory

Clinical specimens or a subset of the clinical specimens may need to be retained for various purposes, such as performing additional tests, for external quality control (EQC), for inter-laboratory comparison (ILC) with a designated reference laboratory, and for sending to the Regional Genome Sequencing Laboratory (RGSL) for whole genome sequencing (WGS). ICMR provided an advisory on sample storage and retention.[12]

- All samples for long-term storage must be appropriately labelled to indicate laboratory identifiers and date of sample collection and must be stored in properly functioning, -80˚C deep freezers
- A full inventory (preferably electronic) of stored samples should be maintained
- Laboratories that serve as validation centres for COVID-19 diagnostic kits should preserve sufficient numbers of positive and negative samples to prepare appropriate panels for validation
- All samples testing positive for SARS-CoV-2 must be retained for a minimum of 30 days from the date of testing before being destroyed

- Depending on the freezer space available in a particular laboratory, one or more aliquots of positive specimens may be retained for the period
- All laboratories 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 the perceived research agenda of the laboratory in relation to COVID-19 in the future
- If the number of samples tested positive at a laboratory is very large and the laboratory is unable to retain all positive samples beyond 30 days, then:
    - A minimum of 10% of all positive samples detected at the laboratory in a month or 40 to 50 positive samples, preferably with equal numbers of high, moderate and low viral loads, should be stored for a period of at least 1 year
    - A single aliquot of a positive sample may be retained, depending on the availability of freezer space in the laboratory
- Considering that the number of samples testing negative at each laboratory will vary depending on the sample load and testing capacity of the laboratory:
    - A minimum of 50 samples or 1% to 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, depending on the availability of freezer space in the laboratory
- All laboratories should send five random positive and five random negative samples per month to QC laboratories, noting the following:
    - All testing laboratories should ensure storage of samples at -80˚C and regular monthly transfer of samples to QC laboratories
    - Include the laboratory name and sample ID; for shipping, samples should be placed in screw-cap vials and correct biosafety and biosecurity precautions should be followed, as per International Air Transport Association (IATA) guidelines

Biomedical waste management in a testing laboratory

- All non-infectious solid waste 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 PPE should be handed over to the autoclave team

Biomedical waste disposal for COVID samples[13 14]

- At the time of sample disposal, VTM with swabs should be discarded in yellow biohazard bags containing 5% freshly prepared sodium hypochlorite solution; VTM

with swabs must be left in contact with the sodium hypochlorite solution for at least 20 minutes

- All dry waste should be discarded in biohazard bags and should be autoclaved before disposal
- Any spillage should be decontaminated using 1% bleach followed by wiping with 70% ethanol
- Any spillages should be reported to the facility manager
- Biomedical waste should be labelled as "COVID-19 waste" before being handed over to a biomedical waste collection agency
- For further details on biomedical waste management, please refer to chapter 3.

4.2 Specimen registration and report management

4.2.1 Introduction

Rapid communication of test results is important for the planning and design of public health and outbreak control interventions. A rapid turnaround time of test results can also have a positive impact on an outbreak. Currently, a maximum of 24 hours is considered reasonable in most settings. Laboratories should follow national and respective state reporting requirements for COVID-19 tests.[2]

At the onset of the COVID-19 pandemic, the challenge was to standardize the format of sample data from various tests conducted across India. ICMR designed a specimen referral form (SRF) for use with every COVID-19 sample; NIC developed the RT-PCR and RATI mobile apps for Android, iOS and Windows mobiles along with the web portal to whitelist phlebotomists (sample collectors) who needed to use the mobile apps and the web portal.[7] It is mandatory to complete this form for every sample collected.

4.2.2 RT-PCR App

The RT-PCR App is a handheld tool for use by medical staff at sample collection centres spread across the country. Sample collection facilities send samples of various types of specimens to ICMR laboratories conducting RT-PCR tests for the confirmation of COVID-19. Advance notification is shared through the app with ICMR. Once a sample's details have been successfully saved, the collection centre staff and the user can view the collection details.

4.2.3 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 are:

- Robust, reliable, cloud-based infrastructure, with redundancy, zero down-time and region-wide databases with a responsive portal and apps, tested for 50 000 concurrent users

- Single sign-on using official government email IDs and mobile number-based access for laboratories (including private laboratories), collection centres and sample collectors
- Extensive training material, videos and FAQs available
- SRF data accessible on a real-time basis by ICMR laboratories, with integrated data analytics, auto alert SMS/emails and GIS
- Option to enter offline data after generation of an SRF ID
- Configurable: skip patient OTP for verification and warning on multiple use of the same patient mobile
- RT-PCR, rapid antigen and rapid antibody tests covered
- Patient mobile and location details, to help tracking of patients and tracing of contacts

Staff responsible

Staff responsible for data management include authorized specimen collection personnel, the data entry team and the person in charge.

Procedures

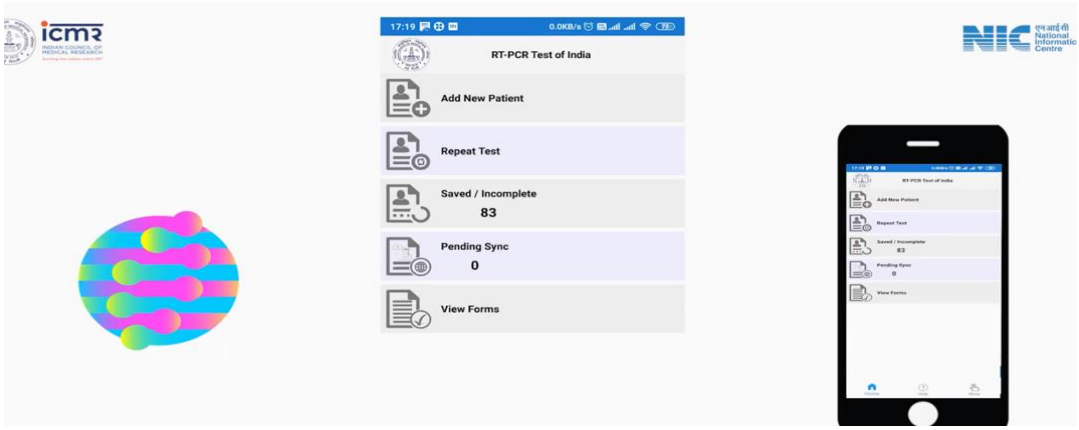
Step-wise SOP (with images) for using the RT-PCR App

While using the app, please ensure your internet connection is active.

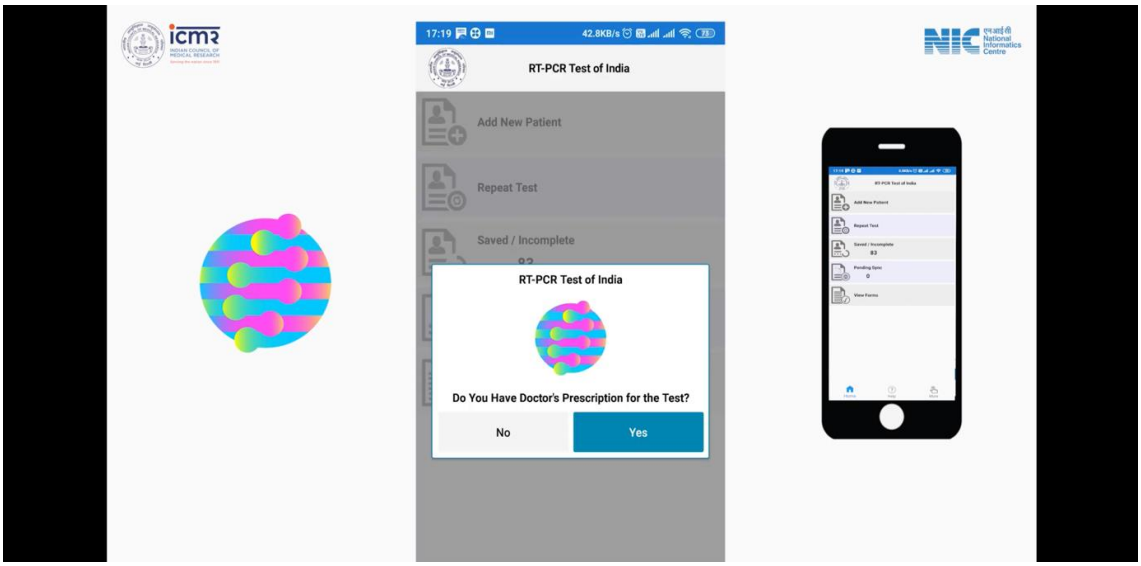
Registering a patient for the first time

STEP 1: Download and install the app from the portal/playstore. Before accepting the terms and conditions, check your mobile number authorization by visiting the portal.

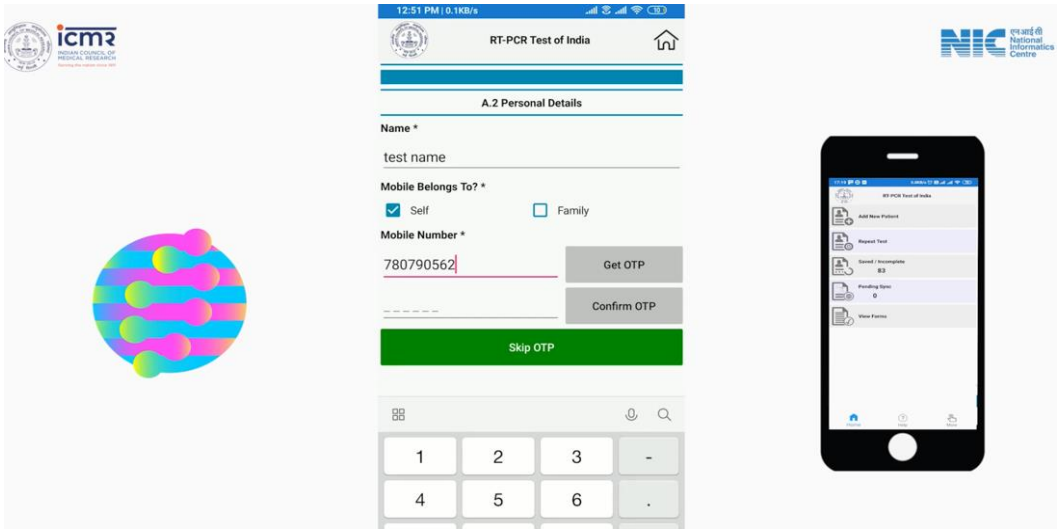
STEP 2: The app will now ask you to enter your mobile number, against which a one-time password (OTP) will be generated. The first menu, at the top, will ask you to "Add New Patient".

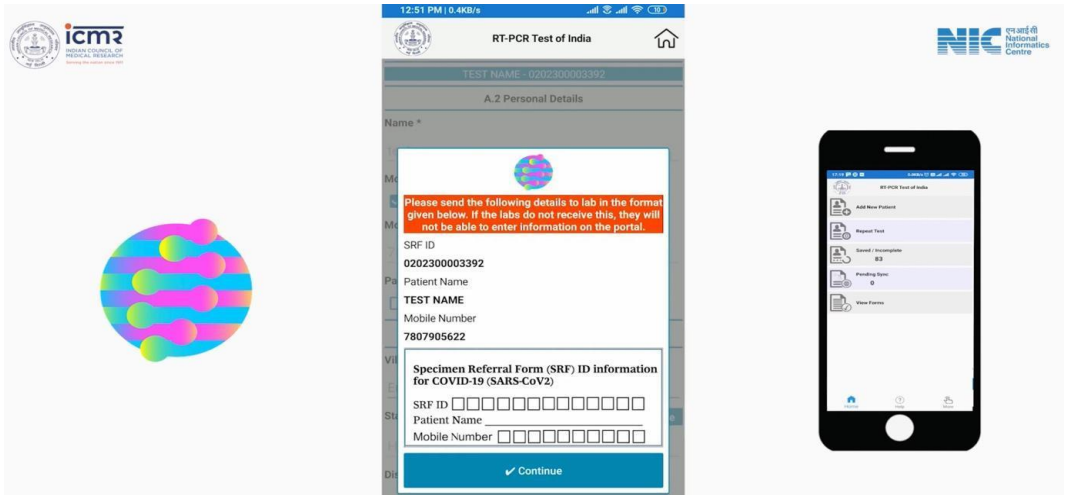


STEP 3: After adding a new patient, the app will ask for a doctor's prescription (this is no longer a mandatory field).



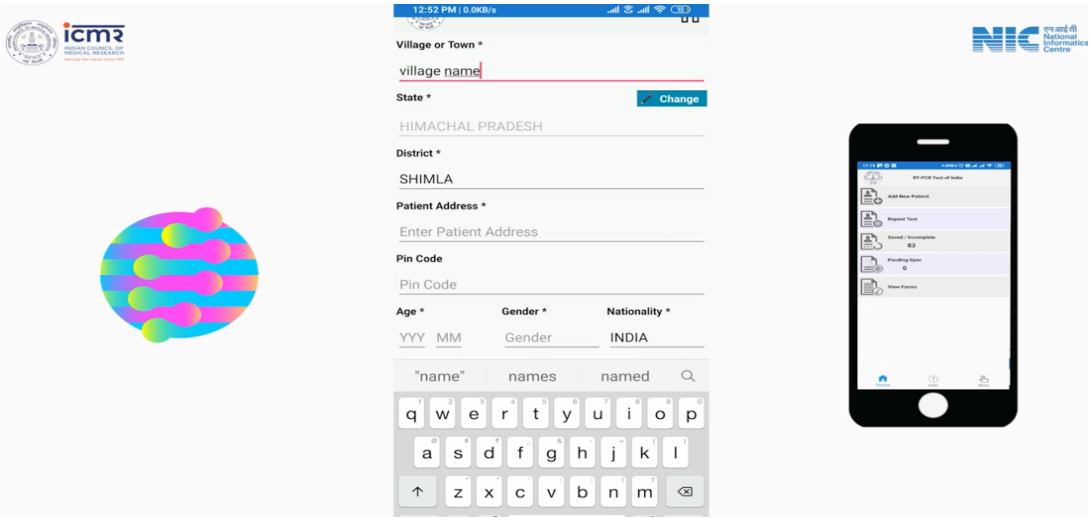
The app will then ask for the name and mobile number of the patient, and an OTP will be sent to the patient's registered phone number provided.

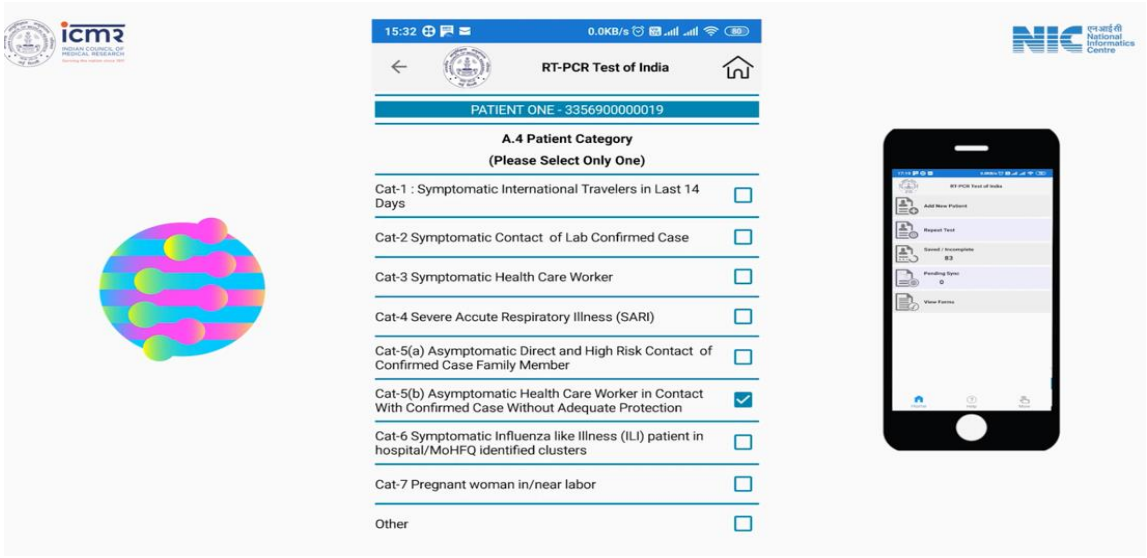




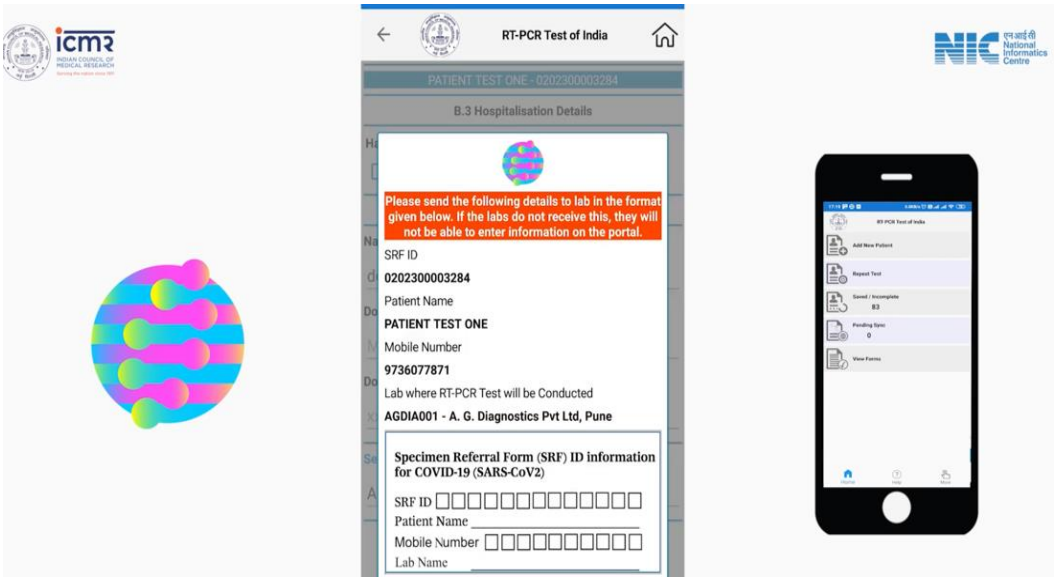
After validating the OTP, part of the form is saved, and the other sections can either be filled in immediately or later.

STEP 4: The app will ask you to enter personal details, patient credentials and clinical presentation questions, as per the latest specimen referral form.

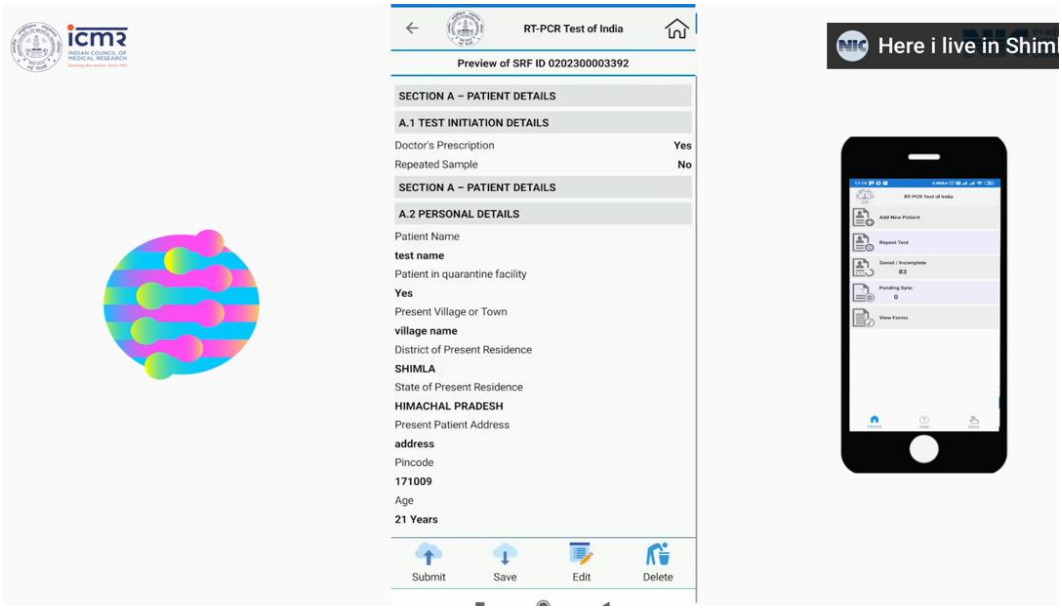




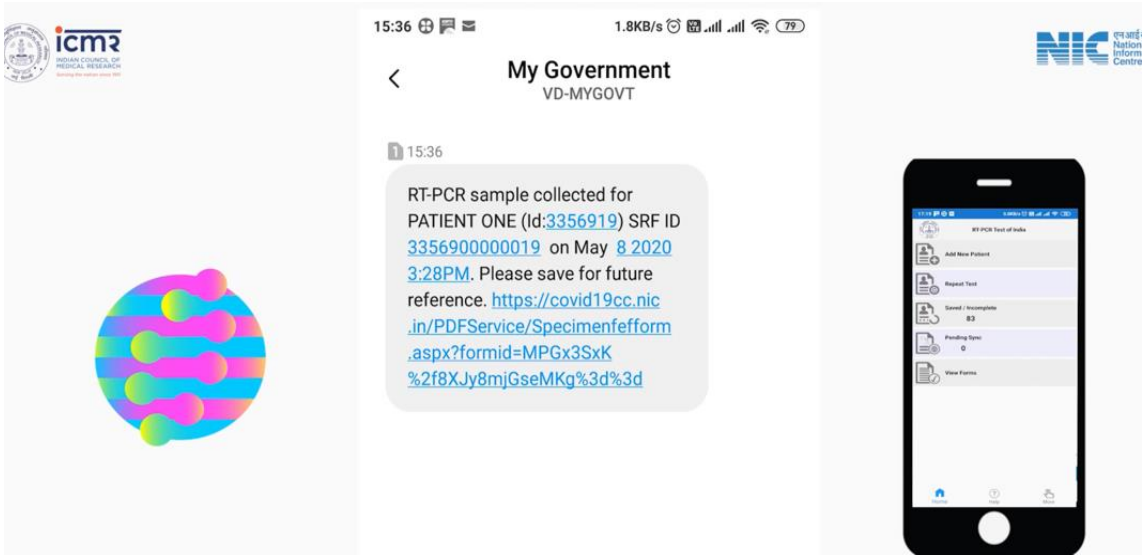
STEP 5: After saving the information and before making final submission, check the pop-up displaying your SRF ID.



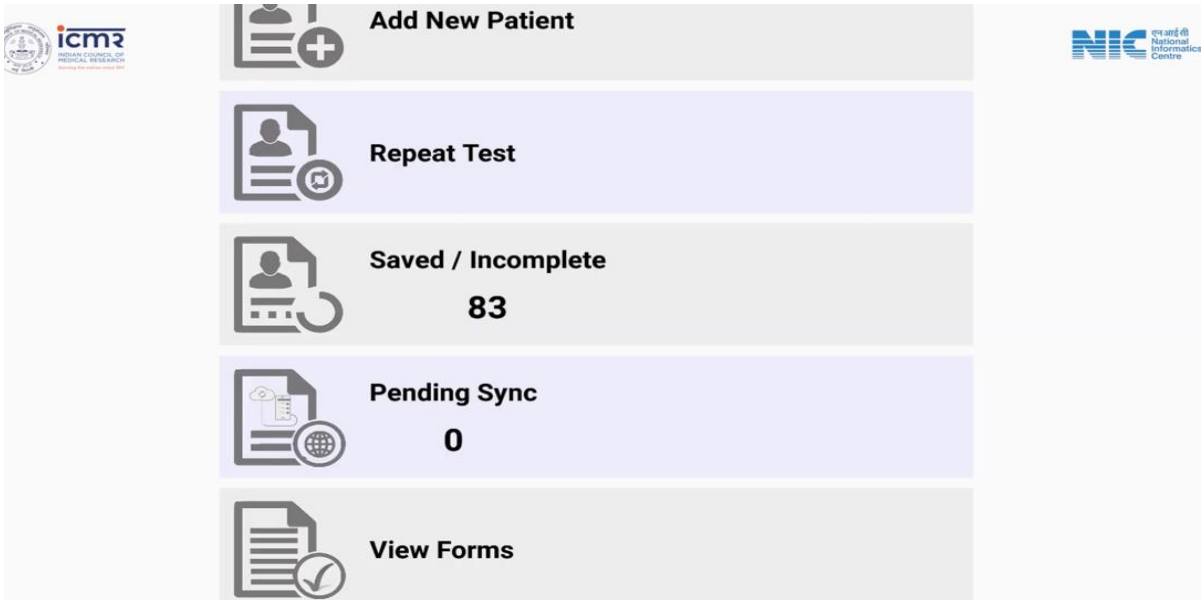
STEP 6: You can edit/continue. You can now also preview your form and proceed with submit/save/edit/delete options.



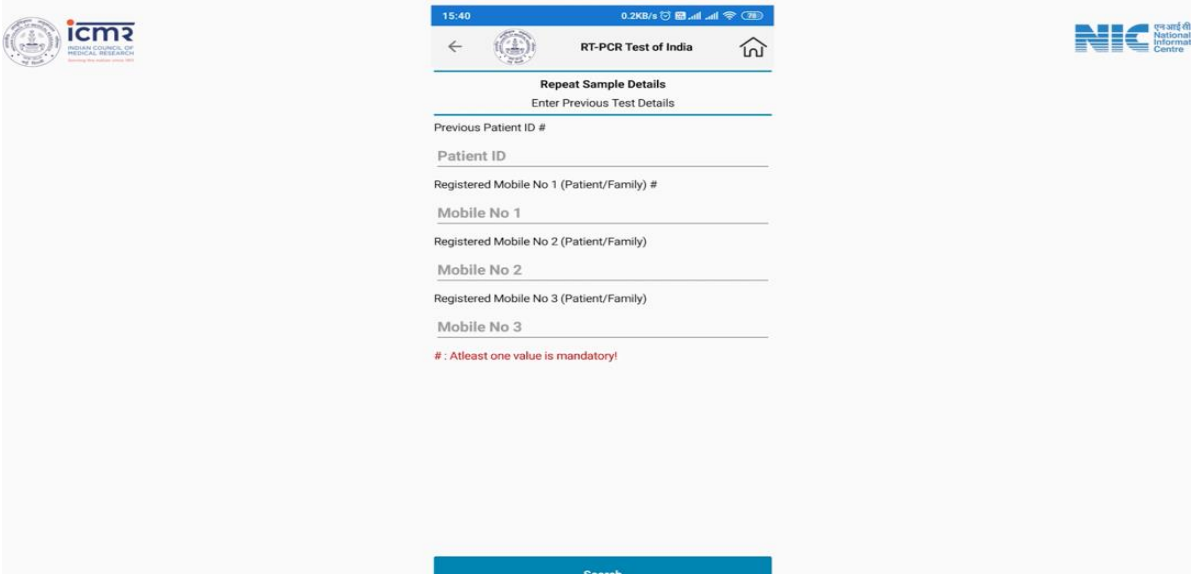
STEP 7: Once submitted, the patient will receive an SMS enabling them to view the report.



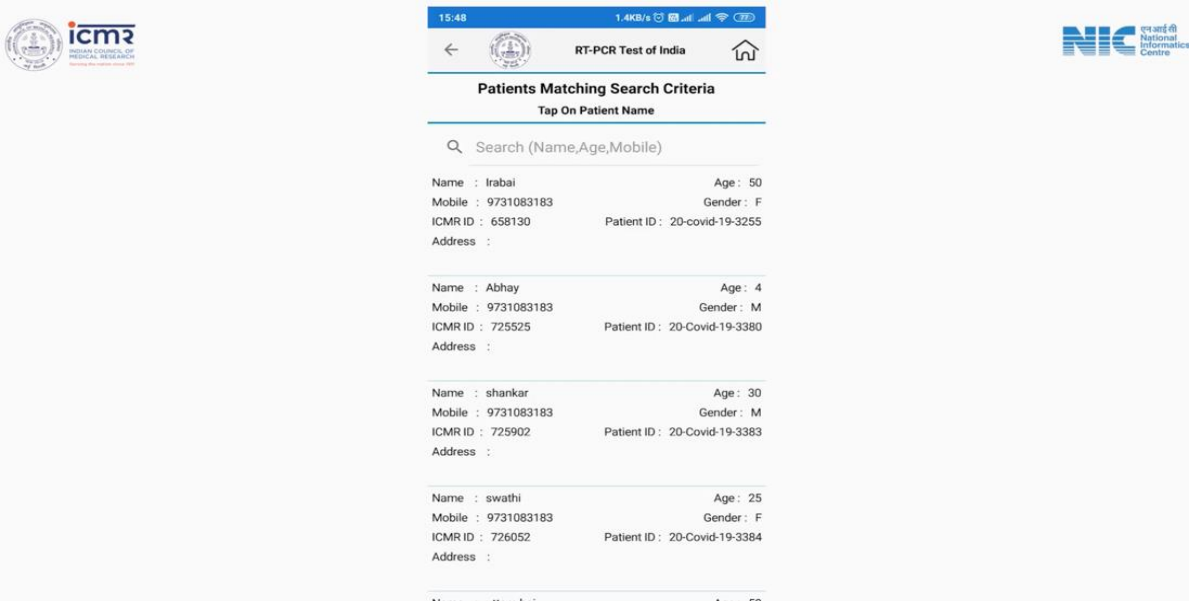
Protocol for repeat tests



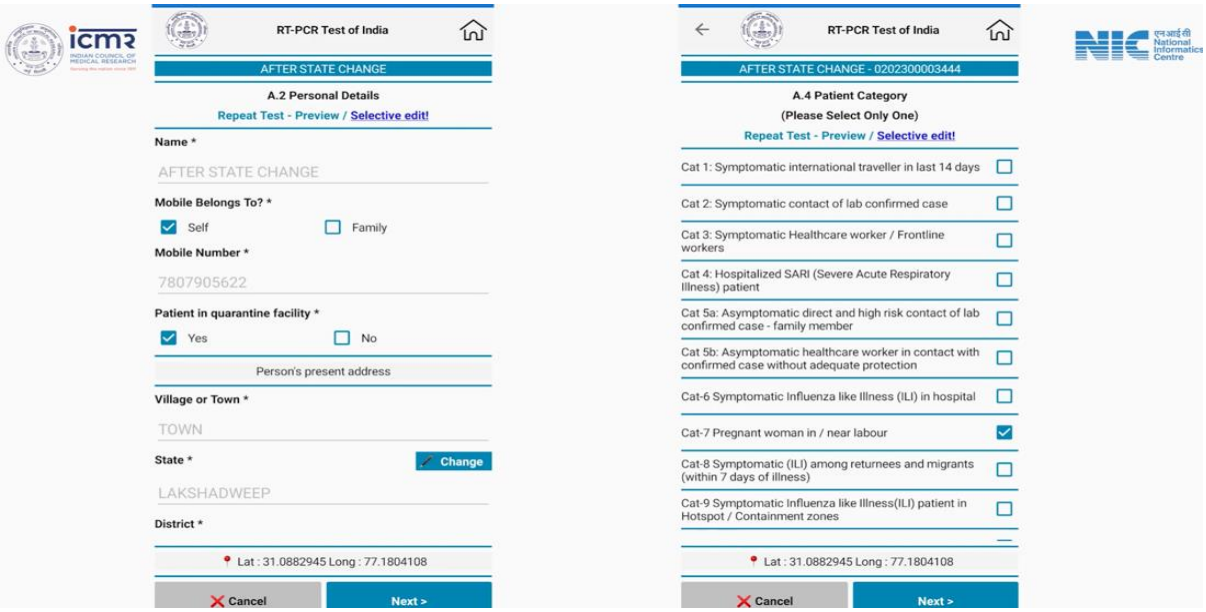
STEP 1: After clicking on the "Repeat test" menu, the screen will ask for the parameters as shown in the image below, and at least one value must be entered.



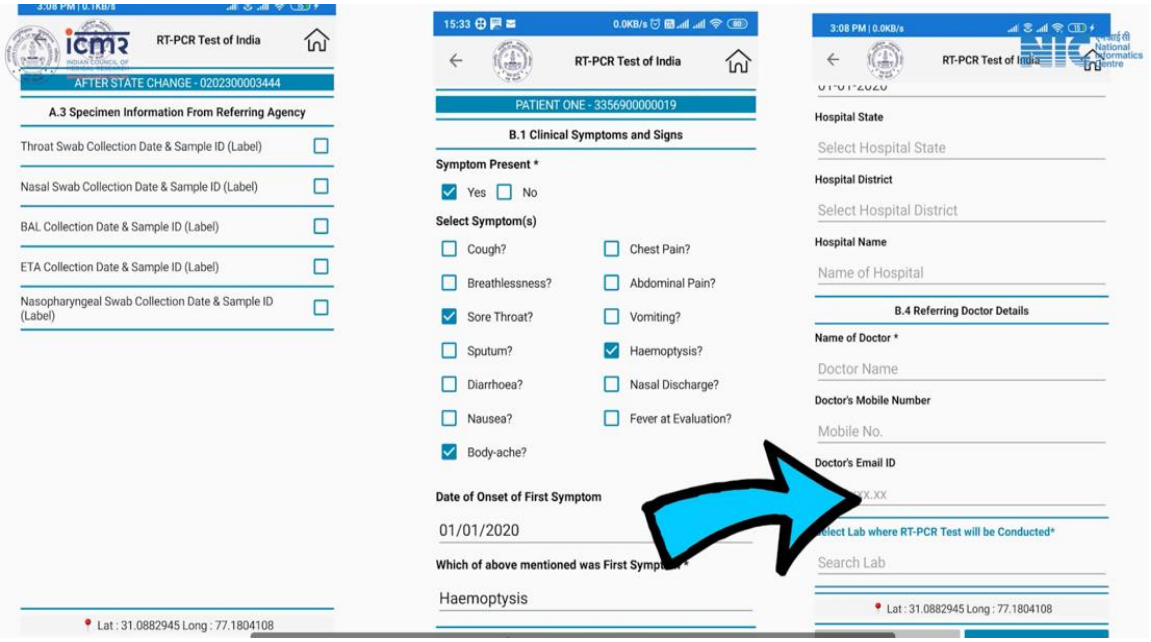
Immediately after entering one of the mandatory fields, you will be able to view patient-matching search criteria.



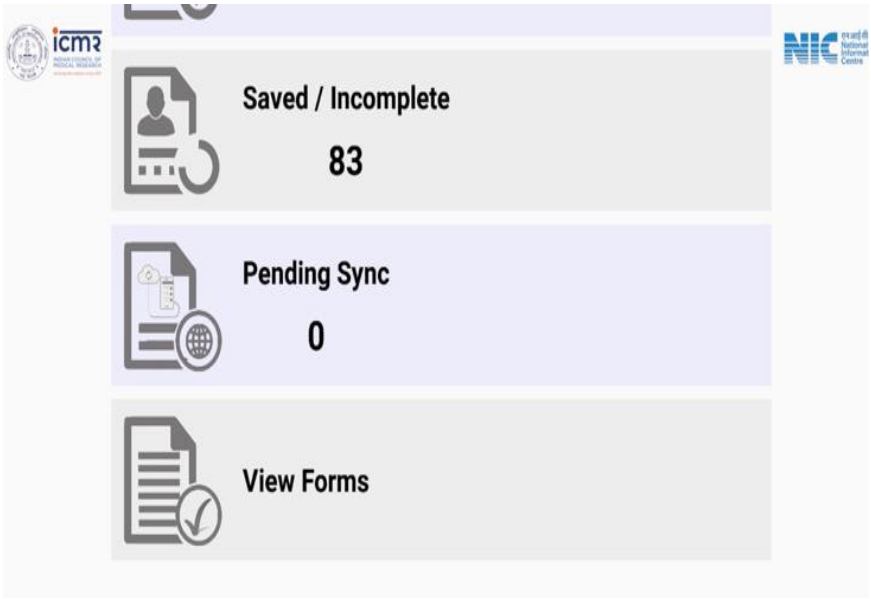
STEP 2: Upon tapping the desired patient record, the app will populate sections A2 and A4 with limited editing privileges.

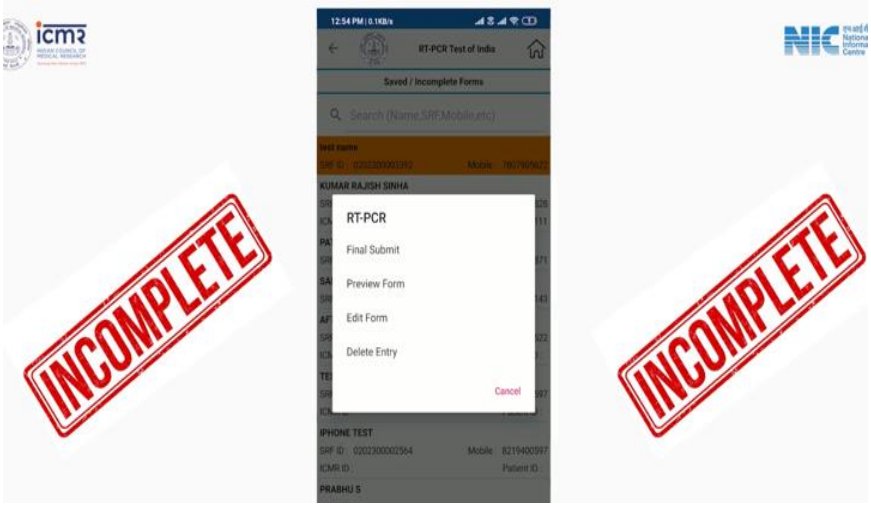


Sections A3 and B1 to B4 may be completed.



Protocol for saved/incomplete registration forms and pending sync

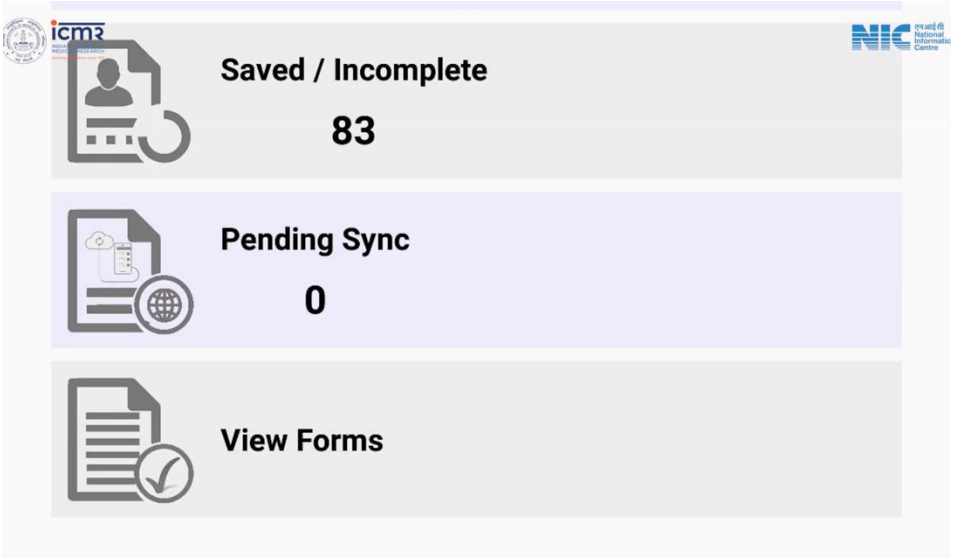


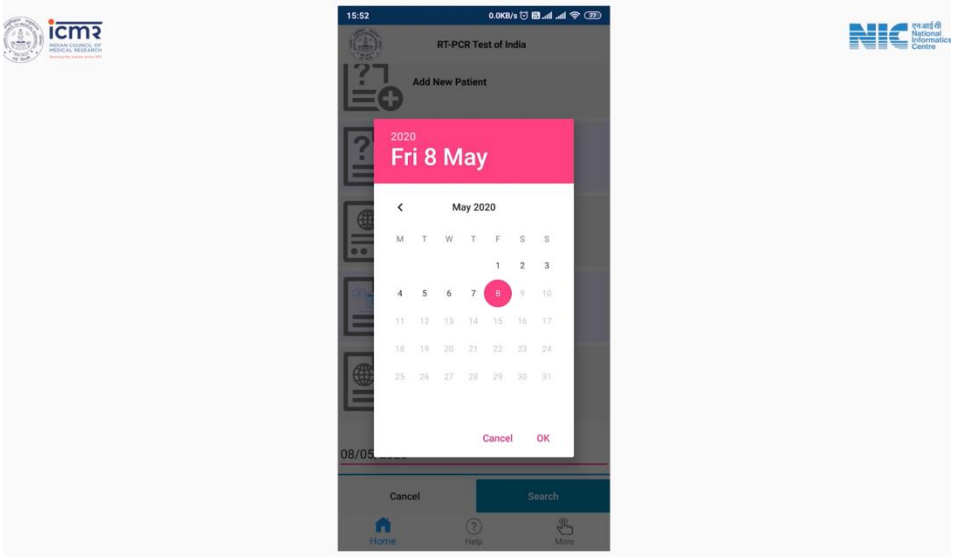


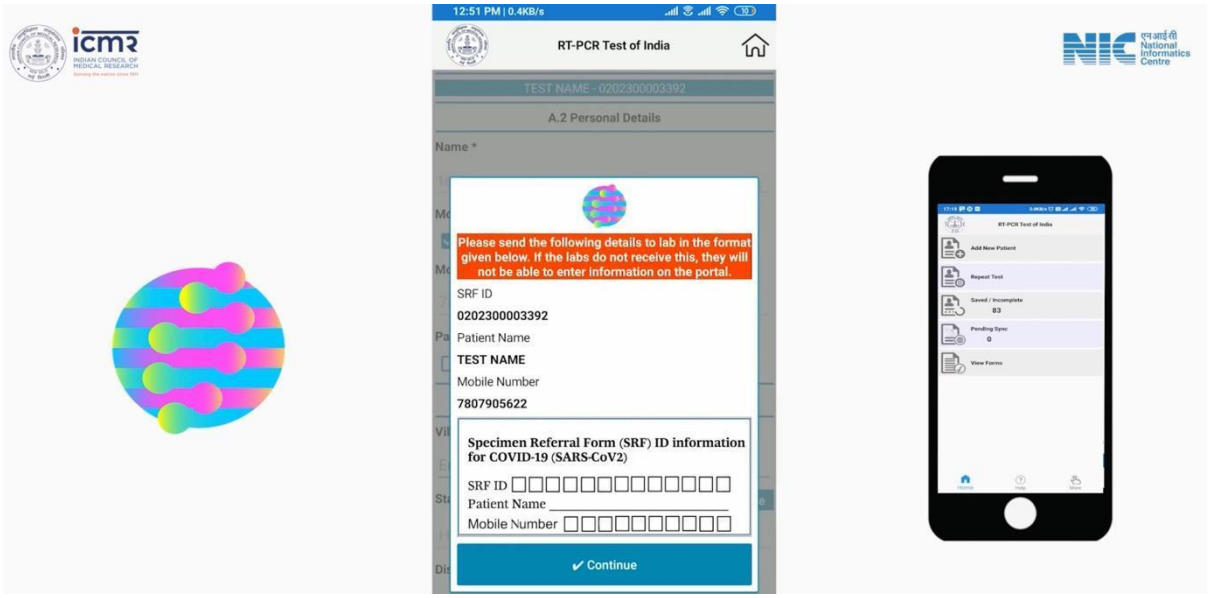
Complete registration of all pending forms and submit information.

VIEW FORMS

There is also an option of a “View forms” menu. You can select a date to view SRF forms submitted by you. Tap on the SRF to view a PDF.







A descriptive video is available at: https://youtu.be/CXTmT6JIXlI

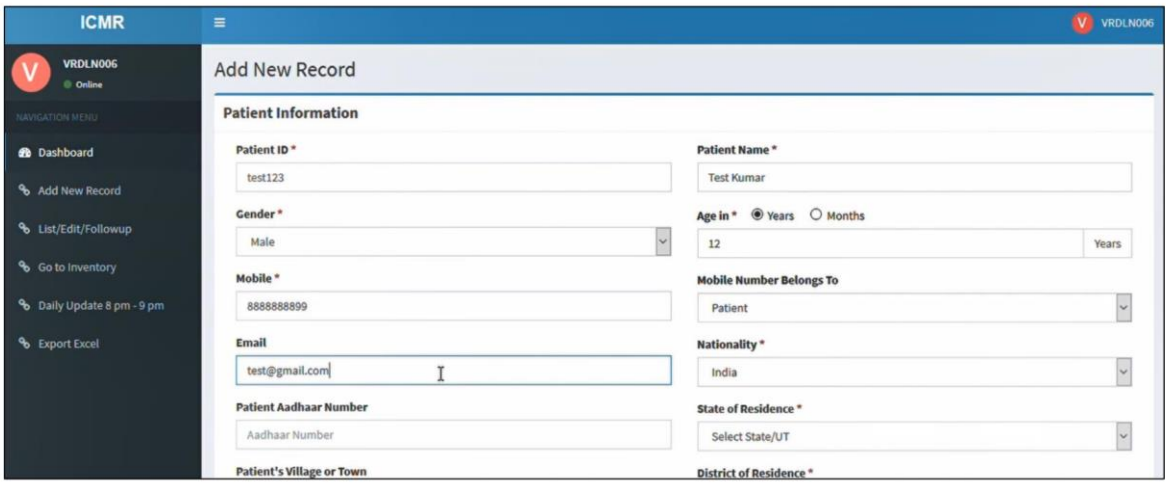
4.2.4 Specimen accessioning in a laboratory information management system (LIMS) (electronic/manual)

- Accessioning of samples in a laboratory information management system (electronic or manual) must be performed for ALL samples.
- Samples must be accessioned ONLY once relevant data have been entered via the RT-PCR app by authorized users (refer to the manual issued from time to time).
- The SRF ID generated through the RT-PCR app must be included in the LIMS registration, along with other details as mandated by in-house SOPs on sample accessioning. Note that whenever required, such as for international travellers, the passport number must be included during accessioning as per travel requirements.
- All records must be maintained for the sample collected, including necessary government-recognized ID, such as Aadhar card, PAN card etc., for positive identification of patients.
- Compliance with all mandated requirements must be ensured from collection until reporting and record keeping. The laboratory reference number is the unique sample ID that is generated through the LIMS and is entered via the ICMR data entry portal along with the SRF ID. Testing batches must be maintained in Google Sheets/Excel/manual registers (whichever is applicable) to allow information exchange for samples tested in a batch, kits/methodology used, time trail etc.
- Records such as sample IDs must be maintained in soft format along with other information in the LIMS. Data must be regularly backed-up as per the laboratory's established procedures.
- All samples collected must be accessioned in the LIMS and updated via the ICMR data entry portal in a real-time manner to ensure compliance with government guidelines.

4.2.5 Data upload via the ICMR portal (https://cvstatus.icmr.gov.in/login.php)

All RT-PCR, rapid antigen and rapid antibody tests for COVID-19 must be reported via the ICMR data entry portal,[15] which helps in developing national estimates for the number of tests conducted, number of positive tests, tests conducted per million population etc.[16] This data portal is the single national source of data entry and can be accessed by all relevant Ministries/Departments to help them define national strategies for COVID-19. ICMR urges all laboratories to continue entering data via the ICMR portal to help in the appropriate guidance of national strategies.

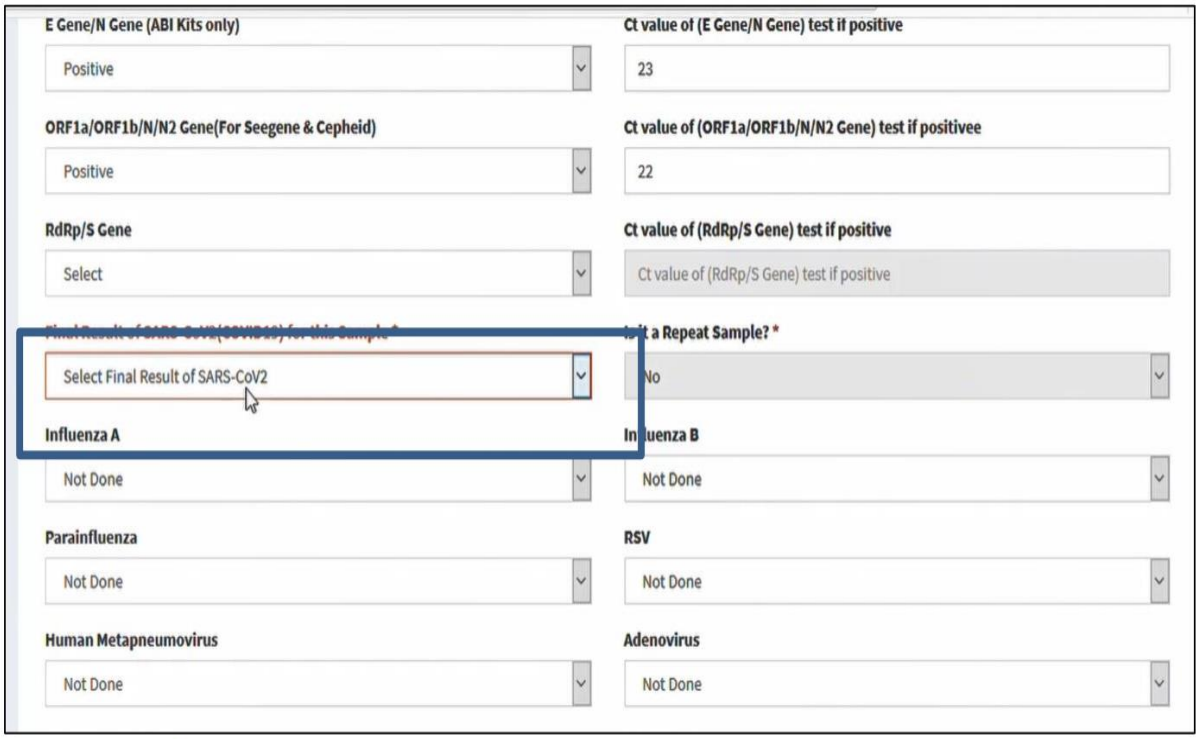
STEP 1: To enter data into the ICMR coronavirus status portal, enter your login credentials and click sign in. The page to add a new record will open with two sections, patient information and clinical data.



STEP 2: Personal and clinical data entry

- All fields marked with an asterisk are mandatory
- Add all required personal details
- In the patient category, select one option
- Add date of arrival in India (if applicable)
- Multiple underlying medical conditions and symptoms can be selected
- In the contact history for a laboratory-confirmed patient, enter details of the contact
- For every positive test result, enter the cycle threshold (Ct) value
- Most important field: enter the final result of SARS-CoV-2 testing (COVID-19) for this sample

Note: Once entered, the value for this field CANNOT BE EDITED. Please fill this in very carefully. Click submit at the end of the page to submit the record. Upon successful submission, the record can be viewed in the List option



For authenticity of reports and traceability

As per a mandate issued by NABL on 19 May 2021, a QR code on the medical test report must be provided, as follows:[17]

- Medical laboratories should note that, in a memorandum issued on 13 May 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, including a QR code linking to the original report.
- Medical laboratories should provide a QR code on all test reports issued, which can be scanned using any QR scanning application available on a mobile or any other device to authenticate and reproduce the test report online. This will prevent the manipulation of test results and the use of fake test reports.
- All Private Laboratories must ensure that all the requirements of the NABL ISO 15189 regulations are met. Authorized test results in a tamper-proof and non-editable test report will build trust in laboratory results.

Observation of compliance

All laboratories must ensure periodic audits of accessioning data and record any mismatched/incomplete information in the occurrence logbook, together with any corrective and preventive action.

4.3 Sample collection, packaging and transport for COVID-19 whole genome sequencing

4.3.1 Introduction

To fully understand the spread and evolution of the SARS-CoV-2 virus and to tackle its future spread, the sequencing and analysis of SARS-CoV-2 genomic data is required. The Indian SARS-CoV-2 Genomics Consortium (INSACOG) was established to expand whole genome sequencing (WGS) of SARS-CoV-2 across the nation, to aid understanding of how the virus spreads and evolves. Currently, the consortium includes 28 INSACOG Genome Sequencing Laboratories (IGSLs) that are mapped to the states and Union Territories to facilitate the smooth flow of samples. INSACOG developed the guidelines upon which this section is based.[18]

4.3.2 Sample collection for WGS

Collection sites

- INSACOG has identified several laboratories across the country to be sentinel sites for the collection and transportation of samples for WGS
- WGS samples may also be collected from non-sentinel sites (laboratories/hospitals) in cases of unusual events, such as vaccine breakthrough, super-spreader events, areas with high mortality/high morbidity trends etc.

Sample selection criteria

- Only those samples that are positive for SARS-CoV-2 by RT-PCR, preferably with a Ct value of 25 or less, should be packaged and transported for WGS
- After carrying out RT-PCR testing, the remaining samples (within 72 hours of collection, stored at 2–8˚C) that are RT-PCR-positive (Ct value <30), should be transported in VTM in a cool pack (4–8˚C) or on ice
- Alternatively, remaining RNA samples may be aliquoted and stored in 1.5-mL microcentrifuge tubes that are correctly labelled and sealed with parafilm (to be stored at -70˚C)
- RNA samples should be placed together in plastic/cardboard cryo-boxes, packed in a thermocol box with dry ice, and shipped to the appropriate RGSL for sequencing
- Sample collection for WGS is shown in Figure 6.

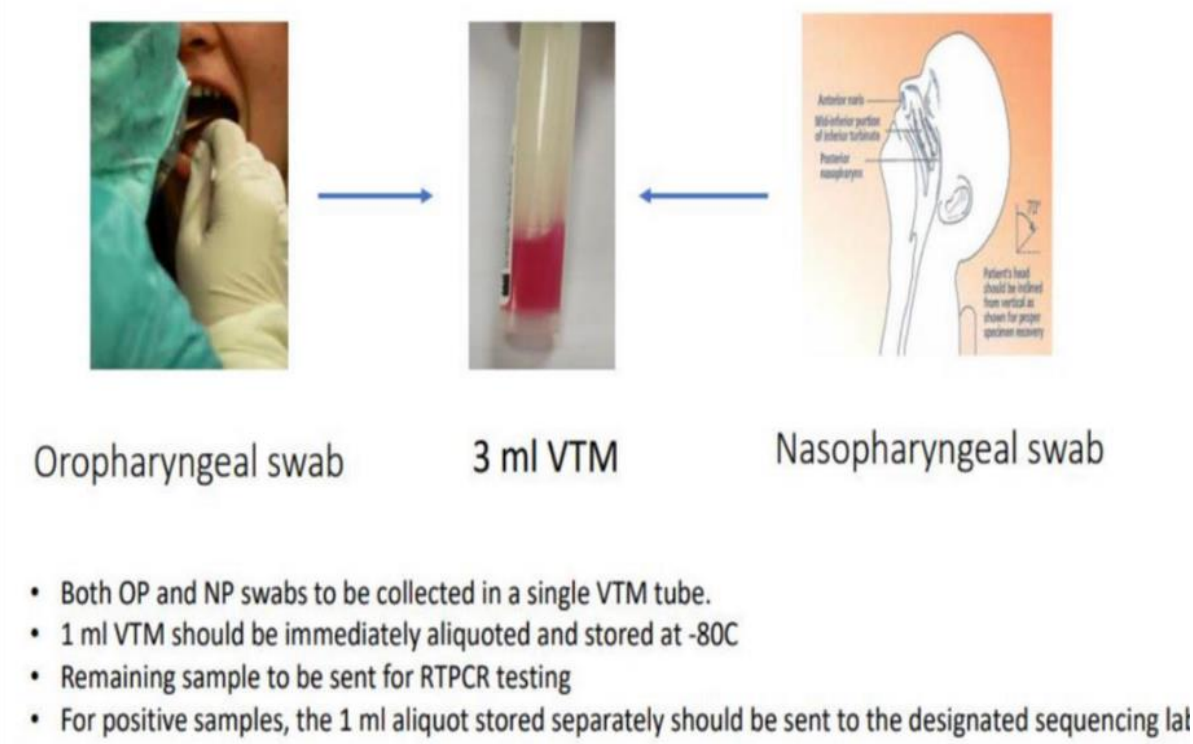


Figure 6: Sample collection for COVID-19 WGS

4.3.3 Sample labelling, packaging and transportation for WGS

Various factors should be considered when packaging and transporting COVID-19 samples for WGS (Figure 7).

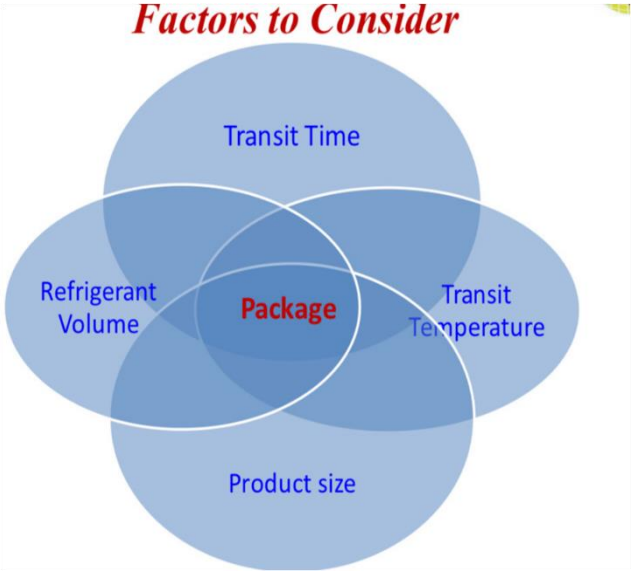


Figure 7: Factors to consider when packaging and transporting samples for COVID-19 WGS

- PPE (apron, gloves, face shield, N95 masks etc.) should be used and correct handwashing procedures should be followed before donning and after doffing PPE
- All biosafety precautions should be followed while carrying out sample packaging and transport
- Samples should be packaged and transported in standard triple packaging with all biosafety precautions and should be accompanied by a line-listing and details of samples including Ct values of all target genes detected
- Sentinel sites must submit a Sample Referral Form via the INSACOG WGS Surveillance module of the Integrated Disease Surveillance Program–Integrated Health Information Platform (IDSP–IHIP) portal, as shown in Figure 8.

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)

Figure 8: Format of the sample reference form for sentinel sites transporting samples for WGS

Figure 9 illustrates the three layers that constitute triple packaging (i.e. primary receptacle, secondary and outer packaging).

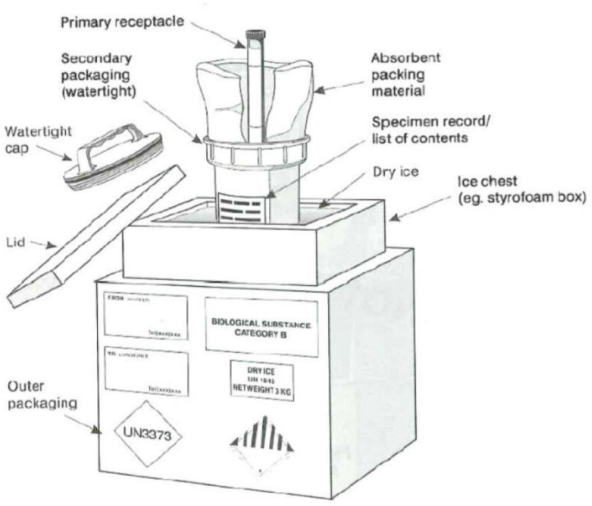


Figure 9: Schematic diagram showing the three layers that constitute triple packaging

- The three layers of triple packaging include:

  i) Primary receptacle: a labelled, watertight, leak-proof primary receptacle containing the specimen, wrapped in sufficient absorbent material to absorb all fluid in case of breakage.
  ii) 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.

  iii) Outer shipping package. The secondary receptacle must be placed in an outer shipping package, which protects it and its contents from outside influences such as physical damage and water while in transit.

- Samples for COVID-19 WGS must be transported to a reference laboratory under cold-chain conditions, with prior notification.
- Figure 10 illustrates the pathway of sample transportation from a sentinel site to a laboratory for WGS.

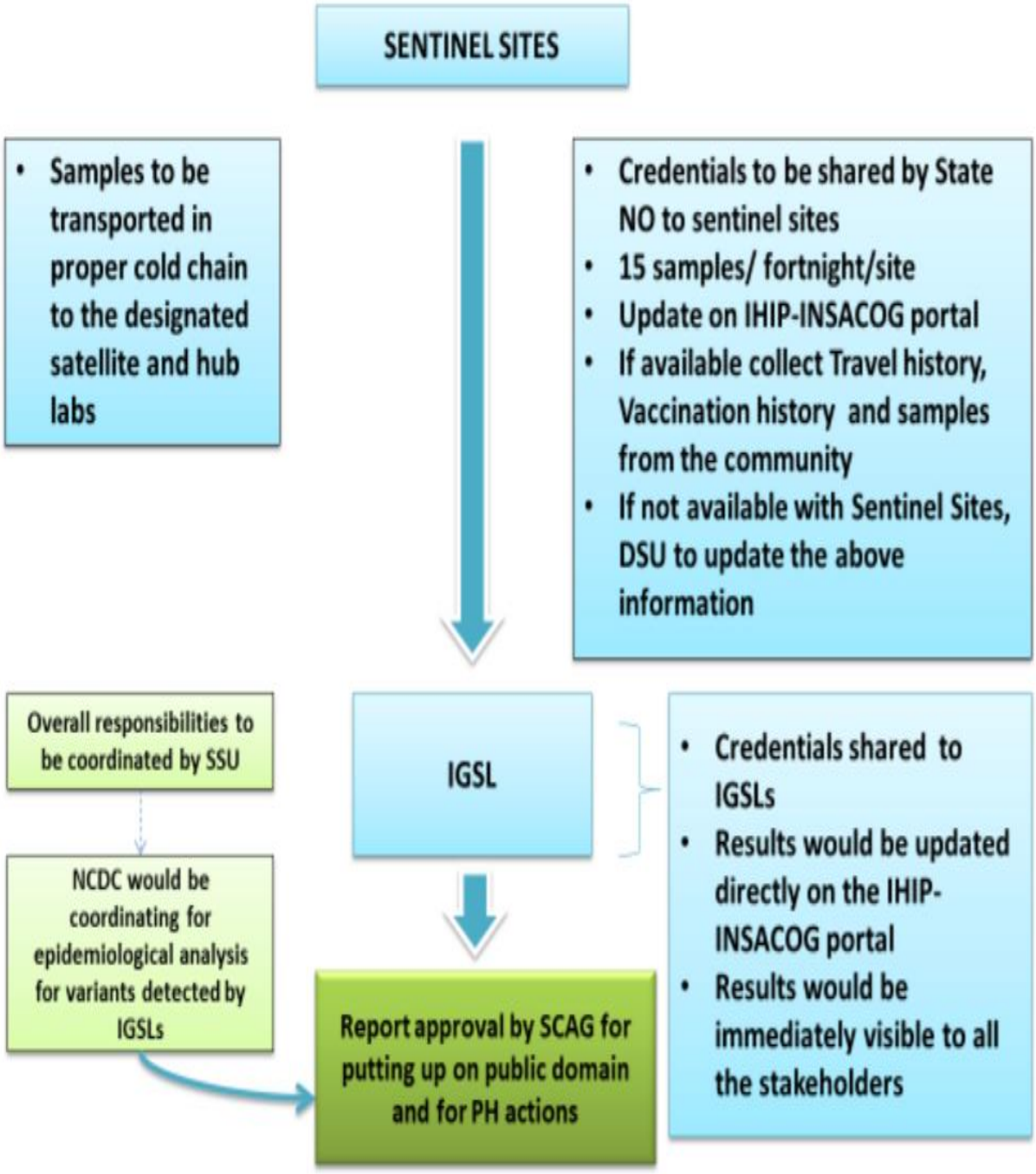


Figure 10: Pathway of sample transportation from a sentinel site to a laboratory for WGS[18]

Figure 11: Specimen referral form (SRF)

ICMR Specimen Referral Form for COVID-19 (SARS-CoV2)

INTRODUCTION

This form is for collection centres/ labs to enter details of the samples being tested for Covid-19. It is mandatory to fill this form for each and every sample being tested. It is essential that the collection centres/ labs exercise caution to ensure that correct information is captured in the form.

INSTRUCTIONS

- Inform the local / district / state health authorities, especially surveillance officer for further guidance
- Seek guidance on requirements for the clinical specimen collection and transport from nodal officer
- This form may be filled in and shared with the IDSP and forwarded to a lab where testing is planned
- Fields marked with asterisk (*) are mandatory

SECTION A – PATIENT DETAILS

A.1 TEST INITIATION DETAILS

*Sample collected first time: Yes ☐ No ☐

If No, Patient ID: .....

A.2 PERSONAL DETAILS

*Patient Name: ..... Father's Name.....

*Age: .... Years/Months/ Days (If age <1 yr, pls. tick months/ days checkbox)

* Gender: Male ☐ Female ☐ Transgender ☐

*Occupation: Health Care Worker ☐ Police ☐ Sanitation ☐ Security Guards ☐ Others ☐

☐ ☐

*Mobile Number: ☐☐☐☐☐☐☐☐☐☐ Mobile Number belongs to: Patient Family

*Nationality: .....

*Present patient address: ..... *Downloaded Aarogya Setu App: Yes ☐ No ☐

..... Pincode .....

*District ..... *State:.....

(These fields to be filled for all patients including foreigners)

Aadhar No. (For Indians): .....

Passport No. (For Foreign Nationals): .....

*Received COVID-19 vaccine: Yes ☐ No ☐

If yes type of vaccine: Covaxin ☐ Covishield ☐

Date of Dose 1 –/–/—— Date of Dose 2 –/–/——

1

Chapter 4 References

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17. QR Code on the Medical Test Reports, - Microsoft Word - QR Code on medical test report (nabl-india.org) dated 19-05-2021.
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Chapter 5
RT-PCR open systems

Chapter 5: RT-PCR open systems

5.1. Introduction

5.1.1 Introduction to the SARS-CoV-2 virus

SARS-CoV-2 is a positive-sense single-stranded RNA ((+) ssRNA) virus. Its genome consists of 29 900 nucleotides (nt) enclosing five open reading frames (ORFs) (5′–3′); ORF1ab polyprotein (P, 7096 amino acids), spike glycoprotein (S, 1273 amino acids), nucleocapsid protein (N, 419 amino acids), envelope protein (E, 75 amino acids), and membrane protein (M, 222 amino acids) (Figure 1). Therefore, several viral genes can be targeted for the detection of SARS-CoV-2 by reverse-transcription polymerase chain reaction (RT-PCR) methods.[1]

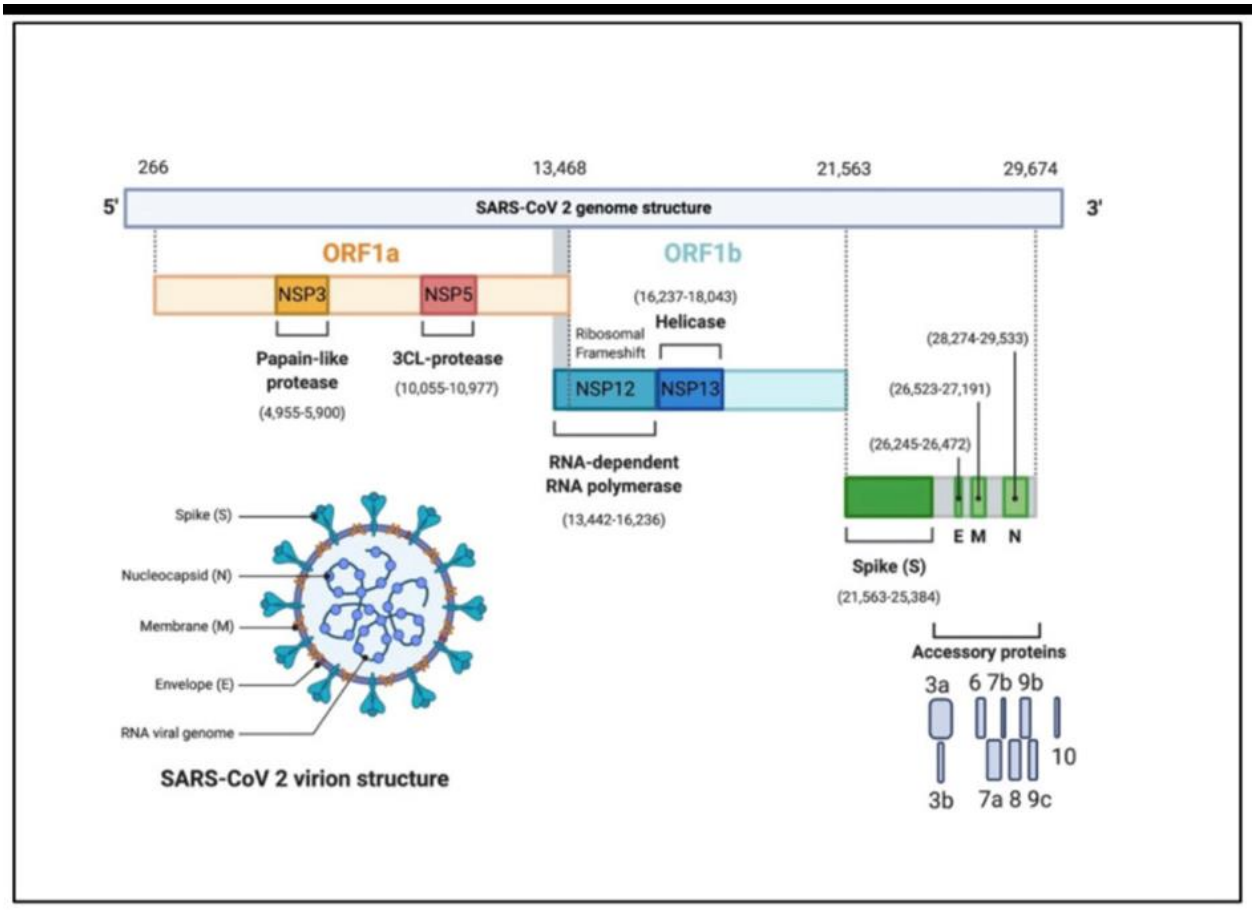

Figure 1: The genomic organization of SARS-CoV-2[2]

5.1.2 Introduction to the molecular diagnosis of SARS-CoV-2

Molecular diagnosis of SARS-CoV-2 is considered to be the optimum diagnostic modality for the early detection of infection. The World Health Organization (WHO) recommends that, wherever possible, suspected cases of active SARS-CoV-2 infection be tested using molecular

nucleic acid amplification test (NAAT) methods. Optimal diagnostics consist of an NAAT assay with at least two independent targets on the SARS-CoV-2 genome; however, in areas with widespread transmission of SARS-CoV-2, a simple algorithm can be adopted with one single discriminatory target. When using a one-target assay, it is recommended to have a strategy in place to monitor for mutations that might affect the performance of probe regions. By routinely testing all specimens with two different primer/probe sets that target different genomic regions it is possible to reduce the risk of obtaining false-negative results.[3]

RT-PCR is an NAAT method that relies on its ability to amplify a small amount of viral genetic material in a sample and is considered to be the gold standard for identification of SARS-CoV-2 virus.[4] [5] Currently, RT-PCR tests for COVID-19 generally use samples collected from the upper respiratory system using swabs. In addition, a few studies have also been done using serum, stool, or ocular secretions.[6] RT-PCR starts with the laboratory conversion of viral genomic RNA into DNA by an RNA-dependent DNA polymerase (reverse transcriptase). This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the viral RNA genome; the reverse transcriptase then generates a short complementary DNA (cDNA) copy of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is achieved using a fluorescent dye or a sequence-specific DNA probe labelled with a fluorescent molecule and a quencher molecule, as in the case of TaqMan assays. An automated system then repeats the amplification process for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or an electrical signal.[6]

RT-PCR has traditionally been carried out as a one-step or a two-step procedure. One-step real-time RT-PCR uses a single tube containing the necessary primers to run the entire RT-PCR reaction. Two-step real-time RT-PCR involves more than one tube to run the separate reverse transcription and amplification reactions but offers greater flexibility and higher sensitivity than the one-step procedure. It requires less starting material and allows for the ability to stock cDNA for quantification of multiple targets. The one-step procedure is generally the preferred approach for the detection of SARS-CoV-2 because it is quick to set up and involves limited sample handling and reduced bench time, decreasing chances for pipetting errors and cross-contamination between the reverse transcription and real-time PCR steps.[6]

5.2 Open real-time (reverse transcriptase) PCR systems

5.2.1 Background to open RT-PCR systems

Among molecular diagnostics, open system real-time RT-PCR was the first approach to be adopted globally and is the gold standard test for detection of SARS-CoV-2.[7]

Open-system RT-PCR refers to test kits and protocols that are used on open PCR platforms, which require the end user to extract the viral RNA in a separate step to the PCR reaction. As

these manual PCR kits and protocols are compatible with multiple PCR machines (thermocyclers), the end user can also choose which thermocycler to use.[8]

There are two types of manual RT-PCR protocols that can be performed on open PCR machines: those that follow laboratory-developed (or in-house) protocols and those that follow commercial kit protocols. ICMR has approved and recommended various commercial and in-house kits that can be used by COVID-19 testing laboratories.[9] The most accessible diagnostic test is RT-PCR for upper respiratory tract samples, such as nasopharyngeal swabs. The various RT-PCR kits available have different clinical sensitivities and specificities. ICMR has established acceptance criteria for both RNA extraction kits and RT-PCR kits (Table 1).[10]

Table 1: Validation of SARS-CoV-2 diagnostic commodities: acceptance criteria

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

A video that shows molecular testing on open platforms, including protocols and technical considerations, is available here: https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing-private/2/steps/1117415)

5.2.2 Advantages of open platforms

Open platforms offer several advantages:[8]

- Tests may be well supported in-country by the manufacturer's distribution network and service staff.
- It may be easier to scale-up testing using multiple kits that are compatible with the same open system, as long as consistent quality can be guaranteed by the laboratory; this may mitigate any supply chain concerns.
- Laboratory staff may already have been trained in testing, troubleshooting and maintaining open platforms.
- Tests are usually less expensive than tests for use on closed platforms.
- Good accuracy of detection as well as the ability to run up to 94 or 382 samples in a single run.

5.2.3 Disadvantages of open platforms

Open platforms also have some disadvantages:[8]

- Ensuring high test performance and quality requires robust quality control and quality assurance procedures in the laboratory.
- Before introducing any new RNA extraction and RT-PCR kits a laboratory must conduct validation of the new kit.
- Sample processing methods may vary for the specimen types recommended and for the type of RNA extraction kit used for COVID-19 diagnosis.
- Well-trained staff are needed to perform the multiple manual steps and to interpret the results.
- Open platforms require additional consumables, equipment or quality controls that may not be supplied with the machine, test or test component.
- The average time of testing is around 2 to 3 hours.

5.3 ICMR Guidelines on RT-PCR testing

RT-PCR is considered to be the first choice of test for routine surveillance in hospital settings and in non-containment areas. For routine surveillance in containment zones and screening at points of entry, RT-PCR/Truenat/CBNAAT are the second choice of tests, rapid antigen tests (RATs) being the first choice of test. (For further details on CBNAAT and Truenat, please see chapters 6 and 7, respectively). A single RT-PCR positive test is to be considered confirmatory, without any need for repeat testing.[11]

5.4 Laboratory space arrangement

There are several important points to consider when arranging a laboratory for RT-PCR testing, as outlined below

- NAATs are extremely sensitive; a typical PCR run will repeatedly amplify a tiny quantity of target nucleic acid (DNA or RNA) sequence in a sample, generating 10^9 copies of the target sequence.
- If the laboratory is not correctly organized properly, or appropriate laboratory processes are not followed, target nucleic acid from amplified product, specimens or control material can contaminate the facility and other diagnostic specimens processed in the laboratory, which can lead to false-positive results.
- Once a laboratory has been contaminated, decontamination is an extremely difficult, expensive and time-consuming process that will render the laboratory inoperable until corrected.
- It is essential, therefore, that an appropriate laboratory process, as recommended by WHO[12 13] and ICMR guidelines, must be set up before any testing is initiated.

- A laboratory performing PCR analyses on diagnostic samples must be divided into four, physically separate rooms, as shown in Figure 2.[14]
    - Area 1: reagent preparation room (positive air-pressure to prevent the introduction of contamination).
    - Area 2: sample processing room (negative air-pressure to keep template nucleic acids in the room).
    - Area 3: template addition room (positive air-pressure to ensure template nucleic acids remain in the room).
    - Area 4: amplification and detection room (positive air-pressure to ensure amplified nucleic acids remain in the room).

Full details on the laboratory design and the workflow processes necessary for RT-PCR testing are provided in chapter 3.

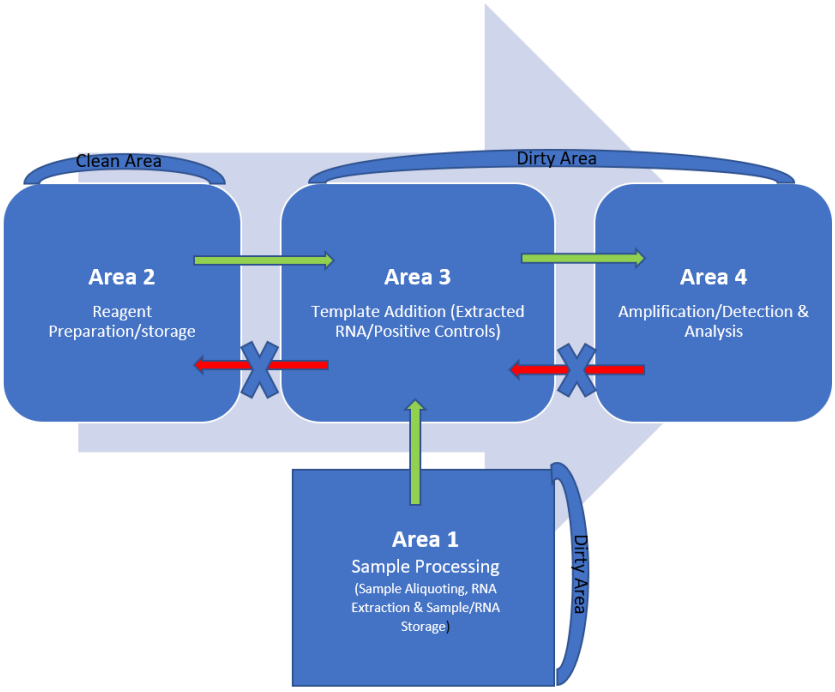

Figure 2: Unidirectional workflow of a molecular testing laboratory

5.5 RT-PCR testing workflow

There are several steps involved in the RT-PCR workflow. The main steps in an open platform for COVID-19 testing are shown in Figure 3.

Figure 3: Overview of COVID-19 RT-PCR testing procedures

Detection of viral RNA from clinical specimens is key to the diagnosis of SARS-CoV-2 infection. RT-PCR diagnosis of COVID-19 is mostly performed on nasopharyngeal swab specimens.[15] This sample is collected in viral transport medium (VTM), which allows the collection, transport and storage of samples.

5.6 Controls for RT-PCR Testing

The laboratory must ensure that the PCR extraction and amplification processes are properly checked for quality using internal quality controls (IQCs) in each test run. IQCs that are commonly employed for PCR testing are described below.

5.6.1 Internal controls

One potential source of false-negative results can occur if there is insufficient sample collection or sample extraction. To mitigate this, an internal control (IC) must be used to monitor whether the process has proceeded correctly for each sample.

The amplification of targets and ICs takes place simultaneously in the same reaction. The IC can be added at the RNA extraction step or the master mix step. There are two different approaches in RT-PCR assay design for internal controls: endogenous and exogenous controls.[16-19]

5.6.2 Endogenous internal controls

Endogenous internal controls comprise a normal cellular gene sequence, which is expected to be present in all specimens, and can be used as an endogenous IC. This type of IC uses housekeeping genes (beta-actin or RNase P) to report the presence of genetic material from the sample. This approach has the advantage of monitoring the integrity of the nucleic acid target; in improperly collected, stored or processed specimens, the endogenous target will be absent (or degraded) and fail to yield a positive result. A disadvantage is that endogenous sequences may not accurately reflect amplification of the primary target due to differences in the primer sequences, size of the amplified product, and the relative amounts of the two targets. This type of control is not placed in a designated well but instead is present in every sample well.

5.6.3 Exogenous internal controls

Exogenous internal controls are a little more complex and comprise synthetic ICs that are used as a proxy for the primary target, which overcomes the inherent limitations of an endogenous IC. They usually contain non-infectious cultured human cell (A549) material or a plasmid DNA or in vitro RNA transcript with primer-binding regions identical to those of the target sequence. They involve adding an exogenous source of encapsulated RNA to each sample prior to extraction.[16 17]

5.6.4 Extraction negative control

- Extraction negative controls consist of RNA that is extracted from a negative sample plus water.
- They indicate whether contamination was introduced during the extraction phase.
- These controls are extracted along with the clinical specimens and are included in the RT-PCR process at the template addition step.[20 21]

5.6.5 No template control (NTC)

- An NTC contains nuclease-free water.
- It is used to check whether there was any contamination during specimen extraction and/or plate set-up. If any NTC reactions are defined as positive, sample contamination may have occurred, and the test must be repeated with strict adherence to the testing procedures.
- An NTC also indicates whether PCR reagents have been compromised when determining the cycle threshold.
- An NTC is added at the template addition step during the RT-PCR process.

5.6.6 Positive template control(s)

- A positive template control is also referred to as a positive control.
- Positive template controls are in vitro-transcribed SARS-CoV-2 RNA, either gene fragment or whole-genome. This control must be handled with caution in a dedicated nucleic acid handling area to prevent possible cross-contamination.
- A positive template control indicates the limit of detection and robustness of an assay.
- A positive control is added during the template addition step of the RT-PCR process.

A failure of any one of these controls (for instance, if the positive control turns out to be negative) invalidates the test result and the assay must be repeated either from stored or newly collected sample after investigating and fixing the cause of the failure (e.g. contamination or degradation of the sample, or expired reagents).[20]

5.7 Steps in RT-PCR testing

5.7.1 Step 1: RNA Extraction

5.7.1.1 Principle

Specimens are lysed to extract the RNA. RNA is subsequently used for reverse transcription and real-time PCR amplification. The major steps in RNA extraction are cell lysis of the tissues, denaturation of the nucleoprotein complexes, inactivation of RNA/DNA nucleases and purification. The lysis buffers required for sample inactivation are provided in the RNA extraction kits.

RNA is a single-stranded nucleic acid, which is highly unstable and has a very short half-life, once extracted from its source. Another reason for its instability is the RNases that are present everywhere, in cells, tissues, bacteria and the environment. RNases degrade RNA and are difficult enzymes to inactivate. RNases resist heat inactivation and thus need strong denaturants for their elimination. Therefore, during RNA extraction, specimens must be lysed under strong denaturing conditions, in an RNase-free environment, to ensure isolation of intact RNA.[3 22]

5.7.1.2 Methods of RNA extraction include:

- Spin column-based methods: kits that use this method include MDS™ Viral RNA Extraction Kit, PATHKITS, QIAMP Viral RNA extraction kit, and HiPurA® Viral RNA Purification Kit (MB615).
- Magnetic bead-based methods: kits that use this method include Maverick Magnetic Bead Based Nucleic Acid Extraction Kit and Coronavirus SARS-CoV-2 RNA Extraction Kit Using Magnetic Beads from PATHKITS.

5.7.1.3 Types of RNA extraction

Manual RNA extraction

Manual RNA extraction kits use spin columns or centrifuges, which enables automation of the binding and purification of nucleic acids. However, the remaining steps of extraction involve a complex series of manual processes that are highly labour intensive. The following points should also be noted.[23]

- Manual extraction takes around 45 minutes to 1 hour to process a batch of five samples.
- Because it is a manual process, there is huge scope for human error and inconsistency in the procedure, especially in laboratories that have a high sample load.

- Manual extraction is not recommended for laboratories that handle more than 200 samples per day.
- The spin column-based method is the most commonly used manual extraction method.

Automated RNA extraction

Automated extractors are specifically designed instruments that help simplify and increase the output of nucleic acid extraction processes using automated liquid dispensers and robotic principles, thus enabling automation in all steps of the extraction process.[24]i

- Most of these extractors use magnetic beads and a few use column-based methods to separate RNA. The reagents required are usually prefilled in cartridges.
- Automated extractors come in a variety of sizes and capacities, ranging from low-throughput to high-throughput (6, 12, 24, 32 or 96 samples per run) Depending on the throughput of the extractor, extractions can be performed within a timeframe of 10 to 60 minutes.
- Automated extractors decrease the working time and labour costs required; they also increase safety, quality, and can produce a high yet reliable yield.
- Automated extraction is recommended for laboratories that handle more than 200 samples per day.

5.7.1.4 Key recommendations for RNA extraction

RNA quality and quantity are the key to the successful testing; therefore, the following points must be carefully considered when performing RNA extraction:

- The specimens to be tested should be stored and processed within the recommended timeframe.
- RNA extraction must be carried out in a BSL-2 or equivalent facility inside a certified biosafety cabinet (BSC Class II Type A2), with staff wearing appropriate personal protective equipment (PPE) as recommended.
- RNase-free reagents should be used, and work surfaces should be free of RNase. The work surface area inside the BSC should be decontaminated with appropriate disinfectants as per the instructions provided in the respective protocols.
- Ensure tip boxes, micropipettes, and tube rack surfaces are RNase free. Use a cleaning agent such as RNase ZAP to remove RNases.
- Extracted RNA is very unstable and should always be stored at 4°C for short periods (4–6 hours). If this is not possible, then the extracted RNA must be stored at -70/-80°C until further use. Repeated freeze–thaw of RNA specimens should be avoided.

- RNA extraction should be carried out as per manufacturer's instructions and the standard operating procedures (SOPs) provided in the laboratory; all work should be documented in the appropriate worksheets.
- If an RT-PCR kit has an indigenous internal control (IC), then the IC must be added after the addition of the carrier RNA, as per the manufacturer's instructions.
- All reagents in the extraction kit must be brought to room temperature before use and must be replaced at their respective storage temperatures to avoid any degradation.
- RNA extraction reagents must not be contaminated. Aliquot the necessary reagents for use. (Remember that PCR is a highly sensitive process and a tiny amount of contamination will negatively impact test results; any contaminated reagents must be discarded.)
- After completing the work, RNA extraction waste along with tips and tubes should be placed in a biohazard bag, which should then be tied and autoclaved.
- The BSC should be cleaned as per the instructions provided in the SOPs, and the UV light should be switched on after the completion of work.

5.7.2 Step 2: Reverse transcription and amplification

RT-PCR technology amplifies small amounts of ribonucleic acid (RNA) from specimens into deoxyribonucleic acid (DNA), which is replicated until SARS-CoV-2, if present, is detectable.[25]

5.7.2.1 Principle

RNA extracted from a specimen is converted to complementary DNA (cDNA). PCR is then used to amplify viral target genes in this cDNA. The real-time detection of amplified targets during PCR is achieved by using fluorescent probes in the PCR reaction and the detection of fluorescence signals by the instrument. The presence or absence of fluorescence signal/s specific to the viral target gene/s is an indicator of the presence of the virus in the specimen. The number of cycles at which the detected fluorescence signal exceeds background levels is called the cycle threshold (Ct). Lower Ct values imply high levels of target RNA in a patient's sample. Conversely, high Ct values imply low levels of target RNA in the sample.

5.7.2.2 Steps in RT-PCR

I. Master mix preparation
II. Addition of templates to the master mix
III. PCR machine set up and data collection

I. Master mix preparation

Master mixes are mixtures containing most of the reagents required for RT-PCR. Typical components of a master mix include a buffer to maintain pH and salt concentrations, magnesium chloride to stabilize double-stranded interactions and act as a cofactor for Taq polymerase, deoxyribonucleotide triphosphates (dNTPs) to build the new DNA strands, and Taq polymerase to synthesize the new DNA.

- Master mix preparation should be carried out inside a PCR hood or laminar flow cabinet in the designated master mix room (reagent preparation room), which is an RNA/DNA-free area.
- PPE, pipettes and consumables used in this area should not be used elsewhere.
- To minimize RNase contamination, it is advisable to change gloves frequently and use RNase-free water and molecular biology-grade tips and tubes.
- Before proceeding with the master mix preparation, the quantities of reagents required must be calculated and documented in a worksheet (please see Annexure 10 – provided for the Covipath RT-PCR kit).

II. Addition of templates to the master mix

The extracted RNA acts as a template that, when added to the master mix, is reverse transcribed into cDNA. This cDNA undergoes exponential amplification during PCR.[19]

- The addition of RNA to the master mix must be performed in a dedicated template addition room inside a laminar airflow cabinet and documented as detailed in the templated represented in the Annexure 11.
- A positive control - a synthetic peptide - is an in vitro transcribed RNA provided with the kit - should be added along with the extracted RNA to the respective labelled master mix tubes as part of the quality control process.
- Any micropipettes, tips or tube racks used for this process must not be used for any other process.
- The extracted RNA samples and the positive control should be maintained at a temperature of 4°C during the entire process of template addition to avoid degradation of RNA in the sample and the positive control.
- Any remaining extracted RNA and the positive control should be immediately stored at -20°C to avoid degradation of RNA in the sample and the positive control.

III. PCR machine set up and data collection (amplification room)

The RT-PCR reactions are set up in RT-PCR machines that are placed in the amplification room.

- A real-time PCR detection system consists of a thermal cycler equipped with an optical detection module to measure the fluorescence signal generated during each amplification cycle as the fluorophore binds to the target sequence.[8 26 27]
- The RT-PCR machine cycles through temperatures that heat and cool the mixture to trigger specific chemical reactions that create new, identical copies of the target sections of viral DNA.
- The cycle is repeated over and over to continue copying the target sections of viral DNA. This is called amplification.
- A standard real-time RT-PCR set up usually goes through 35 cycles, which means that, by the end of the process, around 35 billion new copies of the sections of viral DNA are created from each strand of the virus present in the sample.
- In RT-PCR, the PCR amplification reaction is monitored by the fluorescence signal of the sequence-specific probes and these are captured by the analysis. The PCR curve of a successful amplification shows a smooth sigmoidal shape and high fluorescence intensity (Figure 4).
- The cycle threshold (Ct) is the number of amplification cycles it takes for the fluorescence to go above the signal threshold (i.e. background signal). The higher the viral RNA concentration is in the sample, the lower the Ct value will be.

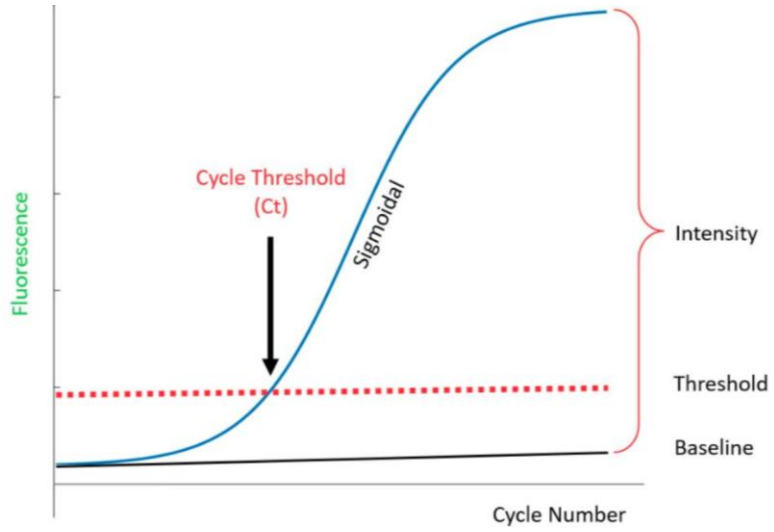

Figure 4: Threshold level and cycle threshold on a real-time PCR amplification curve

5.7.3 Step 3: Interpretation of test results and control data

Once the amplification has completed, the test results data are interpreted by laboratory staff in line with the controls used in the PCR run. Reports are then generated for the samples.

In RT-PCR, the PCR amplification reaction is monitored by the fluorescence signal of the sequence-specific probes. The PCR curve of a successful amplification shows a smooth sigmoidal shape and high fluorescence intensity.

5.7.3.1 Definition of Ct value

The Ct value is the cycle number at which the fluorescent signal of the reaction crosses the threshold. It is the intersection between an amplification curve and a threshold line. The amplification plot in Figure 5 shows the variation of log (ΔRn) with PCR cycle number. The Ct value is inversely related to the starting amount of target DNA/cDNA. If the Ct value becomes too high, it can be difficult to distinguish the real signal from the background signal. For this reason, kits have a cut-off value beyond which results are interpreted as negative. The interpretation of results based on Ct values varies from kit to kit (follow the manufacturer's instructions).

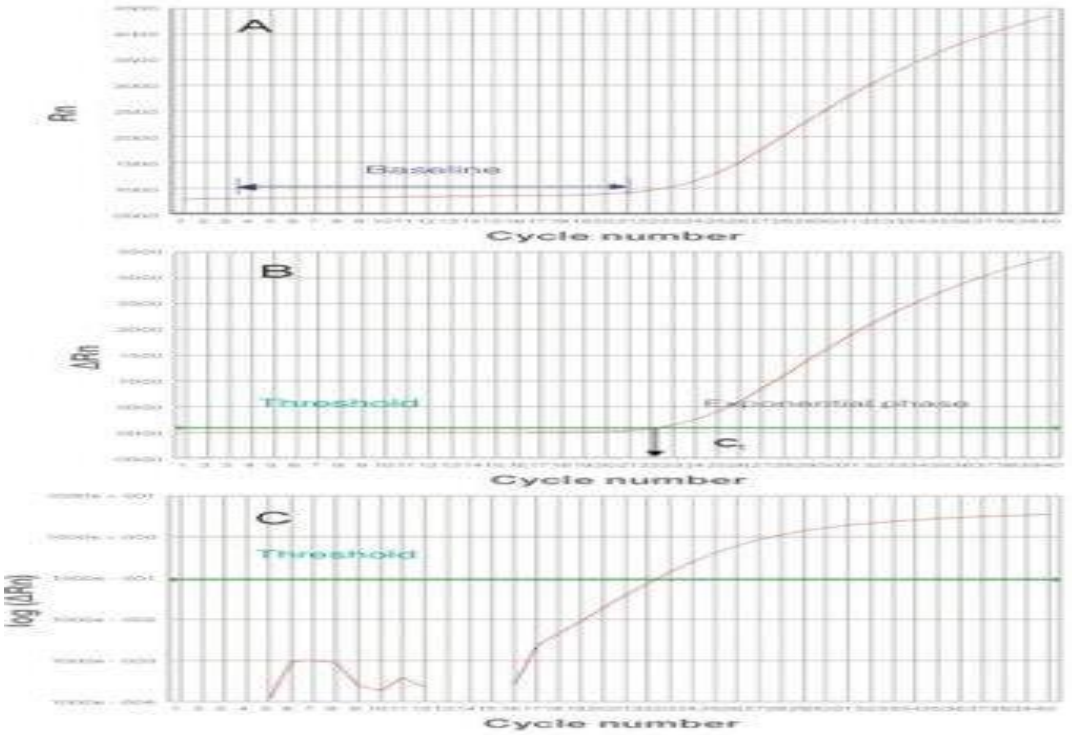

Figure 5: A graphical representation of RT-PCR data (Ct values)

5.7.3.2 Factors that can impact Ct values

There are various factors that can impact the absolute value of Ct besides the concentration of the target; some are outlined below.

- Template-independent factors can influence the Ct value, including
  - Effect of master mix components
  - Efficiency of a PCR reaction
  - Passive reference dye
- Too little template, e.g. very early/late stage of infection or suboptimal nucleic acid isolation

- Poor reverse transcriptase activity during cDNA synthesis (reverse transcriptase is sensitive to degradation).

5.7.3.3 Illustration of Ct values

- In the PCR amplification curve shown in Figure 6, the test has undergone 40 cycles of amplification.
- The Ct value of less than 30 is positive (blue line)
- The result of an inconclusive or weak positive has a Ct value of between 30 and 40 (green line)
- The Ct value of a negative results never exceeds the threshold (red line).
- It is advisable that the reporting authority of the laboratory visualize the curves for any samples that are borderline, to confirm that they are sigmoid curves and whether the fluorescence emitted is sufficiently high in terms of units relative to the positive control.

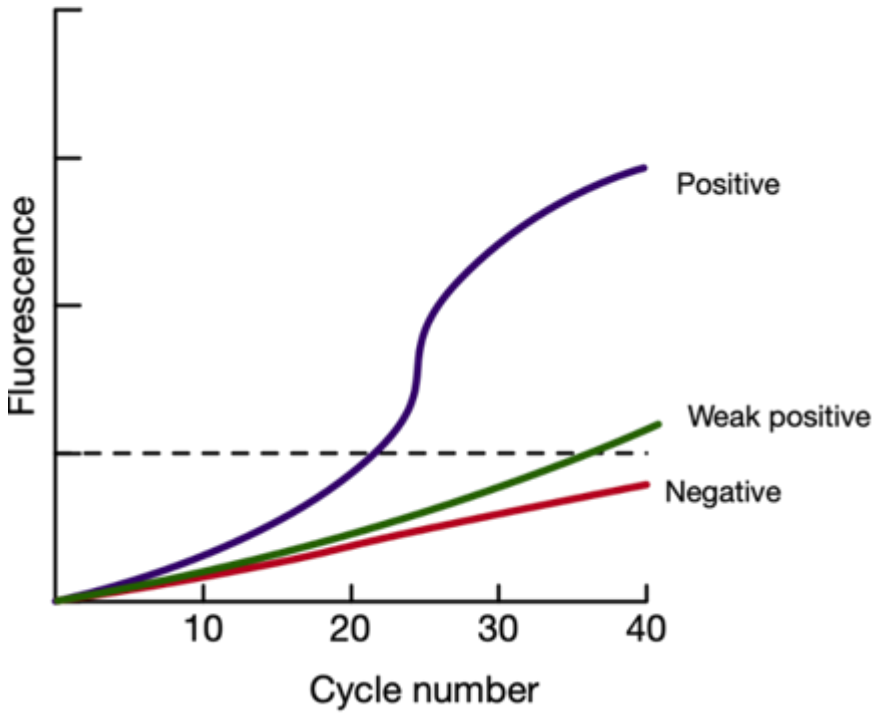

Figure 6: Ct values with varying fluorescence signals

It is important to note that there have been no reliable studies that have definitively proved a direct correlation between disease severity/infectiousness and Ct values.[28] There are also various other challenges in the interpretation of Ct values, as outlined below.

- Comparability of Ct values among different kits is a challenge, as our laboratories are now using a mixture of kits with different Ct cut-offs and different gene targets.
- Ct values are dependent on how a sample has been collected. A poorly collected sample may be reflected by inappropriate Ct values.
- Ct values are determined by the technical competence of the person performing the test, calibration of equipment and pipettes, and the analytical skills of the interpreters.
- Ct values between nasal and oropharyngeal specimens collected from the same individual may differ. Similarly, the temperature of transportation as well as time taken from collection to receipt in the laboratory can also adversely impact Ct values.
- Samples from asymptomatic/mild cases show Ct values similar to those from samples from individuals who develop severe disease.
- Patients in early symptomatic stages may show a high Ct value, which may subsequently change. In such cases, high Ct values will give a false sense of security.
- The severity of COVID-19 disease largely depends on host factors besides the viral load. Some patients with a low viral load may experience very severe disease due to the triggering of immunological responses. Hence, high Ct values may again give a false sense of security.
- The RT-PCR tests currently being conducted are qualitative in nature.
- Ct values may give a rough estimate of viral load. However, more specialized standards are required for quantitative assays, which are currently unavailable for SARS-CoV-2.

In view of the above reasons, it is not recommended to rely on numerical Ct values for determining the infectiousness of COVID-19 patients and deciding on patient management protocols.

There are several steps to be followed during the interpretation of a PCR run,[29] as follows.

- 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 the expected Ct values (this varies with the kit).
- All clinical samples should exhibit internal control reaction curves that cross the threshold line at or before 35 cycles or as instructed by the manufacturer.
- If the results of the internal controls are not within the accepted criteria, then the patient's result should not be released.
- It is advisable that laboratories visualize the curves for samples that are borderline, to confirm that those are sigmoid and whether the fluorescence emitted is sufficiently high in terms of units relative to the positive control.

5.7.4 An example of RT-PCT Ct values and interpretation of results

Every PCR assay has a different pre-defined Ct cut-off value, and the gene targets vary among different kits. The result interpretation tables that are used for the CoviPath COVID-19 RT-PCR Kit (an ICMR-approved kit) are shown as an example (Tables 2 and 3). This kit is a multiplex assay that contains two primer/probe sets specific to different genomic regions of SARS-CoV-2 (ORF1ab and N gene) and one primer/probe set targeting the RNase P gene. The RNase P gene serves as an internal positive control to monitor the sample source.

Table 2: The Ct values the positive control reactions for each probe/primer reactions should give when using the CoviPath COVID-19 RT-PCR Kit

Channel	Positive control	Expected Ct value
FAM	ORF1ab	≤37
VIC	N gene	≤37
JUN	RNase P	≤35

Table 3: Interpretation of test results for clinical specimens when using the CoviPath COVID-19 RT-PCR Kit[30]

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.

5.7.4.1 Implementation of a universal cut-off Ct value for RT-PCR by ICMR

The globally accepted cut-off for Ct values for COVID-19 ranges from 35 to 40, depending upon specific restrictions laid down by individual manufacturers. However, ICMR has taken inputs from different virology laboratories across India to arrive at a single Ct cut-off value, based on individual laboratory experiences (see Annexure 12). ICMR has reported, as per uniform consensus, that a single Ct cut-off value of 35 with a good sigmoidal real-time RT-PCR curve is acceptable. Thus:

- All patients with a Ct value of less than or equal to 35 may be considered positive, while those with a Ct value of more than 35 may be considered negative.
- All samples of with a Ct value of less than or equal to 35 with a poor sigmoidal curve should be retested.

5.7.4.2 ICMR Advisory on Correlation of COVID-19 disease severity with Ct values[28]

The ICMR Advisory on the correlation of COVID-19 disease severity with Ct values is outlined below. In view of this, it is not recommended to rely on numerical Ct values for determining the infectiousness of COVID-19 patients or deciding patient management protocols.

- There are no reliable studies to definitively prove a direct correlation between disease severity / infectiousness and Ct values. Viral load does not have much role in patient management.
- Ct values differ from one kit to the other. Comparability of Ct values among different kits is a challenge as the labs are using a mixed basket of kits now with different Ct cut-offs and different gene targets.
- Ct values also depend on how the sample has been collected. A poorly collected sample may reflect inappropriate Ct values.
- Ct values are also determined by technical competence of the person performing the test, calibration of equipment and pipettes and analytical skills of the interpreters.
- Ct values between nasal and oropharyngeal specimens collected from the same individual may differ.
- Temperature of transportation as well as time taken from collection to receipt in the lab can also adversely impact Ct values.
- Samples from asymptomatic/mild cases show Ct values similar to those who develop severe disease.
- Patients in early symptomatic stage may show a high Ct value which may subsequently change. In such cases, high Ct values will give a false sense of security.
- Severity of COVID-19 disease largely depends on host factors besides the viral load. Some patients with low viral load may land up in very severe disease due to triggering of the immunological responses. Hence, again high Ct value may give a false sense of security

- Moreover, the RT-PCR test presently being conducted is qualitative in nature. Ct values may give a rough estimate of viral load. However, more specialized standards are required for quantitative assays which are currently unavailable for SARS-CoV-2.

5.8 Quality control for RT-PCR

5.8.1 Internal quality control procedures

There are several internal quality control procedures that must be followed for RT-PCR, as outlined below.

- Positive and negative test controls must be included to accurately interpret patient test results.
- A positive control (provided in the kit) must be included in every RT-PCR run as a means of monitoring the reaction setup and reagent integrity.
- A negative control (nuclease-free water)must be included to monitor for any cross-contamination during RNA extraction and reaction set up.
- Known positive and known negative samples with varying Ct values must be included in every batch in the RT-PCR step as a part of the internal quality control procedures.
- A known positive sample and a known negative sample must be included:
    - Each time a new lot of kit is used.
    - Each time a new operator performs the test (i.e. an operator who has not performed the test recently).
    - When problems (storage, operator, instrument or other) are suspected or identified.
    - If otherwise required by the laboratory's standard QC procedures.

5.8.2 External quality assurance (EQA)

The laboratory should participate in a formal proficiency testing programme organized by an accredited proficiency training provider/EQA provider, by signing a formal memorandum of understanding (MOU). As an alternative approach, a laboratory can participate in an interlaboratory comparison (ILC) programme if EQA is not available.

ICMR has mapped COVID-19 testing laboratories in every state as the designated QC laboratory.[31] As part of inter-laboratory quality control (ILQC), once every 6 months all laboratories must send five random positive and five random negative samples to a QC laboratory as part of the ILQC protocol. Further details on quality control procedures can be found in chapter 7.

5.9 Data retention policy

RT-PCR laboratories must have data retention policies in place:[32 33]

- Laboratories should retain a record of all test results for five years, either electronically or as hard copies.
- If data are maintained electronically, data should be backed up on a regular basis and, if possible, stored in a separate location from the original data.
- All data recording should be checked by the laboratory supervisor for correctness and completeness, and each entry should be checked for accuracy of transcription.
- All data storage must be password protected with limited access to protect the patients' information.

5.10 Reagents, consumables and equipment

5.10.1 Reagents

There are some important factors to consider in relation to reagents used for RT-PCR:

- Care should be taken to ensure that all reagents are maintained contamination-free.
- Details of the reagents are listed in the SOPs for RNA extraction and RT-PCR
- All reagents should be clearly labelled with the name, expiration date and relevant safety information.
- All reagents must be stored at the appropriate temperature as instructed by the kit manufacturer.
- The components may vary slightly depending on the kit used and the storage conditions. The appropriate storage conditions for reagents from some manufacturers, for both RNA extraction and RT-PCR, are provided here for reference.

5.10.2 RNA extraction kit components and storage conditions

Manual extraction

The reagents for RNA extraction include those mentioned in Table 4, with slight variations in the labelling of the lysis and other buffers as per the manufacturer.

Table 4: Storage details for reagents used in manual viral RNA extraction

Kit component	Storage conditions	Shelf-life	Other details, if any
Silica spin columns	Dry, at room temperature (15-25°C)	When stored correctly, the components are stable for 12 months until the expiration date printed on the kit box lid	-
Lysis buffer	Dry, at room temperature (15–25°C)	When stored correctly, the components are stable for 12 months until the expiration date printed on the kit box lid	-
Wash buffer concentrates	Room temperature	Stable for 1 year when stored closed at room temperature	-
Isopropyl alcohol or ethanol	Dry, at room temperature (15–25°C)	When stored correctly, the components are stable for 12 months until the expiration date printed on the kit box lid	-
Elution buffer	Dry, at room temperature (15–25°C)	When stored correctly, the components are stable for 12 months until the expiration date printed on the kit box lid	-
Carrier RNA, lyophilized Form	Transported/stored at room temperature for up to 72 hours	Stable for one year from the date of manufacturing when stored at -20°C	Carrier RNA in solubilized form must be stored at -20°C for one year

Automated extraction

The kit components for use with automated RNA extractors usually come as two components based on the storage conditions. Table 5 shows the storage conditions for these components.

Table 5: Storage details for reagents used in with automated viral RNA extraction

Kit component	Storage conditions	Shelf-life
Extraction reagent I	2–8°C for 12 months, protected from direct sunlight and moisture	Can be stored at room temperature for 60 days once opened
Extraction reagent II		
Proteinase K		
Elution buffer		
Magnetic-bead solution		

5.10.3 RT-PCR kit components and storage conditions

Table 6 shows the storage conditions required for RT-PCR kit components from various manufacturers.

Table 6: RT-PCR kit components and storage conditions[34]

Product	Storage
Abbott Realtime SARS-CoV-2 (**Abbott Molecular Inc**)	–15°C to –25°C
RT-PCR kit for detecting 2019-nCoV Kit (**BGI**)	–18°C or lower
Xpert® Xpress SARS-CoV-2 (**Cepheid AB**)	2°C to 28°C
cobas SARS-CoV-2 Qualitative assay (**Roche Molecular Systems, Inc**)	2°C to 8°C
TaqPath Covid-19 Combo Kit (**Thermo Fisher**)	–10°C to –30°C

Some control components may require different storage conditions. These individual components' storage requirements should reviewed as well as the expiration dates on arrival for appropriate storage.

Table 7 shows the storage conditions needed for the CoviPath™ COVID-19 RT-PCR Kit, 200 reactions (Cat. No. A50780).

Table 7: RT-PCR kit components and storage conditions[30]

Component	Quantity	Volume per tube	Unit part number	Storage	Shelf life[1]
CoviPath™ COVID-19 Assay Multiplex (ORF1ab, N gene, RNase P gene)	2 tubes	150 µL	100099244	–30°C to –20°C	12 months
CoviPath™ COVID-19 Control	1 tube	10 µL (2×10^3 copies/µL)	100099245	–30°C to –20°C	12 months
CoviPath™ 1-Step Multiplex Master Mix (No ROX™)	2 tubes	625 µL	100099246	–30°C to –20°C	12 months

[1] The shelf life of the kit is determined by the component with the shortest shelf life.

Additional points to note are as follows:

- Avoid repeated freeze–thaw cycles of unpacked kits (not more than four times).
- RT-PCR kit for detecting SARS-CoV-2 should be transported at -18°C in the dark.
- The opening date, expiration date and the initials of the individual concerned must be present on each reagent that is opened for use.
- Reagents from different lot numbers should not be interchanged without prior functional verification.
- Any reagents from new lots should be tested to ensure that they work properly, by running a PCR positive control using the new reagents. If the PCR positive control fails, the new lots should be tested against the old lots.
- Logbooks should be maintained for all reagents and kits and should document all pertinent information needed to identify possible sources of contamination.

5.10.4 Consumables

Various consumables or disposable materials are used in PCR analysis, including pipette tips, Eppendorf tubes, PCR tubes, PCR plates, and gloves. To reduce contamination and the degradation of target nucleic acids, disposable materials should meet the following standards.

- Special tips that should be used for PCR analysis include barrier tips and aerosol resistant tips, both of which minimize cross-contamination of samples during pipetting.
- These tips can be purchased pre-sterilized and pre-loaded in hinged racks to provide tip protection and easy access.
- Pipette tips for PCR analyses should be lot-certified and RNase-, DNase- and pyrogen-free.
- Laboratories should use polypropylene tubes that are lot-certified DNase-, RNase- and pyrogen-free.
- Disposable, powder-free gloves should be available in each section of the laboratory that is used for PCR analysis.

5.10.5 Equipment

The equipment required for RT-PCR is listed in the SOPs for RNA extraction and RT-PCR.

- Focussed efforts are required to maintain the quality of the following equipment:[35]
    - Biosafety cabinets/laminar flow cabinets
    - RT-PCR machines
    - Centrifuges
    - Pipettes
    - Temperature-dependent instruments: refrigerators/freezers
- User-logs and temperature-logs should be maintained for equipment where applicable.
- All equipment should be calibrated by an NABL-accredited calibration laboratory.
- All RT-PCR machines should be calibrated as part of a regular maintenance regimen and prior to using any new dyes for the first time following the manufacturer's instructions.

5.11 Human resources and training

For human resources in a COVID-19 molecular laboratory, the minimum staff requirements (for 8-hour shifts/day) are outlined below.[36]

- Medical microbiologist: one or more, with experience of working in molecular virology
- Laboratory technician: at least two to three per shift, with relevant experience of working in molecular virology
- Multitasking staff: one or more per shift for washing/cleaning

It should be noted, however, that the total staff requirements will be based on the number of shifts and the sample load of the laboratory. Desired expertise the staff should possess includes:

- A good understanding of laboratory biosafety and biosecurity
- Trained for handling respiratory samples for viral diagnosis, RNA extraction and RT-PCR
- Experience of work in virology and handling clinical specimens, especially respiratory samples

Sample registration and data entry

Dedicated staff for sample registration and data entry should be allocated as appropriate for the testing capacity of the laboratory.

Housekeeping staff

Separate housekeeping staff must be allocated to take care of the clean and dirty areas of the laboratory.

Training

Training regarding biosafety practices should be frequent and stringent for all laboratory staff involved in the use of RT-PCR open systems.

Further details on staffing and training requirements are provided in chapter 3.

5.12 Waste management

This is a brief overview of the waste management procedures associated with the use of RT-PCR open systems. Detailed guidelines on waste management procedures can be found in chapter 3.

- All technical procedures must be performed in a way that minimizes the generation of aerosols and droplets.
- Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be loaded and unloaded in a BSC.
- All materials transported within and between rooms should be placed in secondary packaging, to minimize the potential for a breakage or spill.
- Specimens being removed from the BSC should be surface decontaminated.
- Wastes must be segregated at the point of generation.
- Appropriate methods for decontamination of waste must be available close to the laboratory.

5.13 Dos and don'ts in a PCR laboratory

- Use separate compartment spaces for each step in the PCR process.
- Always use separate consumables and equipment for each step (RNA extraction, master mix preparation, and amplification)
- Avoid any sources of RNase contamination.
- Use RNase-free tubes and tips.
- It is advised to label all instruments, such as pipettes, with the name of the laboratory the instrument is being used in. This will avoid confusion and the

possibility of exchanging pipettes between laboratories, thus reducing the chances of contamination.

- Remember to always keep reagents and components capped whenever possible.
- Correct pipetting technique can minimize contamination between samples that can lead to false-positive results.
- Change gloves often, especially if it is suspected they have become soiled with solutions containing template RNA.
- Always disinfect all instruments, materials and the environment with the correct disinfection methods.
- Use low- or zero-retention filtered tips to avoid cross-contamination.
- Tubes containing stored samples and reagents should be centrifuged briefly before opening to ensure that all liquids are at the bottom of the tubes.
- Always wear a full-length, long-sleeved laboratory coat and other appropriate PPE.

Annexure 10: Master-mix calculation worksheet (96 reaction well plate)

Date: Kit Lot No: Expiry date:

Components	**Volume required per reaction**	**Volume required for N reaction**	
Multiplex Master Mix	6.25 µL	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		

Done by: Verified By:

Annexure 11: Amplification worksheet

Master mix prepared by:_____Template addition 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:			

PCR run by:_____ PCR Checked by: _____

Annexure 12: Letter from ICMR describing cut-off points for Ct values

सत्यमेव जयते

प्रोफेसर (डा.) बलराम भार्गव, पदम श्री
एमडी, डीएम, एफआरसीपी (जी.), एफआरसीपी (ई.), एफएसीसी,
एफएएचए, एफएएमएस, एफएनएएस, एफएएससी, एफ.एन.ए., डी.एस.सी.
सचिव, भारत सरकार
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं
महानिदेशक, आई सी एम आर

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Director-General, ICMR

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Department of Health Research
Ministry of Health & Family Welfare
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D.O.No. VIR/4/2021/ECD-I
Dated: 5th April 2021

Dear Dr Vyas

This is with reference to your letter dated 1st April 2021 regarding CT (cycle threshold) value for RTPCR test for COVID-19.

2. Globally, the accepted cut-off for Ct value for COVID-19 ranges from 35-40 depending upon the instructions laid down by individual manufacturers.

3. However, ICMR has taken inputs from different virology laboratories across the country to arrive at a single Ct value cut-off based on individual laboratory experiences. As per uniform consensus, a Ct value cut-off of 35 with a good sigmoidal real-time RTPCR curve is acceptable. All patients with a Ct value ≤ 35 may be considered as positive while those with Ct value > 35 may be considered as negative. All samples with Ct value ≤ 35 with poor sigmoidal curves should be essentially re-tested.

4. Implementing a Ct value cut-off of 24 is not at all advisable as this will lead to missing of several infectious patients and increased disease transmission.

With best regards

Yours sincerely

Balram Bhargava

(Balram Bhargava)

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Chapter 6

Detection of SARS-CoV-2 using CBNAAT – GeneXpert system

Chapter 6: Detection of SARS-CoV-2 using CBNAAT – GeneXpert System

6.1 Introduction

A closed test system is a system that is designed to be fully integrated and automated to purify, concentrate, amplify, detect and identify targeted nucleic acid sequences. This type of modular system generates test results directly from unprocessed samples without requiring manipulation or handling by the user. The system does not pose a risk for cross-contamination because amplicon-containing tubes and compartments remain completely closed during and after the testing process.[1]

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 by fully integrating and automating the three processes required for real-time (RT)-PCR-based molecular testing (that is, specimen preparation, amplification and detection).[2]

The closed nature of this platform and minimum sample-handling required pose minimal biosafety hazards. Multiple samples can be tested in a single run, depending on the GeneXpert system model used. The systems are simple and robust enough to be introduced and used outside of conventional laboratory settings. The GeneXpert system performs automated specimen processing, RNA extraction, RT-PCR of SARS-CoV-2 RNA, and amplicon detection in a single run with a short turnaround time of 45 minutes.[2 3] The system comprises the instrument, a barcode scanner, a personal computer and preloaded software. The single-use disposable cartridges contain lyophilized reagents, buffers and washes. Target detection and characterization is performed in real-time using a six-colour laser-detection device.

6.2 Intended use of CBNAAT

The Xpert Xpress SARS-CoV-2 CBNAAT is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab and/or nasal wash/aspirate specimens collected from individuals with suspected COVID-19 infection.[4]

6.3 ICMR Advisory on CBNAAT for COVID-19 diagnosis

- CBNAAT platforms such as Cepheid Xpert Xpress SARS-CoV-2, which employ RT-PCR technology, are in use for COVID-19 testing in India.
- As per the ICMR Advisory,[5] 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.

- Cepheid Xpert Xpress SARS-CoV-2 has been approved by the US FDA for use under an emergency-use authorization (EUA).[6 7]
- 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. 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.[7]
- All private laboratories that intend to initiate CBNAAT-based testing for COVID-19 should be encouraged to immediately apply for NABL accreditation.[8] 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.[7]

6.4 Advantages of the GeneXpert system

The GeneXpert system offers several advantages,[2 9-11] as outlined below.

- The GeneXpert system provides testing on a single platform.
- The system is scalable and provides full flexibility, with faster results. Xpert® Xpress SARS-CoV-2 tests on a system(s) comprising eight or more modules meets the definition of a high-throughput technology capable of running more than 200 specimens per day. The GeneXpert® Infinity System is a fully automated system with robotic handling of samples. These systems can process up to 2074 test results in 24 hours.
- The cartridges are self-contained, hence cross-contamination among samples is minimized.
- GeneXpert systems are easy to use and have minimal training requirements.
- GeneXpert instruments can also be interfaced with most laboratory information systems and the delivery of results can be achieved via an SMS message or other means of delivering the results.

6.5 Specimen collection, storage and transport

Nasopharyngeal swabs, nasal swabs and nasal wash/aspirate specimens can be stored in viral transport medium or saline, at room temperature (15–30°C) for up to 8 hours and refrigerated (2–8°C) for up to 7 days until testing is performed on a GeneXpert Instrument System.[12] Proper

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

6.6 Principles of the GeneXpert system

The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for the qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on a GeneXpert Instrument System. GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and use RT-PCR assays to detect target sequences in simple or complex samples.

These systems require single-use disposable cartridges that contain the RT-PCR reagents and host the RT-PCR process. 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. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument.

6.6.1 Assay design

The Xpert Xpress SARS-CoV-2 test contains primers, probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper-respiratory specimens. This test targets/detects the E gene and also the SARS-CoV-2 specific N2 region of the N gene.[6]

6.6.2 Interpretation of results

The results for CBNAAT are interpreted as positive, presumptive positive, or negative (Table 1).[6 13]

- A positive result is indicative of active infection with SARS-CoV-2, when either both the N2 and E genes of the SARS-CoV-2 nucleic acid target are detected or just the N2 gene is detected and the E gene is not detected. 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. The agent detected may not be the definite cause of disease.
- A negative result is declared when both the N2 and E genes are not detected. It does not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. A negative result must be considered with clinical observations, patient history, and epidemiological information.

- A presumptive positive result indicates that SARS-CoV-2 nucleic acid may be present. Only one of the SARS-CoV-2 nucleic acid targets is detected (the E gene), while the other SARS-CoV-2 nucleic acid target (the N2 gene) is not detected.

Possible reasons to repeat an assay:

- A presumptive positive result.
- An instrument error result, which could be due to, but not limited to, the maximum pressure limits being exceeded.
- A no result – repeat test indicates that insufficient data were collected. For example, the PCC failed or a power failure occurred.

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

6.6.3 Quality control (QC)

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.

Cartridge controls (internal 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. These internal controls should be checked when interpreting the results.

External controls

It is recommended that external controls are performed each time a new shipment or of the same lot of Xpert Xpress SARS-CoV-2 assay reagents is received; each time a new operator is performing the test (i.e. an operator who has not performed the test recently); when problems (e.g. with storage, the operator, an instrument, or other) are suspected or identified; or if otherwise required by an institution's standard QC procedures.

There are commercially available external controls, as shown in Table 2.

Table 2: Commercially available external controls[14]

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

6.6.4 External quality assurance (EQA)

EQA is a process that allows COVID-19 testing laboratories to assess their performance by comparing their results with results from other laboratories within their network (testing and reference laboratories) via panel testing and retesting. Various EQA methods or processes are in common use, as outlined below.[15]

Inter-laboratory comparison (ILC): ICMR has mapped COVID-19 testing laboratories in every state that are designated QC laboratories.[16] Laboratories should participate in an ILC programme when EQA is not available. A laboratory should integrate ILC samples into the routine workflow in a manner that follows, as much as possible, like handling of patient samples. These ILC samples should be examined by personnel who routinely examine patient samples, using the same procedure as those used for patient samples.

All testing laboratories should liaise with the recommended QC laboratories and must ensure regular participation in QC activity.

Proficiency testing: All COVID-19 testing laboratories should participate in proficiency testing every three months (quarterly). Laboratories should select proficiency testing providers with a track record in delivering proficiency testing panels within their region.

6.6.5 Limitations of the test system

There are some limitations of the Xpert Xpress SARS-CoV-2 system that should be noted.[12]

- 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.

6.6.6 Performance characteristics

The performance characteristics of the Xpert Xpress SARS-CoV-2 are as follows.

- Analytical sensitivity (limit of detection, LoD): verification of the estimated LoD claim was performed on one reagent lot in replicates of 22, prepared in nasopharyngeal swab clinical matrix. The claimed LoD for the assay is 0.0100 plaque-forming units (PFU)/mL.[12]
- Analytical reactivity (inclusivity): The inclusivity of the Xpert Xpress SARS-CoV-2 test was evaluated using in silico analysis of the assay primers and probes in relation to 324 SARS-CoV-2 sequences available in the GISAID gene database[17] for two targets, E and N2. 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): an in silico analysis for possible cross-reactions with 39 organisms was conducted by individually mapping primers and probes used in the Xpert Xpress SARS-CoV-2 test to sequences downloaded from the GISAID database. 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. For the list of organisms please see the Xpert Xpress SARS-CoV-2 test instructions for use.

6.7 GeneXpert system models

There are various GeneXpert system models with different capacities in terms of the number of modules. The entire GeneXpert family of instruments is based on the same fundamental principle, and each individual module functions in the same way, regardless of instrument cabinet size.[3] Each module processes one specimen at a time. A specimen is loaded into the cartridge, the cartridge is loaded into a module, and the specimen is processed. For all

instruments included in the GeneXpert family, specimen processing includes fully automated nucleic acid extraction, amplification, amplified probe detection, and result reporting. The GeneXpert Dx software is installed on a computer supplied by the company and can accommodate a variety of applications. The system is also equipped with a barcode scanner, which facilitates data entry in the system.

The range of GeneXpert instruments includes systems with 1, 2, 4, 16, 48 or 80 modules. The modules function independently so that batching is not required and individual tests can be started at different times. The total time to results is about 45 minutes for any Xpert Xpress SARS-CoV-2 test. Allowing for an additional 5 minutes to unload and re-load a cartridge (total 50 minutes), each module is capable of running 28 Xpert Xpress SARS-CoV-2 tests per 24-hour day. For sites that initially expect low throughput but are unsure whether this will increase later, a GeneXpert IV can be ordered with fewer modules, leaving the remaining bays empty. This allows for the possibility of increasing the throughput later by installing additional modules, which can be ordered separately from the manufacturer. If additional instruments are required in a laboratory to increase throughput, instruments may be spliced together with a cable to allow data to be stored on a single computer.[2]

The various GeneXpert instruments available, along with the number of modules and throughput capacities, are shown in Table 3.

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.

6.8 Infrastructure requirements

The GeneXpert system requires limited infrastructure compared with other RT-PCR setups. The selection of a site will depend on the testing workload and the efficiency of referral

networks, and should take into consideration the infrastructure requirements, the human resources capacity and running costs. To meet infrastructure requirements and optimize the throughput of an instrument and running costs, machines are often placed at a level above the peripheral level, which requires establishment of reliable specimen- or patient-referral networks. Once the GeneXpert instrument is available, it does not require additional laboratory equipment, but the sophisticated nature of the device requires that certain conditions and infrastructure be present to ensure its efficient use. These considerations may limit where it can be positioned:

- The device needs a stable and continuous electrical supply to avoid interruptions to the procedure and the subsequent loss of results, waste of cartridges and possible damage to or failure of the modules.
- A BSL-2-level laboratory facility is required, including a molecular biology setup for virological diagnosis and a functioning and calibrated biosafety cabinet (BSC) type 2A/2B in the laboratory.

6.9 Laboratory layout and conditions

There are a number of recommendations for the layout of a laboratory and appropriate operating conditions for the GeneXpert instrument:

- There should be two rooms (or one room with a partition), for the BSC and for the GeneXpert machine, respectively. Additionally, it is important to have separate rooms for donning PPE, doffing PPE, autoclave(s) and a staff/reporting room.[2 7] For more details on the laboratory design and workflow processes, please refer to chapter 3.
- The manufacturer's recommended ambient operating temperature for the GeneXpert instrument is between 15°C and 30°C. The room where the test is performed may need air conditioning or heating to ensure that the ambient temperature is maintained in the recommended range. Ignoring the recommended temperature range may increase error rates, because extreme temperatures interfere with thermo-cycling during the test.[2]
- It is important to store all cartridges and the specimen reagent at 2–28°C or as per the manufacturer's recommendations. It has been stated by the manufacturer that the cartridges are stable if kept at 2 to 45°C for less than 6 weeks at 75% relative humidity. The cartridges are bulky when packed and require substantial storage space. An average household refrigerator can hold the supplies needed for 2 weeks in a laboratory performing 12 to 16 tests per day.[2]

6.10 Human resources requirements

The Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems; there are also other specific requirements for the Indian context.[12 18 19]

Availability of the following minimum staff is required (for 8 hour-shifts/day) for a CBNAAT laboratory:

- Medical microbiologists: one or more with experience of work in molecular virology.
- Technicians: Between four and six technicians (two to three per shift) with relevant experience of work in molecular virology.
- Multi-task staff: one or more for washing/cleaning.
- The staff requirements will change based on the number of shifts.

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.

6.11 Maintenance of equipment

Although the GeneXpert system is designed to prevent cross-contamination and ensure accurate results, the instrument should be checked and cleaned periodically as a precautionary measure.[20] 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.

6.12 Calibration of equipment

Calibration of the GeneXpert instrument is not required during the initial system setup. Cepheid performs all the necessary calibrations before the system is shipped. However, as recommended, the system should be checked for proper calibration on an annual basis. The system is designed to measure module performance with the internal assay controls. In the event of a module failure, the replacement module provided will have been calibrated prior to shipment.[21]

6.13 Verification of equipment

Each module in the GeneXpert instrument should be evaluated as being "fit for purpose" through verification with known positive or negative material prior to commencing routine testing of clinical specimens. A single verification test should be performed per module upon instrument installation and following calibration of instrument modules. Verification panels are now routinely distributed by Cepheid with each new instrument with recalibrated modules.[2]

6.14 Biomedical waste management

A robust institutional policy on the management of biomedical waste of human origin should be in place; there should also be well defined arrangements for the segregation and disposal of biomedical waste.[7 22 23] Proper waste disposal is important and should be followed meticulously every time an assay is performed. All biological specimens, transfer devices and used cartridges should be considered capable of transmitting infectious agents and require the use of standard precautions. These materials may exhibit characteristics of chemical hazardous waste, requiring specific disposal procedures. More details are provided in chapter 3, on Biosafety and Waste Management Protocols.

6.15 Monitoring and evaluation

Monitoring and evaluation of Xpert Xpress SARS-CoV-2 implementation is necessary to ensure the effective and efficient use of resources and to measure the impact in order to guide and justify further scale-up. Routine monitoring at the site level ensures that established diagnostic algorithms are being followed, detects whether a particular instrument module is functioning suboptimally and whether any users require additional training, and allows supplies to be effectively managed.

Site-level information should be shared with the supervising regional or national reference laboratory; this will allow the relevant laboratory to provide guidance on any actions that need to be undertaken to improve effectiveness, efficiency or user performance, and to strengthen the supply-management process to prevent stock-outs or cartridges from expiring, by exchanging cartridges among sites.

The key data that are recommended to be collected monthly or quarterly fall into three main categories:[2]

1) Monitoring the groups of patients tested and the test results.

2) Monitoring the operation of the GeneXpert platform and the performance of users. This should include:

- The number and types of errors. Identifying the most frequent types of errors can help troubleshoot the process, given that certain errors may be associated with the technique used to process specimens; other errors may be related to mechanical problems with the instrument's modules or other issues, such as the room temperature.
- The number of errors occurring by instrument module. If a particular module produces more errors over time compared with other modules, it may require repair.
- The number of errors occurring by user. If a particular user has an unusually high number of errors, further investigation of the specific types of error is warranted, as some errors may be caused by the technique used to process specimens.
- The number of tests lost due to power outages or surges.
- The number, duration and causes of routine interruptions in the testing service. Common causes of service interruptions include cartridge stock-outs, expired cartridges, no staff available, instrument breakdown and computer breakdown.
- The number of instrument modules not functioning and the duration (in days) of module failure during the reporting period.
- The number of instrument modules overdue for calibration at the end of the reporting period.

3) Monitoring supply management:

- The number of cartridges in stock at the beginning of the reporting period.
- The number of cartridges received during the reporting period.
- The number of cartridges used during the reporting period.
- The number of cartridges that were lost or damaged during the reporting period.
- The number of cartridges in stock at the end of the reporting period.
- Whether there were any stock-outs during the reporting period and, if so, the duration of the stockout (in days).
- The number of cartridges that expired before being used.

Chapter 6 References

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Chapter 7
Detection of SARS-CoV-2 using Truenat

Chapter 7: Detection of SARS-CoV-2 using Truenat

7.1 Introduction

SARS-CoV-2 is the causative agent of the novel coronavirus disease, COVID-19, in humans. Early and correct identification of SARS-CoV-2 infection is important for effective isolation, treatment and case management of COVID-19. In line with World Health Organization (WHO) recommendations, molecular diagnostics are currently the method of choice for SARS-CoV-2 virus detection and differentiation. However, molecular tests have so far been restricted to centralised reference laboratories, as they require skilled staff and elaborate infrastructure. The introduction of rapid nucleic acid amplification tests (NAATs) has accelerated COVID-19 diagnosis in India. One such indigenously developed, chip-based, real-time PCR testing system is Truenat, which has been developed by MolBio Diagnostics Pvt. Ltd. Technical data for this system can be found in the various product guides produced by the company.[1-3] It is a rapid, simple, robust and user-friendly test that can be used as a semi-quantitative viral detection method, with a very short test duration of 1 hour. This is achieved through a combination of portable, lightweight, battery-operated, real-time micro-PCR analyzers, a universal cartridge-based sample preparation device and room temperature-stable micro-PCR chips and kits. Hence, this technology offers "sample to result" capability, even in resource-limited settings such as point-of-care (POC) testing sites.

7.2 ICMR Advisory on Truenat for COVID-19 diagnosis

As per the ICMR Advisory on Strategy for COVID-19 Testing in India,[4] Truenat is considered to be the first choice of test on a par with RT-PCR for routine surveillance in hospital settings and in non-containment zones. For routine surveillance in containment zones and screening at points of entry, a rapid antigen test is the first choice of test and RT-PCR/Truenat/cartridge-based NAATs (CBNAATs) are the second choice. For further details on the testing strategy, please refer to chapter 1.

A single Truenat-positive test is to be considered confirmatory, with no repeat testing required. No re-testing is recommended prior to discharge from a COVID-19 facility following clinical recovery, including for transfer from a COVID area/facility to a non-COVID area/facility. No further RT-PCR-based confirmation is required for samples that are confirmed to be "true positives" by a Truenat assay. All positive and negative results must be reported through the ICMR portal on a real-time basis.

ICMR has recommended Truenat's two-step singleplex assay, comprising screening (E gene) and confirmatory (RdRP gene) assays, as well as the multiplex assay, comprising screening (E gene) and confirmatory (Orf1a gene) assays.[5] This fully indigenous diagnostic platform offers a reliable and affordable option to augment SARS-CoV-2 testing capacity in India. The

platform comprises a Truenat machine, in-built RNA extraction system, RT-PCR chips, collection swabs and viral lysis medium (VLM). A single assay has a turnaround time of 35 to 50 minutes for 1 to 4 samples, with a possible total of 12 to 48 samples being tested per day, depending upon the type of machine used. The biosafety and biosecurity requirements are minimal by virtue of the samples being collected in VLM, which inactivates the virus. The test can be used at a variety of healthcare levels, including district hospital and primary health centre levels.[6] Testing of nasopharyngeal swabs and oropharyngeal specimens using the Truenat system 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. All laboratories who have applied can reach out to ICMR with a copy of their NABL application, and ICMR will provide expedited approval for laboratories performing Truenat-based testing, subject to NABL approval,[7] which can be submitted within a maximum time span of four weeks from the date of approval.

7.3 Advantages of the Truenat system

The Truenat system offers several advantages, as outlined below.

- The Truenat system enables decentralisation and near-patient diagnosis and monitoring and is useful even in resource-limited settings.[8] Additionally:
    - It is portable, lightweight, battery-operated and fully automated
    - The technology is rapid, simple, easy to use, robust and user-friendly
    - The equipment is a "laboratory in a suitcase" and can be used in remote areas where there is poor infrastructure, power supply or connectivity
    - The Truenat SARS-CoV-2 testing kit can be stored at room temperature (2–30°C)
- The Truenat system has considerably lower biosafety requirements than other NAAT-based methods and is thus suitable for POC use.[9]
    - The VLM in which the sample is collected and transported inactivates the virus, minimizing the risk of infection and posing a minimal biosafety hazard
    - Safety is further augmented by the closed nature of the Truenat platform and minimal sample handling, further minimising biosafety and biosecurity requirements
- The device has an automated reporting system and is GPRS/Bluetooth enabled, to aid in data transfer
- Using the Truelab micro PCR printer, results can be printed for record-keeping
    - Test results are automatically stored and can be retrieved at any time

- Results can be printed using a Truelab micro PCR printer or transferred via a WiFi network or 3G/GPRS network to a laboratory computer or any remote computer

7.3.1 Intended use of Truenat testing

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.

7.3.2 Target genes for Truenat testing

Three target genes of SARS-CoV-2 are used in Truenat testing, namely the envelope protein (*E)* gene, the RNA-dependent RNA polymerase *(RdRp)* gene and the open reading frame 1a (*Orf1a)* gene. Detection of human RNase P serves as a full process internal positive control (IPC), which ensures proper swab collection, nucleic acid extraction and PCR.

7.4 Types of Truenat COVID-19 assays

Currently, there are three different types of Truenat assays available for COVID-19 diagnosis:

- A two-step singleplex assay, comprising assay 1 and assay 2:
  - Assay 1 (Truenat Beta CoV *E* gene screening assay*) is an *E* gene-screening assay. All samples with suspected COVID-19 should first be tested with this 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) is an *RdRp* gene confirmatory assay. All samples that test positive by this assay must be considered to be "true positives".

*As per the revised ICMR guidelines,[5] Assay 1 must be followed by Assay 2 for confirmation of the results.

- A multiplex assay comprising assay 3:
  - Assay 3 is a single assay comprising both the screening (*E* gene) and confirmatory (*Orf1a*) targets in a single test. All samples of suspected COVID-19 cases can also be tested using this assay. All negatives are to be considered as "true negatives". All samples that test positive by this assay must be considered to be "true positives".

7.5 Sample collection, storage and transportation for Truenat testing

Oropharyngeal or nasopharyngeal swab specimens must be collected as per standard procedures using standard nylon flocked swabs. Trueprep Transport Medium for Swab Specimen[10] is used as a medium for collection, decontamination and transport before pre-treatment using lysis buffer, followed by extraction and purification of nucleic acid. The transport medium for the swab specimen decontaminates the specimen and maintains the integrity of the nucleic acid, hence it can be used for the safe collection and transport of microorganisms from the site of collection to the Truenat testing laboratory. Specimens collected in the transport medium are stable for up to 3 days at 40°C and up to 1 week at 30°C. Details are provided under the chapter on sample collection, packaging, transport and storage.

7.6 Principle of Truenat testing

Truenat SARS-CoV-2 testing kits work on the same principle as real-time reverse-transcription polymerase chain reaction (RT-PCR),[11] based on Taqman chemistry. Viral RNA from a patient sample is first extracted using a Trueprep AUTO Universal Cartridge-based sample preparation device and preparation kit. The purified RNA is mixed with the PCR reagents containing the reverse transcriptase (RT) enzyme. This mixture is added to the reaction well of the Truenat chip, and the chip test is inserted into the Truelab RealTime Quantitative micro PCR Analyzer, where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. Ribonuclease P (RNase P), a human constitutive gene, is used as an internal control in every assay.

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.

A video about Truenat (sample collection and testing) can be found by following this link: https://youtu.be/5kJUw7gJlAQ

7.6.1 Truenat performance characteristics

For the two-step singleplex assay, the limit of detection (LoD) of Truenat Beta CoV *E* gene is 486 genome copies/mL, while for Truenat SARS-CoV-2 *RdRp* gene the LoD is 407 genome copies/mL. For the Truenat COVID-19 Multiplex assay, the LoDs for the E and Orf1A genes are 487 and 480 genome copies/mL, respectively.

A study conducted under the supervision of ICMR-NIV, Pune, India, concluded that all three types of Truenat assays showed concordant results in comparison with reference gold standard

RT-PCR. The sensitivity, specificity, positive predictive values and negative predictive values were shown to be 100%.[11]

Another study conducted in Lucknow, India, which involved 1807 patient samples, showed that in comparison with RT-PCR, Truenat had a sensitivity of 69.5%, a specificity of 90.9%, negative predictive value of 97.2% and diagnostic accuracy of 89.2%.[8]

7.7 Truenat workstation and accessories

7.7.1 Truenat workstation

The Truenat workstation consists of a nucleic acid extraction device (Trueprep AUTO v2), a real-time PCR analyzer, a printer, and various accessories, including RNA cartridges, Truenat chips, fixed volume (6 µL) precision micropipettes, and a micro-tip holding stand. The Truelab workstation includes a sample preparation kit, an RNA extraction system, an RT-PCR machine, and disposable kit components (Figures 1 and 2). The Truenat SARS-CoV-2 testing kits are disposable, room temperature-stable, chip-based real-time PCR tests with dried $MgCl_2$ in the reaction well and freeze-dried RT-PCR reagents in the microtube for performing real-time RT-PCR tests for viral infection. The test is performed on the portable Truelab Real-Time micro PCR analyzer. A volume of 6 µL of purified RNA is required to perform the PCR test. The intelligent chip also carries test- and batch-related information and captures information about used tests to prevent any accidental re-use of the chip.

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

Figure 2: Truenat devices

7.7.2 Trueprep AUTO Universal Cartridge-based sample preparation device

This device is a fully automatic sample preparation device that works in tandem with Trueprep AUTO cartridges and Trueprep AUTO reagent kits for the extraction and purification of nucleic acids from a clinical specimen.

The number and models of Trueprep sample preparation devices and Truelab micro PCR Analyzers should be selected based on the site-level demand. All reagents and consumables required for the test procedures are provided by the manufacturer, with the exception of personal protective equipment (PPE), a timer, and hypochlorite-based disinfectant.[12]

7.7.3 Truelab Real-time Quantitative micro PCR analyzer

The Truelab Analyzer is a fully automatic device and is available with one (Uno), two (Duo) or four (Quattro) chip ports,[13], which allow for independent testing of one, two and four samples per run, respectively (Table 1). Up to 20 000 results can be stored on the analyzer for future recall and reference.

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

7.7.4 Truelab micro PCR printer

The printer connects via Bluetooth and can wirelessly print the results of PCR tests performed by any of the Truelab Real-time Quantitative micro PCR Analyzers.

7.7.5 Trueprep AUTO Cartridges

These are disposable plastic cartridges for the automated extraction of nucleic acid from clinical specimens.

7.7.6 Truenat Chips

These chips are target-specific, ready-to-use and disposable micro PCR chips that run on any of the Truelab Real-time Quantitative micro PCR Analyzers, to obtain semi-quantitative real-time PCR results.

7.8 Interpretation of results

The amplification curves are displayed on the Truelab real-time micro PCR Analyzer screen, to indicate the progress of the test. 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 time taken identified by the cycle threshold (Ct) of the specimen will depend on the number of virus copies in the sample. 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. In general, in RT-PCR technologies, Ct values less than or equal to 35 are considered to be positive, however in Truenat the cut off is 32.

While IPC will co-amplify in most positive cases, 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. Tables 2 and 3 provide guidance on the interpretation of the test results.[2 14 15]

Table 2: Truenat COVID-19 test results based on Ct values

E gene	Orf1A	
<32 Positive	Negative	Presumptive Positive
>32 Above target cutoff	Negative	Negative
Negative	<32 Positive	Positive
Negative	>32 Above target cutoff	Negative
<32 Positive	<32 Positive	Positive
>32 Above target cutoff	<32 Positive	Positive
<32 Positive	>32 Above target cutoff	Presumptive Positive
>32 Above target cutoff	>32 Above target cutoff	Negative
Negative	Negative	Negative

Table 3: Truenat COVID-19 multiplex assay result interpretation

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

7.9 Uploading results to the ICMR portal

ICMR requires all laboratories to enter data via the ICMR portal (https://cvstatus.icmr.gov.in/login.php) to help in guiding national strategies.[9] Further details on uploading data to the ICMR portal can be found in the chapter on Sample Collection, packaging, transport and storage.

7.10 Truenat quality control and quality assurance

7.10.1 Internal process control

Ribonuclease P (RNase P), a human constitutive gene, is used as an IPC in all test chips. Known positive and negative controls (Truenat Universal Control kit) are run alongside samples from time to time. It is advisable to run these controls under the following circumstances:[2]

- Whenever a new shipment of test kits is received, even if they have the same lot number
- 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

7.10.2 External quality assurance (EQA)

There is no NABL-approved EQA programme for COVID-19 testing in India. Therefore, an 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.

7.11 Infrastructure requirements

The infrastructure requirements for Truenat testing are minimal. One room with a partition, or two rooms – one for RNA extraction and one for the Truenat PCR analyzer – are required to perform Truenat testing.[12] 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

Further details on laboratory design are provided under the chapter on workflow process in a molecular testing laboratory.

Due to the portability of this testing platform, Truenat can be utilized in peripheral healthcare settings and mobile laboratories. Truenat devices have in-built batteries that can run without external power for up to 8 hours, hence the Truenat system can be used on any flat, stable surface at an ambient temperature (between 15°C and 40°C).

7.12 Storage of kit and reagents

Truenat kits are stable for up to 2 years from the date of manufacture if stored between 2°C and 30°C. They are also stable for up to 1 month at temperatures of up to 45°C. Care should be taken not to expose the kits to light or elevated temperatures (above recommended levels), and these kits should not be stored frozen.

Trueprep AUTO Universal Sample Pre-Treatment Pack and Trueprep AUTO Transport Medium for Swab Specimen Pack are stable for up to 2 years from the date of manufacture if stored between 2°C and 40°C. They are also stable for up to 1 month at temperatures of up to 45°C.

7.13 Maintenance of the Truenat system

The Truenat system can be used in a laboratory or as a POC test. The maintenance 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 must be cleaned before and after use and in case of spills (appropriate spill management procedures must be followed as per standard protocols.) Daily, monthly and need-based maintenance should be performed and documented as per the Truenat Preventive Maintenance Log (please refer SOP for Truenat testing). The fixed volume (6 µL) pipettes must be replaced by the company every 6 months, free of charge.

7.14 Human resource requirements

Availability of the following minimum staff[16] is required (for 8-hour shifts/day):

- Medical microbiologists: one or more with experience of work in molecular virology
- Technicians: at least four to six (two to three per shift) with relevant experience in molecular virology
- Multitasking staff: one or more for washing/cleaning

These staff requirements will vary based on the number of shifts.

Desired expertise of the staff:

- Good understanding of laboratory biosafety and biosecurity
- 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

7.15 Truenat device errors

Truenat devices exhibit specific “errors” or “alerts” on the display, for various reasons, as outlined below.

Trueprep Auto v2 Universal Cartridge-based sample preparation device errors

Trueprep Auto sample preparation device errors may be related to thick specimens (that have not liquified properly), the cartridge not being detected, problems with the reset card/QR code reader, device heating plates not working, a damaged cartridge valve or pressure drop errors.[17]

TruelabReal-Time Quantitative micro PCR Analyzer errors

- The Truelab micro PCR Analyzer can show error messages due to an 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.[18]
- The Truelab micro PCR Analyzer also shows alert messages when it is unable to read the chip information or establish an internal connection, or if the chip used has expired or has already been used.

7.16 Biosafety and biomedical waste management

The Truenat system has minimal biosafety requirements and is thus suitable for POC testing. The standard procedures for handling biological material should be followed, including the use of protective clothing and gloves.[19] A robust institutional policy on the management of biomedical waste of human origin should be in place, and there should be well defined arrangements for the segregation and discarding of biomedical waste.

The biological specimens, chips, transfer devices, cartridges and gloves used for Truenat testing should be disposed of immediately following the run, as per standard biowaste disposal procedures. A regular laboratory cleaning protocol should be maintained to prevent the occurrence of contamination.[20] 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. For biosafety considerations, including spill management, the details are provided under the chapter on Biosafety and waste management protocols.

7.17 Limitations of the Truenat assay

The Truenat assay does have some limitations to be aware of, and these are described below.

- The optimal performance of Truenat testing 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.
- A specimen for which the Truenat assay reports “not detected” cannot be concluded to be negative for SARS-CoV-2. As with any diagnostic test, results from the Truenat assay should be interpreted in the context of other clinical and laboratory findings.

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Chapter 8
Quality management in COVID-19 testing

Chapter 8: Quality management in COVID-19 testing

8.1 Introduction to quality management

To ensure that laboratories provide accurate and reliable results and to reduce the risk of errors, it is essential to implement a quality management system (QMS).[1] In a quality management system, all aspects of the laboratory operations, including the organizational structure, processes and procedures, need to be addressed to assure quality (Figure 1).

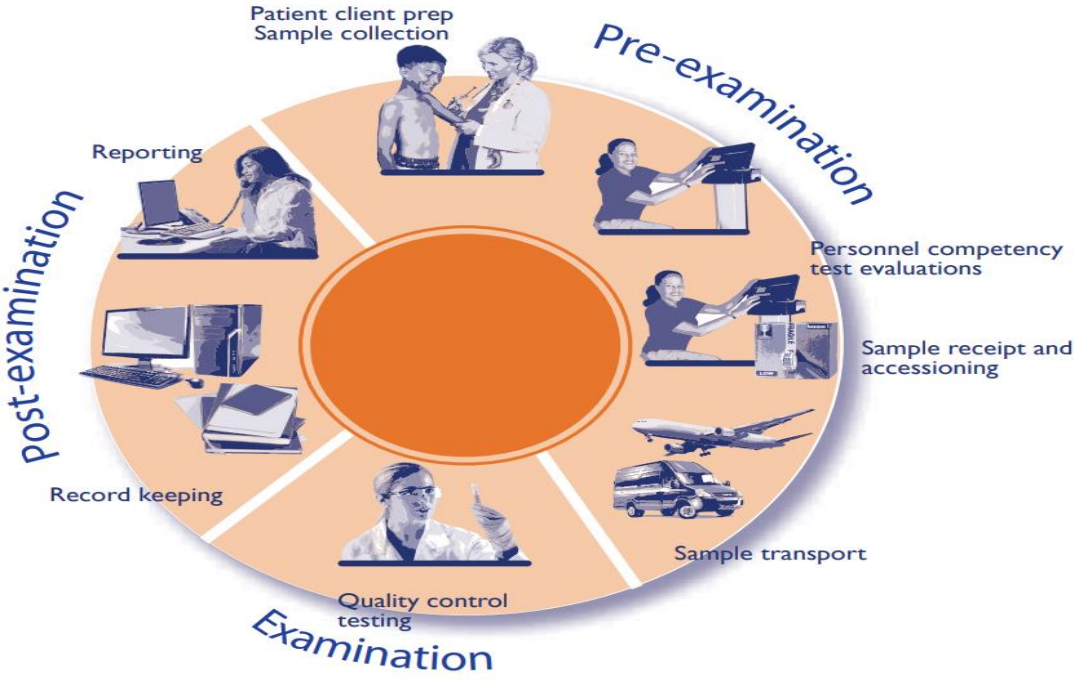

Figure 1: Laboratory procedures and processes

8.2 Quality management system model

The quality management system model is a way to ensure that all laboratory procedures and processes are organized into an understandable and workable structure. In a quality management model, there are 12 quality system essentials that must be addressed (Figure 2). Laboratories that do not implement a good quality management system may not guarantee an error-free laboratory, whereas having a good quality management system can yield a high-quality laboratory that detects any errors and prevents them from recurring.[2]

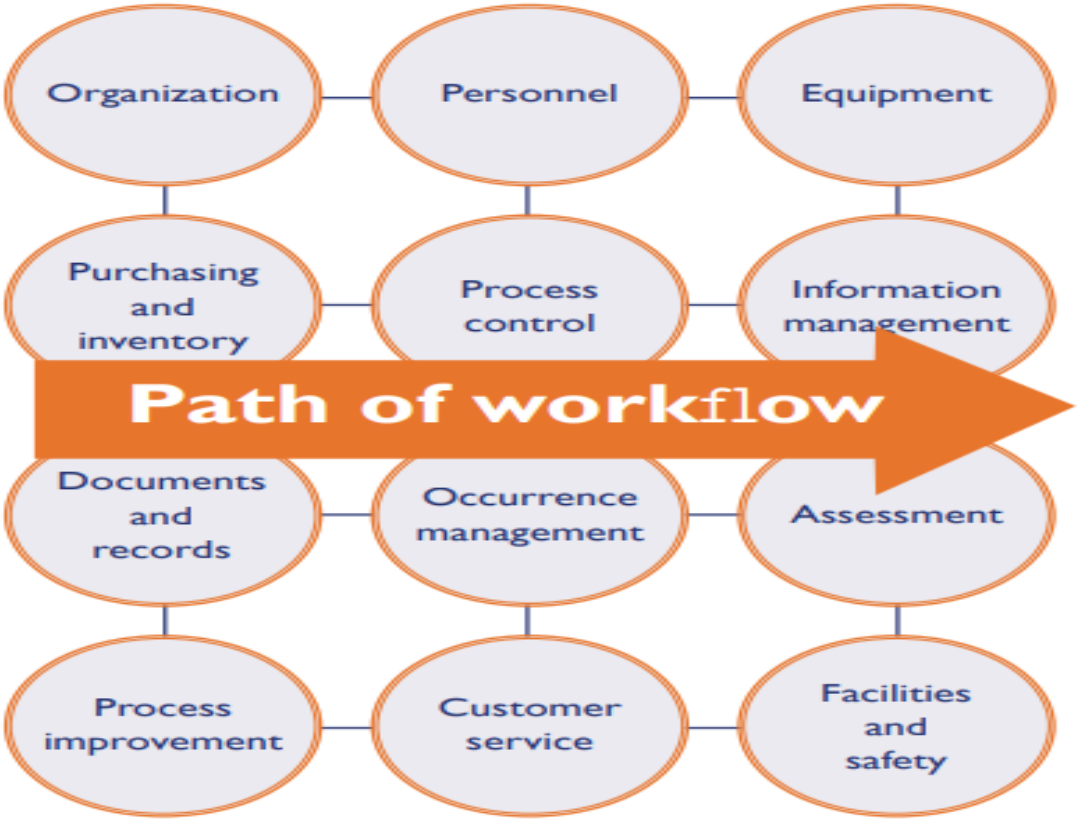

Figure 2: Overview of the quality management system model

8.3 Quality assurance

Quality assurance (QA) refers to the overall process implemented by a laboratory that aims to ensure that the final results reported are as accurate and reliable as possible. QA is a system designed to continuously improve the reliability and efficiency of laboratory testing services.[1]

The main elements of QA include:

- Standard operating procedures (SOPs) should be available, and staff should be trained in their use. SOPs should cover all procedures, from managing incoming specimens to authorizing and issuing test reports.
- Documentation: all laboratory forms and registers should be standardized and staff trained to fill out all documentation consistently and fully.

- Quality control (QC), which refers to procedures used in each assay to assure that a test run is valid and the results are reliable.
- Quality indicator monitoring, which refers to the collection and analysis of data that can serve as indicators for the correct performance of the entire testing process.
- External quality assurance (EQA), which aims to analyze the accuracy of the entire testing process from receipt of sample to testing of sample to reporting of results.

8.4 Quality control

QC is part of the quality management system and is used to monitor the examination (analytic) phase of testing. The goal of QC is to detect, evaluate and correct any errors due to test system failure, environmental conditions or operator performance, before patient results are reported.[2]

Important aspects of QC procedures are outlined below.[1]

- QC can involve monitoring of an entire test system or just a single aspect of a test.
- QC processes are a means of systematic internal monitoring of the performance of bench work in testing laboratories, including instrument checks and verifying new lots of test kits.
- QC procedures can be used to validate the competency of testing laboratories by assessing sample quality and monitoring test procedures, test kits, and instruments against established criteria.
- QC includes the review of test results and documentation of the validity of testing methods.

8.5 Internal quality control

Internal quality control (IQC) is a key aspect of QC, with the general important points to note outlined below. Please also refer to the relevant QC sections in relation to RT-PCR, CBNAAT and TrueNat (chapters 5, 6 and 7, respectively, for further details of relevant QC and SOPs).

- IQC should be performed routinely as per the recommendation of the test manufacturer for a particular assay.
- Built-in controls are those that are integrated into the design of a test system, such as a test kit device. Most built-in controls monitor only a portion of the examination phase, and they vary from one test to another as to what is being monitored. Although these built-in controls give some degree of confidence, they do not monitor for all conditions that could affect test results. It is advisable to periodically test traditional control materials that mimic patient samples, for added confidence in the accuracy and reliability of test results. Examples of test kits with built-in controls are rapid tests that

detect the presence of antigens or antibodies and the TrueNat and CBNAAT closed RT_PCR systems.

- IQCs must be included with each batch of clinical specimens. If the results of the controls are not within the acceptance criteria, the supervisor must be informed to review and troubleshoot the issue.
- A COVID-19 molecular testing laboratory must ensure that the extraction and amplification processes for PCR are properly checked for quality by using IQC in each test run. IQCs that are commonly employed for PCR testing are listed below.

8.5.1 Internal controls

A potential source of false-negative results could stem from insufficient sample collected or sample extracted. To mitigate this, an internal control (IC) is used to ensure that the process has proceeded correctly for each sample. The amplification of targets and ICs takes place simultaneously in the same reaction, with the ICs added at the RNA extraction step. There are two different approaches that can be used in RT-PCR assay design for ICs: endogenous and exogenous controls.[3-6]

8.5.2 Endogenous internal controls

Endogenous internal controls comprise a normal cellular gene sequence, which is expected to be present in all specimens, and can be used as an endogenous IC. This type of IC uses housekeeping genes (beta-actin or RNase P) to report the presence of genetic material from the sample. This approach has the advantage of monitoring the integrity of the nucleic acid target; in improperly collected, stored or processed specimens, the endogenous target will be absent (or degraded) and fail to yield a positive result. A disadvantage is that endogenous sequences may not accurately reflect amplification of the primary target due to differences in the primer sequences, size of the amplified product, and the relative amounts of the two targets. This type of control is not placed in a designated well but instead is present in every sample well.

8.5.3 Exogenous internal controls

Exogenous internal controls are a little more complex and comprise synthetic ICs that are used as a proxy for the primary target, which overcomes the inherent limitations of an endogenous IC. They usually contain non-infectious cultured human cell (A549) material or a plasmid DNA or in vitro RNA transcript with primer-binding regions identical to those of the target sequence. They involve adding an exogenous source of encapsulated RNA to each sample prior to extraction.

8.5.4 Extraction negative control

- Extraction negative controls consist of RNA that is extracted from a negative sample plus water.
- They indicate whether contamination was introduced during the extraction phase.
- These controls are extracted along with the clinical specimens and are included in the RT-PCR process at the template addition step.

8.5.5 No template control (NTC)

- An NTC contains nuclease-free water.
- It is used to check whether there was any contamination during specimen extraction and/or plate set-up. If any NTC reactions are defined as positive, sample contamination may have occurred, and the test must be repeated with strict adherence to the testing procedures.
- An NTC also indicates whether PCR reagents have been compromised when determining the cycle threshold.
- An NTC is added at the template addition step during the RT-PCR process.

8.5.6 Positive template control(s)

- A positive template control is also referred to as a positive control.
- Positive template controls are in vitro-transcribed SARS-CoV-2 RNA, either gene fragment or whole-genome. This control must be handled with caution in a dedicated nucleic acid handling area to prevent possible cross-contamination.
- A positive template control indicates the limit of detection and robustness of an assay.
- A positive control is added during the template addition step of the RT-PCR process.

8.5.7 Procedure in the event of an IQC failure

- A failure of any one of these controls (for instance, if the positive control turns out to be negative) invalidates the test result and the assay must be repeated, either from stored or newly collected sample after investigating and fixing the cause of the failure (e.g. contamination or degradation of the sample, or expired reagents).[17]
- No patient results must be released if IQC does not satisfy the acceptance criteria.
- Once troubleshooting has been performed and any necessary corrective action taken, when samples are re-run, the last two samples from the previous run should be included to ensure the continuity of valid results.
- In cases where patient results have already been issued, they should be re-called immediately (giving an explanation of the reason), and the patient should be retested as a priority.

Several third-party commercial companies supply full process controls for the extraction and amplification steps of SARS-CoV-2 testing. Some of the current providers include those listed below, with prices ranging from 50 to 550 US dollars for 100 tests.

- ZeptoMetrix
- SeraCare
- European Virus Archive – Global
- BIO-RAD

Commercial QCs are preferred but absence of commercial controls, laboratories can use the substitutes outlined below.

- Negative control: water/universal transport media/viral transport media
- Positive control: a patient sample with a known and preferably low Ct value of between 25 and 30

8.6 External quality assessment

External quality assessment (EQA) is a process that allows laboratories, including COVID-19 testing laboratories, to assess their performance by comparing their results with results from other laboratories within their network (testing and reference laboratories) via panel testing and retesting.

One or more of the following three EQA methods can be applied for COVID-19 molecular testing laboratories, namely rechecking or retesting, proficiency testing, and on-site evaluation.

8.6.1 Rechecking or retesting

With rechecking or retesting, samples that have been tested at one laboratory are retested at another laboratory, allowing for inter-laboratory comparison (ILC). Important points to note are outlined below.

- When EQA is not available, a laboratory must participate in an ILC programme with another NABL-accredited laboratory at least once every six months.
- CBNAAT/TrueNat COVID-19 testing laboratories may arrange their own inter-laboratory quality control (ILQC).
- Laboratories must integrate ILC samples into their routine workflow in a manner that follows, as much as possible, the way patient samples are handled.
- ILC samples must be examined by the same personnel who routinely examine patient samples, using the same procedures as those used for patient samples.

ICMR guidelines on ILQC testing for all Covid-19 laboratories using open-system RT-PCR

ICMR ILQC activity began in September 2020. Initially it was scheduled to take place quarterly; this was changed to bi-annually with effect from 1 March 2021. ICMR has mapped COVID-19 testing laboratories in each state and has designated QC laboratories. Figure 3 shows the structure for ILQC.

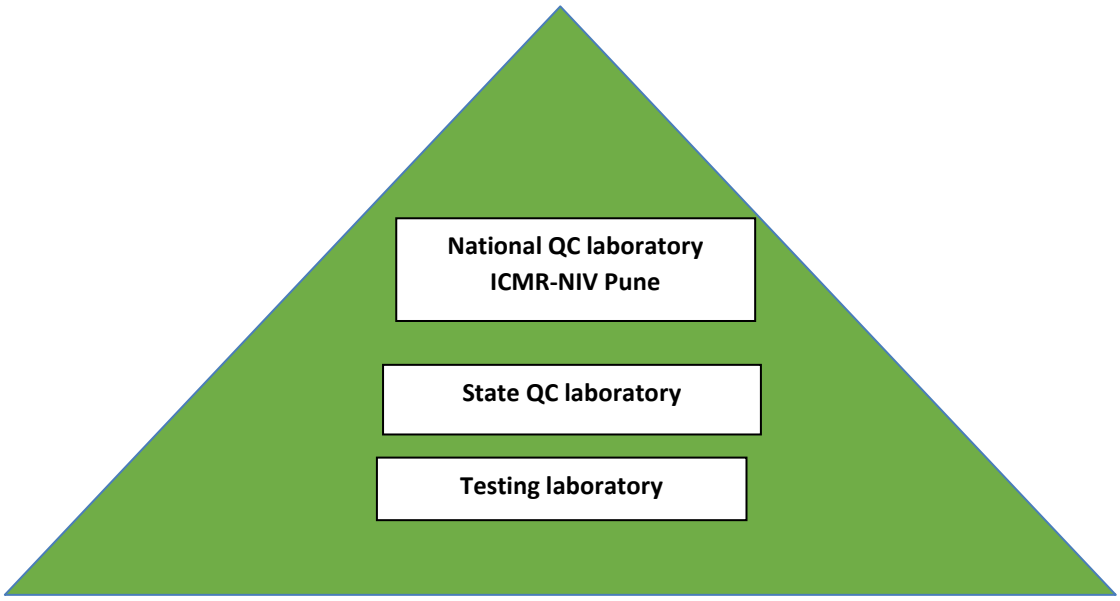

Figure 3: The structure of inter-laboratory quality control

As part of ILQC, all laboratories that use open-system RT-PCR must send five random positive and five random negative samples, once every 6 months, to the designated QC labs as part of the ILQC protocol. To facilitate the entire ILQC process, ICMR has launched an online portal.[8] A summary of the procedure is outlined below.[9 10]

- Five negative samples randomly collected over 1 week.
- Five positive samples randomly collected over 1 week (preferably with Ct values between 25 and 35).
- All testing laboratories should liaise with the recommended QC laboratories and must ensure regular participation in QC activity.
- All testing laboratories must ensure proper storage of samples at -80°C.
- Proper labelling of all samples as per the instructions given in the ICMR QC portal must be followed.
- For shipping, all samples must be aliquoted in screw-cap vials and proper biosafety and biosecurity precautions should be followed as per International Air Transport Association (IATA) guidelines.

- All QC results must be entered by the individual laboratories only via the ICMR QC portal and should not be shared with the QC laboratory. Laboratories can log in with the username and password that they already use for patient data entry. Laboratories are required to enter the results of the SARS-CoV-2 target genes along with the results of housekeeping genes.
- A passing score for ILQC is 90% concordance. If a score is ≥90%, re-participation in the same 6-monthly ILQC cycle is not required.
- If testing laboratories fail the ILQC exercise (by scoring less than 90% concordance), they must participate again by sending a further five samples (three positive and two negative samples), after an interval of 31 days, to the QC laboratory during the same 6-month period of ILQC.
- Positive and negative samples must be sent by the testing laboratory and the testing laboratory must enter all relevant entries via the portal. Without entry via the portal, samples will not be accepted by the QC laboratory. No results of submitted samples are sent to the QC laboratory.
- The testing laboratory should ensure that the samples for QC reach the assigned laboratory.
- If less than 10 samples are sent during the first attempt of the 6-monthly ILQC cycle, the exercise will be considered null and void and testing laboratories will need to re-participate within the same 6-monthly cycle by sending a further 10 samples (as per the sample characteristics mentioned earlier).
- Discordant results will be issued if:
    - Sample results are not uploaded via the portal (positive as well as negative samples).
    - Housekeeping genes and Ct values are not entered.
    - Ct values for positive samples are not uploaded via the portal.
    - Positive samples have Ct values <25, (irrespective of the results generated by the QC laboratory).
- If Ct values of SARS-CoV-2 target genes for all five positive samples are <25 in the first attempt in the 6-monthly ILQC cycle, the exercise will be considered null and void and testing laboratories will need to re-participate within the same 6-monthly cycle by sending a further 10 samples.
- Only the results of the SARS-CoV-2 target genes that are detected by the PCR kit should be entered via the portal, along with results of housekeeping genes (genes not detected by the PCR kit in use should not be labelled as negative).
- Once the QC laboratory receives the samples from the testing laboratory, the QC laboratory must be sure to enter the date of receipt of samples from the testing laboratory. The laboratory assigned the responsibility for QC must run the samples and enter the results via the portal.

- Results (as percentage concordance) will be sent automatically to the laboratory concerned via the portal.
- No results will be sent from QC laboratories to testing laboratories.

8.6.2 Proficiency testing

The details of the proficiency testing process are outlined below.

- An external proficiency testing (PT) provider will send a set of simulated SARS-CoV-2-positive and -negative clinical samples for testing in various laboratories, and the results from all of the laboratories will be analyzed, compared and reported back to the participating laboratories. [1]
- The positive panels should contain different genetic lineages of SARS-CoV-2.
- To assess the quality of COVID-19 laboratories in India, ICMR was provided with PT panels by the World Health Organization (WHO) – India, through the Royal College of Pathologists of Australasia Quality Assurance Programs, Australia. The main objective of proficiency testing is to provide independent demonstrations of laboratory competence. A total of 779 laboratories participated in the proficiency testing, which included 410 government-operated laboratories and 369 privately-operated laboratories. Of these laboratories, 739 (almost 95%) achieved passing scores in the proficiency testing panel.[11]
- Some examples of proficiency testing panels for COVID-19 and their providers include the following:
    - QCMD, https://www.randox.com/coronavirus-qcmd/
    - INSTAND, (https://www.instand-ev.de/en/instand-eqas/)
    - WHO Health, Emergencies and Global Influenza Programme (https://www.who.int/teams/global-influenza-programme/laboratory-network/eqa-project)
    - ECDC/EVD-LabNet/ERLI-Net, https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-influenza-lab-quality-control. Laboratories can enrol for free as part of the influenza laboratory network or at a cost not exceeding USD 420 but this may vary by country.
- Laboratories should choose providers experienced in delivering EQA panels within their region.

8.6.3 On-site evaluation

On-site evaluation is usually employed when it is difficult to conduct traditional proficiency testing or to use the rechecking/retesting method. On-site evaluations should be performed by

experienced subject-matter experts, who observe and assess the quality management systems of COVID-19 testing laboratories across the three testing phases.

On-site evaluation should be conducted at least annually, but preferably every three to six months. On-site evaluation includes:

- Patient management
- Biosafety adherence
- Quality control procedures
- Staff competency
- Sample collection procedures
- Standardized testing policies
- Documentation and maintenance of records

Periodic on-site evaluation may not be feasible during the COVID-19 pandemic. However, on-site evaluation should be conducted when selecting laboratories that will carry out COVID-19 testing. For the on-site evaluation of a laboratory, the WHO laboratory assessment tool can be used and the details for the same are provided in chapter 11 on audit preparedness.

8.7 Validation and verification of tests for COVID-19

Commercially available tests undergo rigorous performance evaluations by the manufacturer (validation). A laboratory then performs their own examinations (verification) and compares the data they obtain in their setting with the data provided by the manufacturer, thereby confirming if the performance claims made by the manufacturer (acceptance criteria) have been met (Table 1). If no performance data are available from the manufacturer or the test is performed using an in-house assay protocol, the laboratory should perform its own validation study.

Table 1: Validation and verification process for COVID-19 tests

	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

8.8 Definition of acceptance criteria for the performance measurements

The validation of any test must establish pertinent performance characteristics (e.g. accuracy, precision, sensitivity, limit of detection, etc.) that demonstrate or confirm a method is suitable for its intended purpose.

- Accuracy is the ability of a test to measure the real value of an analyte. It is usually reported as analytical sensitivity and specificity. It should be determined using a panel of positive and negative samples or spiked materials.
- Precision is the ability of a test to generate consistent results from replicates of the same sample.
- Three types of precision should be determined:
    - Inter-instrument reproducibility
    - Daily reproducibility
    - Operator reproducibility

Table 2 shows the analytical sensitivity and specificity of RT-PCR tests as recommended by ICMR.[12]

Table 2: Validation of SARS-CoV-2 diagnostic commodities: acceptance criteria

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).

ICMR has validated a variety of COVID-19 tests, including RT-PCR kits, CBNAAT and TrueNat kits (both two-step singleplex assays and multiplex assays). The COVID-19 RT-PCR diagnostic kits that have been validated by ICMR are provided in the links below:

RT_PCR_Tests_Kits_Evaluation_Summ_26022021 ICMR.pdf

https://www.icmr.gov.in/pdf/covid/kits/RT_PCR_Tests_Kits_Evaluation_Summ_20072021.pdf

https://www.icmr.gov.in/ckitevaluation.html

https://www.icmr.gov.in/pdf/covid/kits/Guidance_COVID_testing_commodities_18062021.pdf

8.9 Verification protocol by laboratories

ICMR validates RT-PCR kits and displays details of the validated kits on its website. It is mandatory for laboratories to use only those kits that have been approved by ICMR. Verification of RT-PCR kits is not mandated by ICMR. Laboratories may take advantage of the WHO recommendation of confirming the first 5 positive and first 10 negative specimens (collected from patients that fit the case definition) by referring them to one of the WHO reference laboratories that provides confirmatory testing for COVID-19.[13] It is also mandatory for a laboratory to have a robust QC system before reporting on each batch of samples Table 3 shows the processes used by laboratories to perform verification.

Table 3: Processes used by laboratories to perform verification

Verification parameter	Acceptance criteria
External controls (n=2) •one positive control •one negative control	Both results should agree with the respective positive and negative controls
Accuracy controls (n=20) •five undiluted positives • five diluted positives (where one volume of positive sample is to be diluted into 2 volumes of molecular grade water) • five negatives • five clinical samples	The undiluted and diluted positive samples should be positive for SARS-CoV-2 •Negative samples (for example AccuPlex reference material and patient samples) should be negative for SARS-CoV-2 •The clinical samples to be selected should consist of two with low Ct-values, two with high Ct-values and one sample that tested negative for COVID-19 o 100% of test results should agree with the expected results for the undiluted positive samples and negative samples o ≥80% (≥4/5) of test results should be in agreement with the expected results for the diluted positive samples
Reproducibility controls (n=4) •one positive and one negative sample for each staff member	All replicates of the positive samples should be 100% positive, and all replicates of the negative samples should be 100% negative

8.9.1 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. The frequency with which lot to lot verification should be performed is as follows; at least one known positive sample and one known negative sample must be included and tested each time:

- A new lot of a kit is received.

- A new operator performs the test (i.e. an operator who has not performed the test recently).
- 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.

8.9.2 Failed verification/validation

If the verification or validation test results do not meet the acceptance criteria:

- Consult the manufacturer's documentation for potential reasons why the test failed.
- Investigate potential sources of error, including:
    - mislabelling of controls and/or samples, or transcription errors in recording results.
    - testing personnel deviating from standard operating procedures.
    - environmental contamination.
- Omission or misinterpretation of initial external control material results. This can identify reagents that are not performing as expected and procedure errors.
- Test the discordant controls and samples in a third laboratory or compare with results obtained in another laboratory with the same batch of control samples to confirm whether the expected results are accurate.
- Correct any errors identified and retest discordant samples. Do not retest discordant samples until there is evidence that the source of error has been eliminated, for example QC material performing as expected.
- Re-analyze data:
    - if acceptance criteria are met, present the findings.
    - if acceptance criteria are still not met, continue to investigate and correct any sources of error.

8.9.3 Quality improvement

- Quality improvement (QI) is a process by which the components of laboratory testing services are analyzed to identify areas requiring improvement, to plan and undertake improvements, and to evaluate the effectiveness of improvements.
- QI is also recognized as process improvement and involves continuous monitoring, identification of defects, and remedial action, such as refresher training, to prevent recurrence of problems.
- Data collection, data analysis and creative problem-solving are the key components of this process. It may require data from audits, participation in EQA schemes, and on-site evaluation to improve testing processes.

- The ultimate target of QI is to take corrective action against the identified problem, remove its root cause, and reduce or eliminate its recurrence. Implementing preventive action reduces the likelihood of recurrence.

8.10 Corrective and preventive action

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.[14 15]

8.10.1 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

8.10.2 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.

Please refer to Annexures 13 and 14 for an example of a CAPA form and further elements of QC, respectively.

8.11 Key performance indicators

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.[1] KPIs should be analyzed 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)

Laboratories should also monitor the percentage of results reported within the target TAT, and the average and range of TAT.

The total TAT is the time between specimen collection and result reporting to the clinician. In addition to monitoring total TAT, the laboratory should also monitor the time from specimen collection to receipt at the laboratory and the time from receipt at laboratory to result reporting (within-laboratory TAT), to identify and address any bottlenecks at the various stages of the diagnostic process.

Annexure 13: 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.....

Annexure 14a: Elements of quality control - administrative

Quality Control	Recommendations
Workplace	• SARS-CoV-2 molecular testing should be performed in a secure, dedicated workspace • Manual PCR requires a three-room set-up (extraction, addition, and detection) • Laboratories should be organised to allow efficient testing workflow
Staff Competency	• Staff should have technical knowledge and skills appropriate for laboratory work • Staff should receive training on relevant technical and safety practices for SARS-CoV-2 molecular testing • Staff should take part in regular competency assessments, and if required, consider retraining. e.g., online course on COVID-19 diagnostics and testing *https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing*
Standard operating procedures (SOPs)	• Laboratory should have SOPs for COVID-19 molecular testing • SOPs should comply with current WHO recommendations or national guidelines • SOPs should be kept up to date and written exactly as practiced in the laboratory • SOPs and manuals should be located in the laboratory for easy access for all staff
Laboratory register	• All tests performed should be recorded in a standard format in the laboratory register • Use an approved register format in COVID-19 testing laboratories throughout the country network • Laboratory registers should be located in the laboratory work area at all times and stored in a secure location • Test results should be written directly into the register or electronic registry rather than transcribed from a worksheet

Data collection	• Laboratories should collect and analyze data monthly
	• Data should be collected on key performance indicators:
	  ○ Sample rejection rate
	  ○ Number of samples tested by sample category
	  ○ Number of positive, negative and invalid test results
	  ○ Turn-around time
	  ○ Number of failed IQC results
	  ○ EQA/PT performance (pass/fail or % score)

Equipment	• All laboratory equipment must be maintained in safe and working condition • Laboratory records should show supplier, date of purchase, serial number, and cost of each piece of equipment • Instrument manuals should be located near the equipment • Staff should be trained on the use and maintenance of PCR instruments • Equipment should be serviced as per the recommendation of the manufacturer, and service records should be kept in the laboratory

Quality Control	Recommendations
Supplies	• Procure diagnostic kits as per the WHO Emergency Use Assessment and Listing (*https://www.who.int/diagnostics_laboratory/eual/emergency/en/* and/or that have granted Emergency Use Authorization from national authorities • Prioritise diagnostic kits with high performance characteristics in independently evaluated data using a large sample size (e.g., *https://www.finddx.org/covid-19/pipeline/*) • Select suppliers that have local distributors or supply network in-country • Carefully consider ancillary items during forecasting and procurement (e.g., extraction buffers, sample collection materials, etc.)
Biosafety	• Initial sample processing (before inactivation) should take place in a validated biological safety cabinet (BSC) or primary containment device • Non-propagative tests (sequencing and Nucleic acid amplification tests should be conducted at a facility using procedures equivalent to Biosafety Level 2 (BSL-2) ○ For GeneXpert, non-propagative tests can be performed on a bench without using a BSC, when the risk assessment so dictates, and proper precautions are in place. This includes wearing of appropriate PPE and working in a well-ventilated area. • Laboratories should conduct risk assessment for intended testing and subsequently, based on the findings, decide on safety control measures to put in place (e.g., personal protective equipment)

Annexure 14b: Elements of quality control – sample management

Quality Control	Recommendations
Collection	• All samples collected from COVID-19 suspect patients should be considered as potentially infectious. Hence, biosafety must be emphasised when handling and collecting samples. • Use appropriate PPE (gloves, solid front or wrap-around gown, face masks or respirators, if available) • Test request forms should capture all required information from the person being tested, for proper handling, reporting, and clinical care. More info about developing a request form is available at: *https://extranet.who.int/lqsi/content/develop-request-form-laboratory-testing* • Upper respiratory samples (nasopharyngeal swab, oropharyngeal (throat) swab, or nasopharyngeal aspirate, or nasal wash) and lower respiratory samples (bronchoalveolar lavage, endotracheal aspirate, and expectorated sputum) are recommended for COVID-19 testing. **Please note that expectorated sputum is ONLY for patients with a productive cough. Sputum induction is NOT recommended.** • Use Dacron or polyester-flocked swabs for sample collection. Calcium alginate swabs and cotton swabs with wooden shafts are not recommended. • Label sample tubes with patient details, date and time of collection
Transport	• Laboratories that are unable to meet biosafety requirements should consider referring samples to reference laboratories • All samples should be stored at 2–8°C for up to 48 hours after collection. For handling or shipping after 48 hours, storage at -70°C is recommended. • Use viral transport medium (VTM), if a delay is unavoidable. VTM can be locally prepared; see US CDC protocol (*https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf*). Minimum essential media, sterile phosphate buffered saline, or 0.9% saline can be used as alternatives to VTM for SARS-CoV2 tests. • Samples should be transported as UN3373, 'Biological Substance Category B' using triple packaging (*https://www.un3373.com/category-biological-substances/category-b/*). If shipping is within national borders, comply with national regulations. For overnight shipment, use shipment in an ice pack (temp 2–8°C).
Laboratory	• Samples must be handled efficiently to ensure prompt and accurate reporting of results • Details of submitted samples should be recorded on the laboratory register or entered into an electronic laboratory information system before tests are carried out • Samples should be evaluated as per the acceptance criteria, such as leakage, inadequate sample volume, and sample integrity, and reasons recorded if samples are rejected

Annexure 14c: Elements of quality control – SARS-COV-2 molecular testing

Quality Control	Recommendations
Assay validation or verification	• Validation or verification should be conducted to ensure test performance for intended use as indicated by the manufacturer. However, under emergency conditions, validation and verification studies may be limited. • Five positive and 10 negative samples should be referred to WHO reference laboratories for confirmatory testing • Alternatively, new or less experienced laboratories should be mentored by reference or more experienced laboratories for their initial test results confirmation and performance improvement • Lot-to-lot verification should be conducted for a newly received lot or batch of test kits. Each lot should be tested using well-characterised samples to verify performance against existing lots in use.
Reagent	• Reagent reconstitution should be done in a PCR hood or BSC following the product insert and brought to the right temperature conditions before use (use cold blocks or ice) • Do not substitute or mix reagents from different kit lots or other manufacturers • Minimise freeze-thaw cycles • Maintain primer/probe integrity. After suspension and dilution, aliquot immediately into volume enough for one run. • Do not use expired reagents
Sample processing	• RNA extraction must be performed in a BSL-2 or equivalent facility • Sample must be allowed to thaw completely before use • Purulent or clotty sputum should be treated with dithiothreitol before aliquoting • Test tubes should be labeled with sample details to enable traceability. Always use a new aerosol-barrier or positive-displacement pipette tip for each sample.
Testing	• SARS-CoV-2 molecular testing procedures should be readily available (manuals, SOPs, job aids) • Testing should be performed as per the SOPs of the laboratory • Leftover samples should be stored serially at -70 OC for retesting by an EQA program
Interpretation	• Test result interpretation should follow the testing algorithm of the country or available guidance • Discordant results should be resolved by repeat testing on a newly collected sample and possibly by sequencing • Any unexpected result should be reported and related samples sent to WHO reference laboratories for confirmation *https://www.who.int/who-documents-detail/who-reference-laboratories-providing-confirmatory-testing-for-covid-19*
Reporting	• Test results should be reviewed independently by a laboratory supervisor to confirm accuracy before release. Independent review involves confirming patient details with the test result and validity test by control results.

Chapter 8 References

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Chapter 9
Troubleshooting COVID-19 testing

Chapter 9: Troubleshooting COVID-19 testing

9.1 Introduction

The total testing process in the laboratory is a cyclical process that is divided into three phases: pre-analytical, analytical and post-analytical.[1]

- The pre-analytical phase is the phase where the laboratory has no direct control over the process. Pre-analytical factors that can affect results include sample type, sampling time, sample handling, patient preparation and the nutritional status of the patient.
- The analytical phase involves the actual running of the test.
- The post-analytical phase includes recording the results, interpreting the results, reporting the results to the ordering physician, and filing the report.

9.2 Pre-analytical errors affecting the diagnosis of COVID-19

Pre-analytical errors include any errors that occur prior to analysis. More than 60% of errors occur in the pre-analytical phase of any diagnostic process, with relatively few analytical and post-analytical errors.[2] Figure 1 shows analytical factors to consider when conducting real-time reverse-transcript PCR (rRT-PCR).

The false-negative rate for RT-PCR COVID-19 tests has been reported to be as high as 41%, and there have been several reports of swab-negative patients who subsequently were found to be positive on repeat testing.[2 3] It is hypothesized that the high rate of false-negatives with SARS-CoV-2 testing may be associated with pre-analytical factors, including:

- Timing of swabbing - a swab should be taken at the time of symptom onset when the highest viral load occurs in COVID-19 infection. This will be logistically challenging for both patients and healthcare systems, but swabs taken at later time points may be falsely negative.
- Swabbing practices - swabbing is a complex task requiring training and competency assessment. It will be associated with anxiety due to the uncertain immune status of the healthcare professional in the context of a potentially lethal infection. These factors coupled with a lack of a standard swabbing practice may contribute to the high false-negative rate.
- RNA instability and laboratory practices - RNA instability may be an important cause of false-negative results in COVID-19 testing, compounded by low viral loads. Delays from time of sampling to extraction due to transport or processing issues may also exacerbate false negativity.

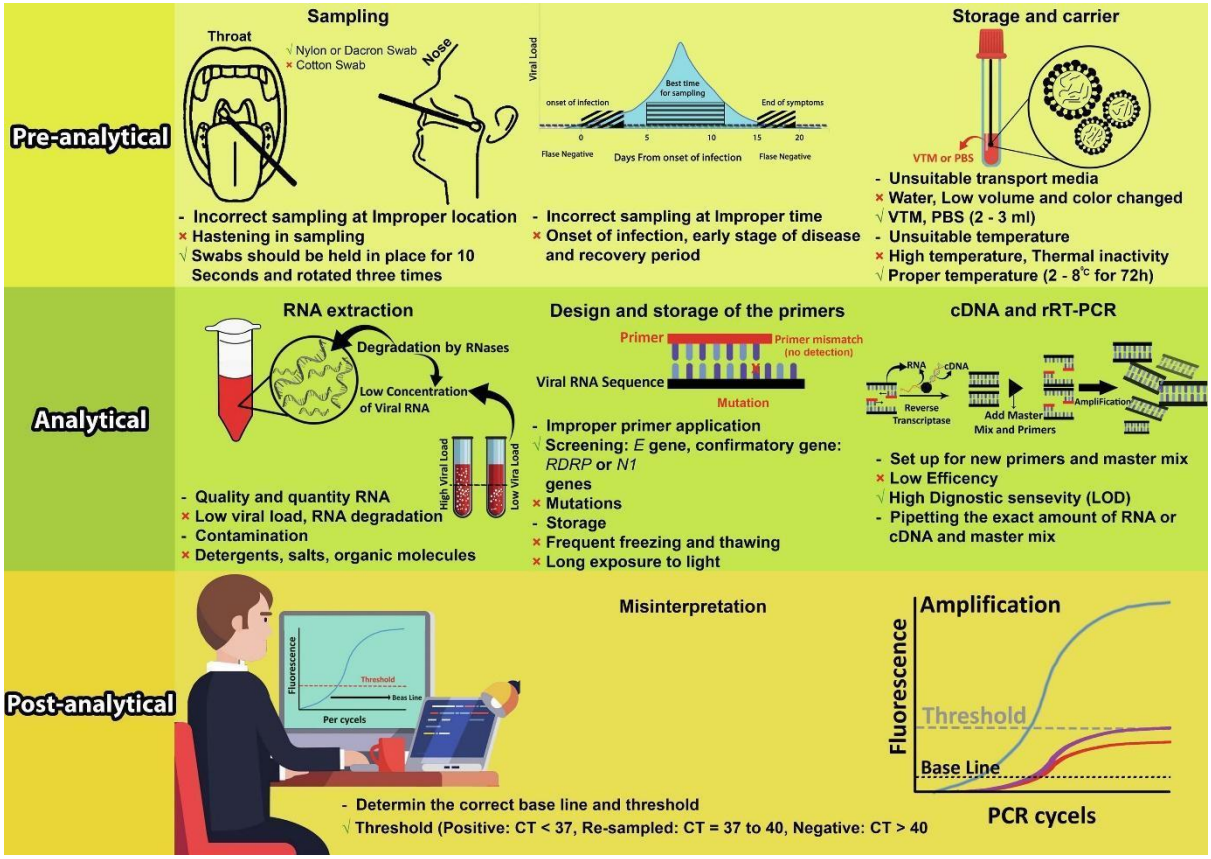

Figure 1: Analytical considerations when conducting rRT-PCR for SARS-CoV-2[4]

Other aspects of pre-analytical errors include:

- Lack of identification or misidentification of samples
- Inadequate procedures for specimen collection, handling, transport and storage
- Collection of inappropriate or inadequate material in terms of quality or volume
- Presence of interfering substances
- Manual (pipetting) errors
- Sample contamination

9.3 Analytical errors affecting the diagnosis of COVID-19

The analytical process involves the detection of SARS-CoV-2 by real-time RT-PCR, typically from nasopharyngeal swab samples. The analytical process comprises viral inactivation, extraction of nucleic acids, preparation of PCR mix, the reverse transcription reaction (RT), PCR (or amplification) and, finally, validation of results. The example used in this risk analysis is a PCR method comprising both manual and automated steps, with inactivated samples placed

in ready-to-use extraction plates and undergoing automated extraction. In the example configuration, preparation and distribution of the mix are carried out manually, and the patient samples are also manually distributed in PCR strips that are specifically designed for thermal cyclers. These files then require technical validation followed by biological validation before the diagnostic test report can be compiled and sent out. Any risk analysis is specific to the particular method implemented, but a certain number of risks are common to all comparable methods used to detect SARS-CoV-2 by means of PCR. Some common analytical errors that are observed are as follows:[5]

- Testing carried out outside of the diagnostic window
- Active viral recombination
- Use of inadequately validated assays
- Lack of harmonization of primers and probes
- Instrument malfunctioning
- Insufficient or inadequate material
- Non-specific PCR annealing
- Misinterpretation of expression profiles

9.4 Post-analytical errors affecting the diagnosis of COVID-19

Post-analytical processes concern the communication of the results. This final stage plays a role in the external quality of the result (i.e. the ability of the laboratory to provide a result that is compliant with the actual status of the patient: infected or not.)[5]

Post-analytical errors include:

- Failure to report test results
- Delay in reporting
- Incorrect calculation
- Critical results not reported or delayed
- Results sent to the wrong patient

9.5 Troubleshooting exercises for RT-PCR open systems

Successful real-time PCR should have the characteristics shown in Figure 2. Any deviation from this is an indication of an improper RT-PCR process. Figure 3 shows the various problems

that can be encountered during RT-PCR testing. Any of these problems can lead to incorrect reporting, i.e. either false-positive or false-negative reports.

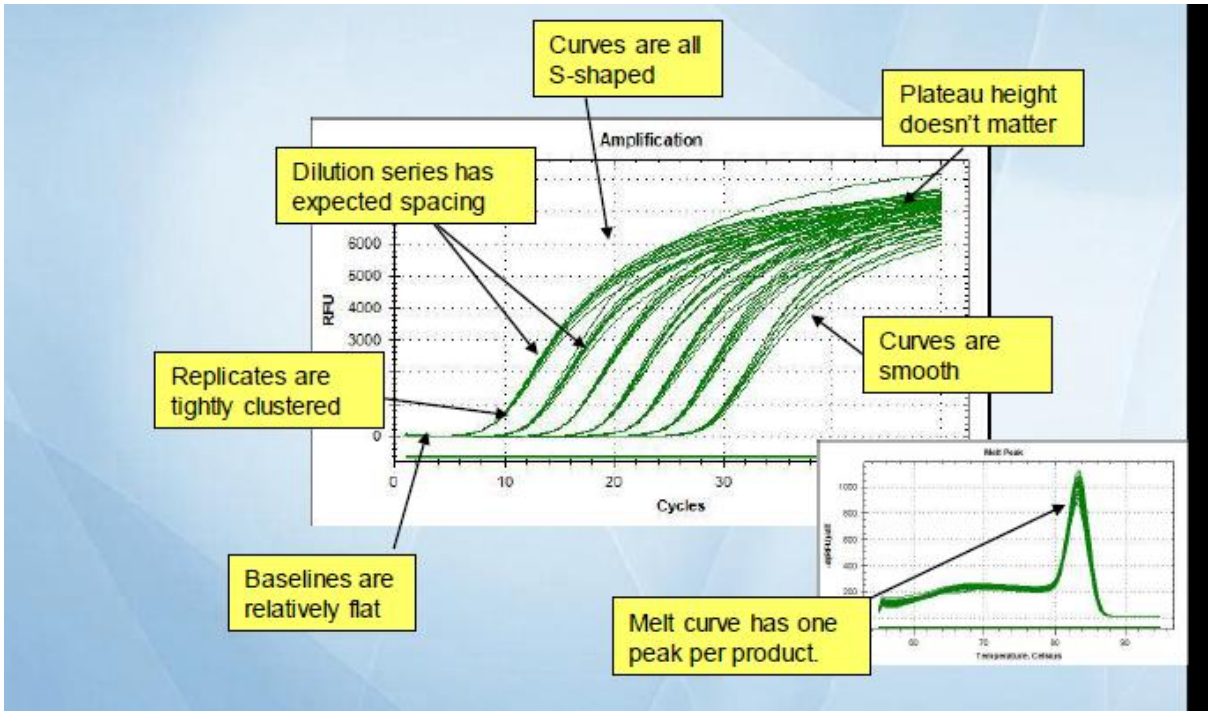

Figure 2: Characteristics of successful real-time PCR

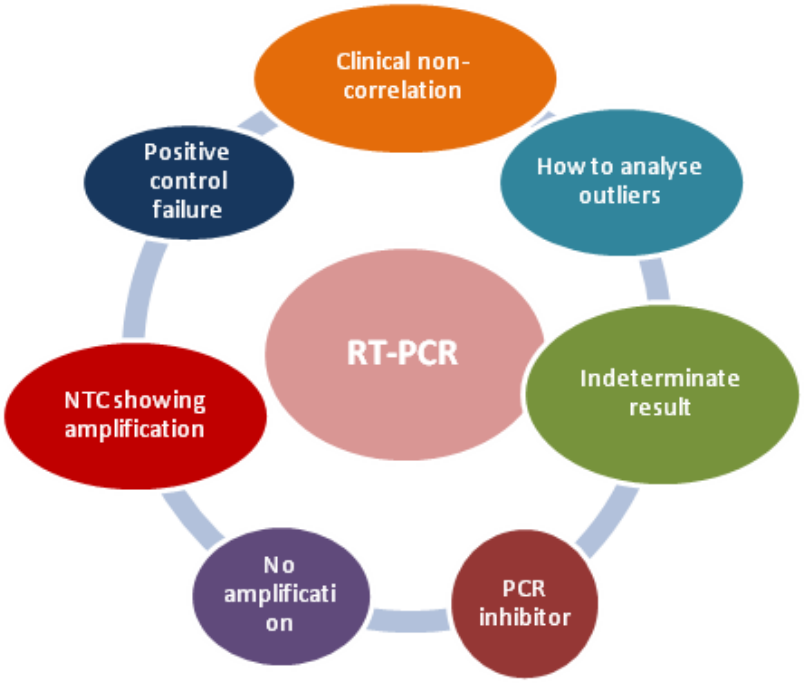

Figure 3: Problems that can be encountered during RT-PCR testing

Any problem must be identified, and a root cause analysis should be performed. Possible solutions should also be documented under corrective and preventive action (CAPA).

Some possible problems that may be encountered, and their solutions, are outlined below.

9.5.1 No amplification

No signal is detected in any of the fluorescence channels, as shown in Figure 4

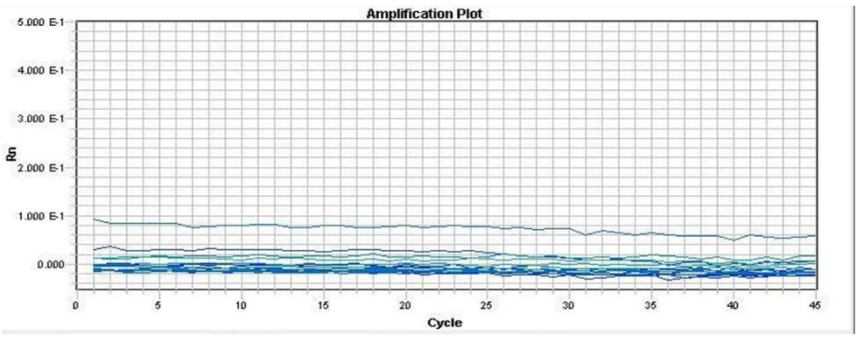

Figure 4: Flat curves due to no signal being detected in any of the fluorescence channels

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 conclusive results can be determined, and the assay must be repeated from the extract or fresh sample must be obtained.

No signal with positive controls in fluorescence channel FAM/VIC

Possible causes:

- Degradation of the positive controls
- Improper aliquoting or storage of the controls
- Improper dispensing of the required quantity of the controls
- Incorrect selection of fluorescence channel

- Incorrect temperature profile
- Incorrect configuration of PCR

Corrective action: use a new aliquot of positive control and repeat the test.

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

Possible causes

- PCR conditions did not comply with the protocol
- The PCR was inhibited

Corrective action: repeat the test.

9.5.2 Limitations and interfering substances (PCR inhibitors)

- The internal control is not amplified when added during the extraction phase of the RT-PCR process
- The specimen's internal control cycle threshold (Ct) value is >4 higher than that of the negative extraction control

Possible causes

- Presence of inhibitors in the specimen
- Residual alcohol from extraction

Corrective action: re-extract RNA and repeat PCR; if the problem recurs, collect a repeat sample and repeat the test.

9.5.3 Indeterminate result

Possible causes:

- No smooth sigmoidal curve
- Very high Ct values
- Discordance between screening and confirmation assay

Corrective action: repeat the test.

9.5.4 Contamination in the no template control (NTC)

NTC shows contamination, as illustrated in Figure 5.

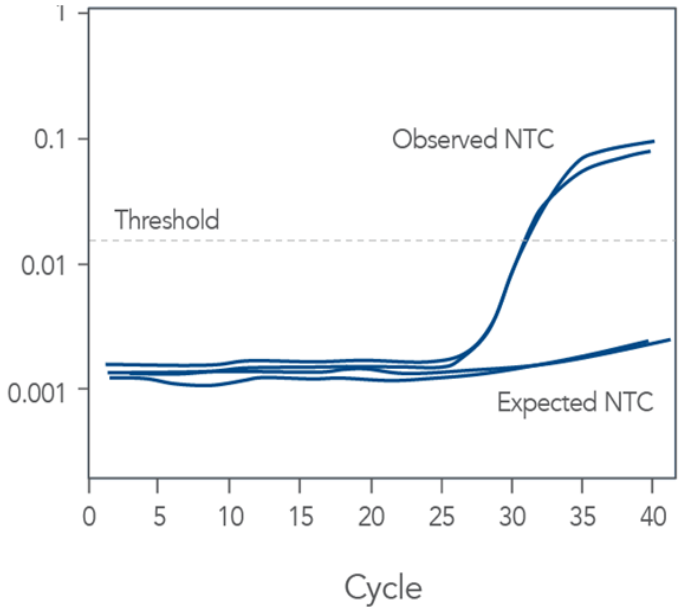

Figure 5: Graph showing contamination in the NTC

Possible causes:

- The DNase- or RNase-free water may have been contaminated
- Improper decontamination of the pipettes or the biosafety cabinet
- Improper testing protocol carried out by laboratory technicians; requires proper training protocols and unidirectional workflow
- A lack of regular fumigation of the laboratory

Corrective action:

- Results should not be released for the entire batch of samples
- The laboratory should be thoroughly decontaminated, tip boxes should be changed, and the run should be repeated.

9.5.5 Examples of RT-PCR problems and solutions/preventive measures

1. NTC contamination and amplification seen in all wells

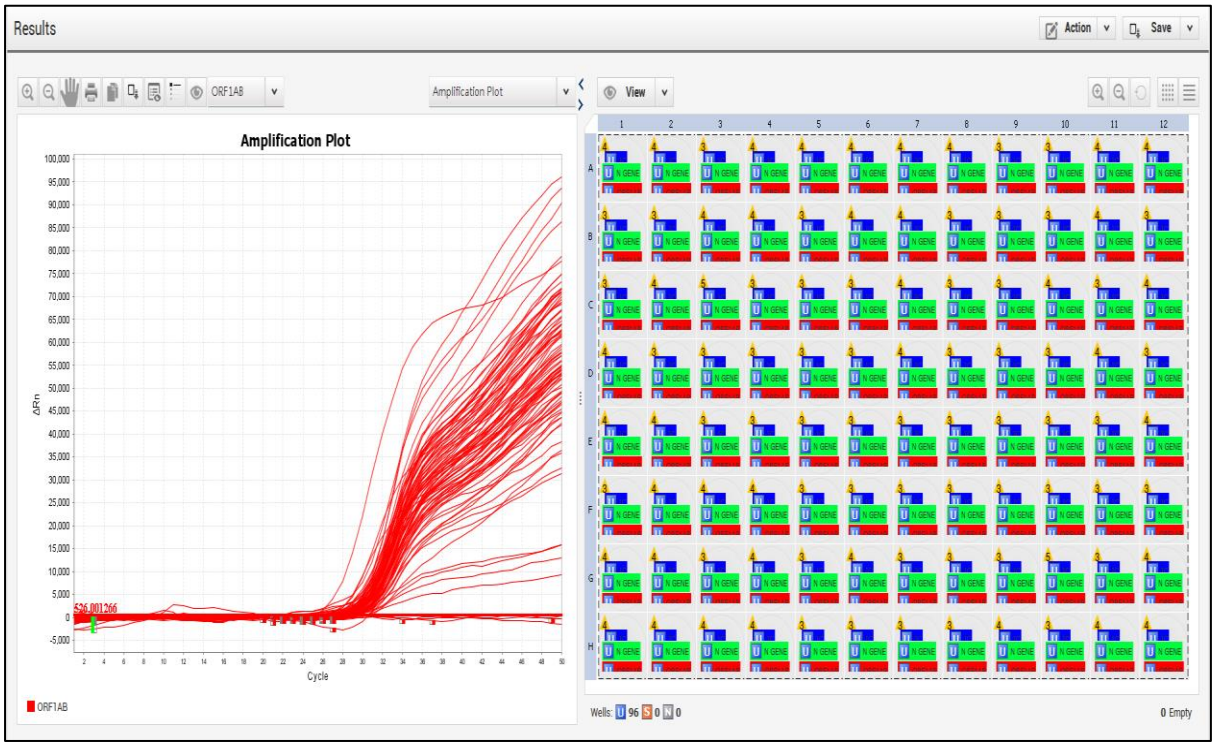

Figure 6: Graph showing contamination in the NTC – Example 1

- Always use good PCR-grade nuclease-free water (NFW) throughout the process.
- If non-sterile NFW is used while preparing reagents, contamination of PCR reagents may occur, resulting in amplification of entire wells.

2. Reagent contamination

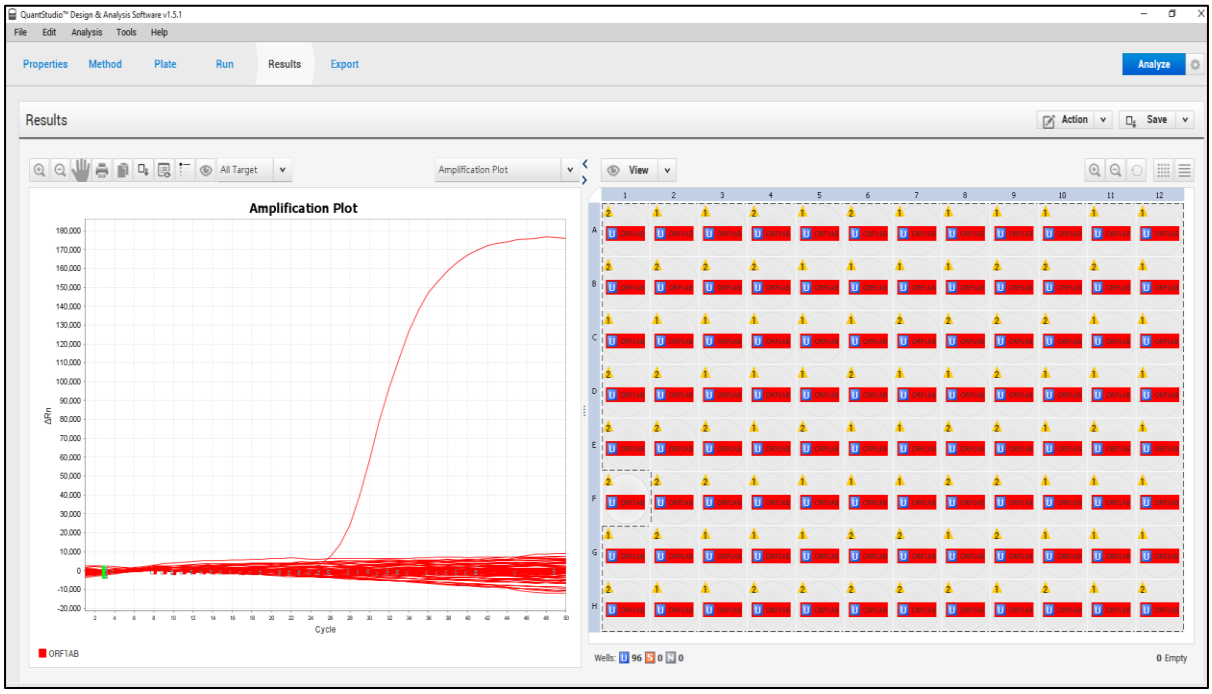

Figure 7: Graph showing contamination in the NTC – Example 2

- Introduce a new lot for each repeated PCR assay
- Before starting, clean bench surfaces and pipettes with a DNA decontamination reagent and 30 minutes of UV exposure in the laminar airflow cabinet
- Use a clean, separate set of pipettes and use barrier/filter tips for all work, including stock reagent preparation

3. Internal control failure

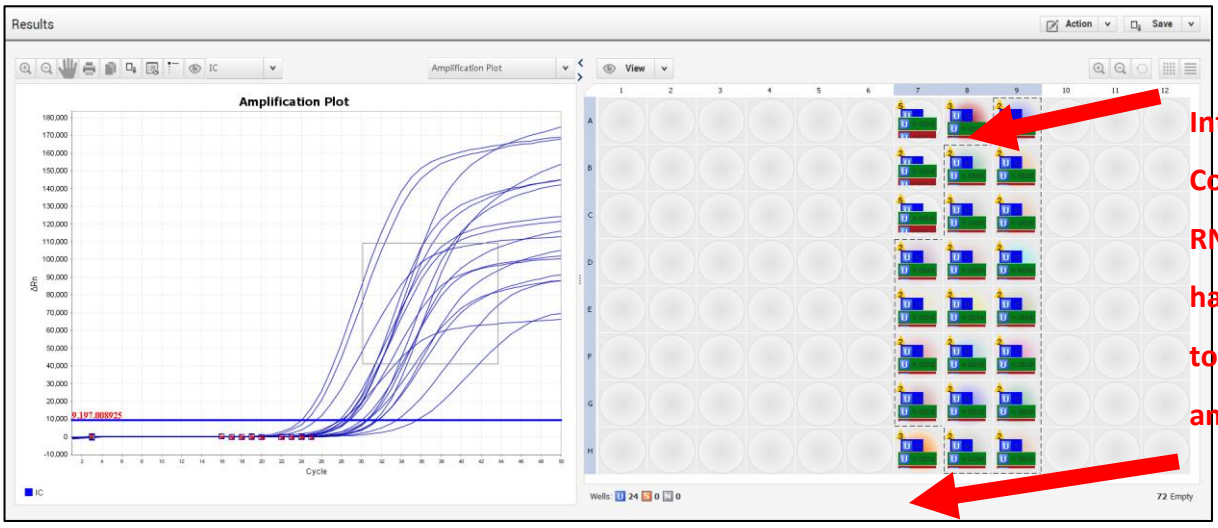

Figure 8: Graph representing an internal control failure – example 1

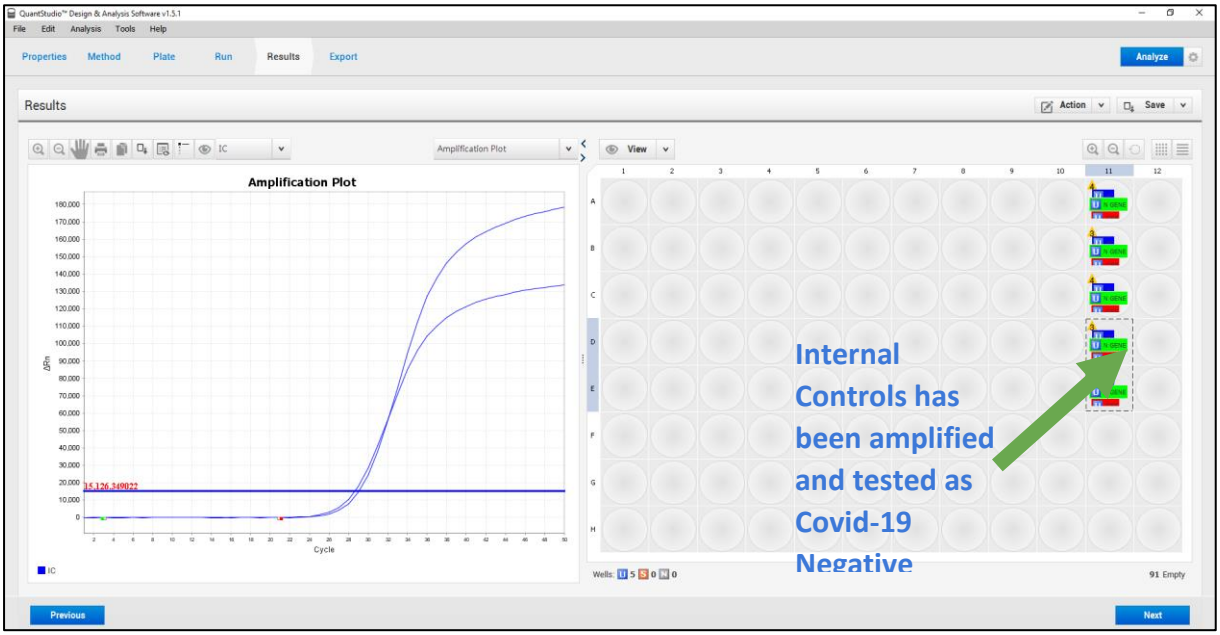

Figure 9: Graph representing an internal control failure – example 2

As per ICMR guidelines, an IC failure sample should be submitted as inconclusive via the ICMR portal. It is suggested to collect a repeat sample from the patient concerned to obtain an accurate result for them.

How to interpret a borderline result:

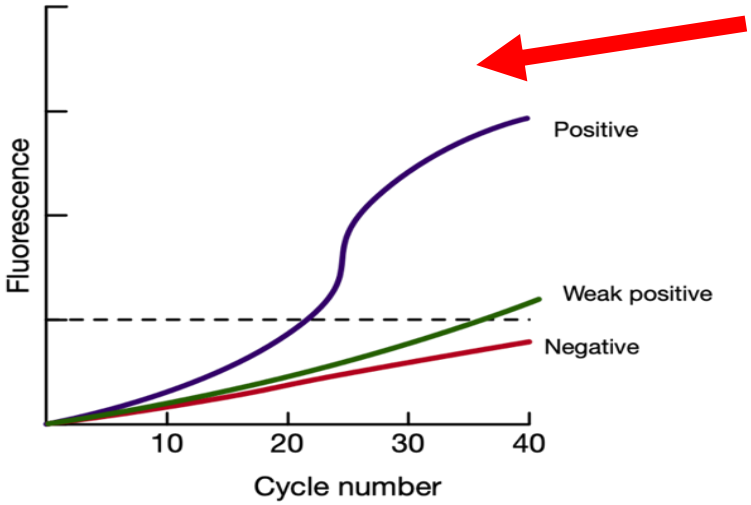

Figure 10: Graph representing a good amplification plot for borderline positive result interpretation

It is advisable that laboratories visualize curves for samples that are borderline, to confirm whether they are sigmoid and whether the fluorescence emitted is sufficiently high in terms of units with respect to the positive control.

Chapter 9 References

1. WHO. Laboratory Quality Management System Handbook 2011 [Available from: https://www.who.int/publications/i/item/9789241548274.
2. Lippi G, Chance JJ, Church S, et al. Preanalytical quality improvement: from dream to reality. *Clin Chem Lab Med* 2011;49(7):1113-26. doi: 10.1515/cclm.2011.600 [published Online First: 2011/04/27]
3. Payne D, Newton D, Evans P, et al. Preanalytical issues affecting the diagnosis of COVID-19. *J Clin Pathol* 2021;74(4):207-08. doi: 10.1136/jclinpath-2020-206751 [published Online First: 2020/07/08]
4. Rahbari R, Moradi N, Abdi M. rRT-PCR for SARS-CoV-2: Analytical considerations. *Clin Chim Acta* 2021;516:1-7. doi: 10.1016/j.cca.2021.01.011 [published Online First: 2021/01/25]
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Chapter 10
Equipment Management

Chapter 10: Equipment management

10.1 Introduction

Equipment management is one of the essential elements of a quality management system. Proper management of the equipment in a laboratory is necessary to ensure accurate, reliable and timely testing. The benefits of a good equipment management programme are shown in Figure 1. Information in this chapter is based on WHO guidelines for the management of laboratory equipment.[1 2]

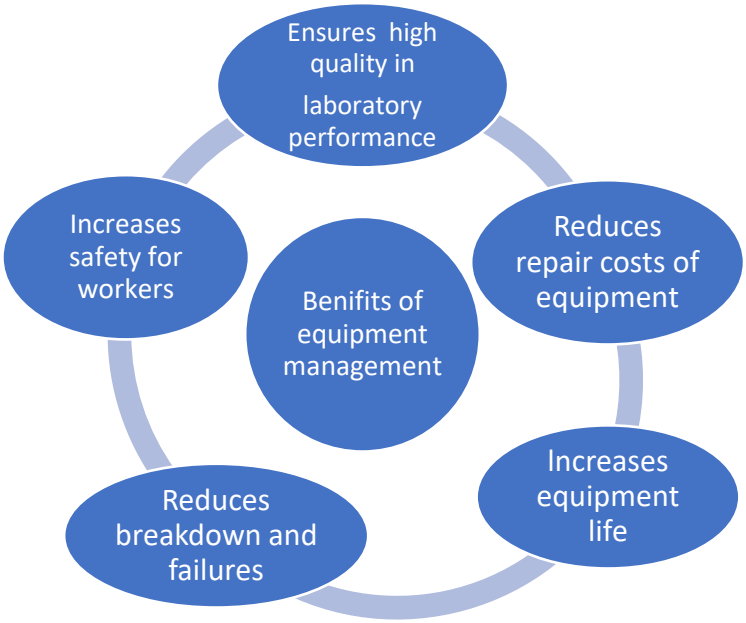

Figure 1: The benefits of good equipment management

10.2 Elements of equipment management

As a laboratory puts an equipment management programme in place, the elements shown in Figure 2 should be considered.

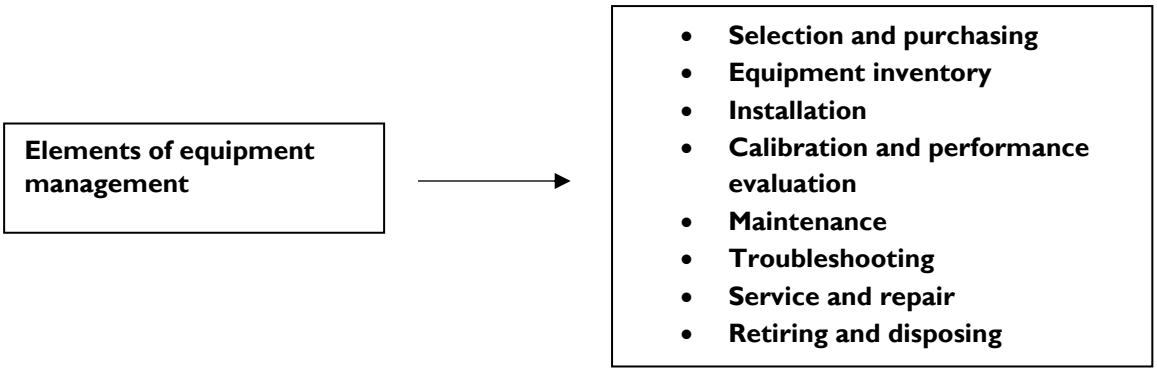

Figure 2: Elements of equipment management

10.3 Selecting and purchasing equipment

10.3.1 Selection of equipment

When selecting equipment for purchase, laboratories must take into account:

- The proposed use of the equipment
- How well the equipment accords with the service provided
- Performance characteristics
- Available space and accessibility
- Cost
- Availability of reagents and consumables, and arrangements for the supply of these materials
- Ease of operation
- Warranty
- Availability of technical support from the manufacturer; service contracts
- Location in the laboratory
- Safety

10.3.2 Purchasing equipment

The initial decision to be taken is whether to purchase or lease equipment. When making this decision, it is a good idea to factor in repair costs. The manufacturer should provide all necessary information on the operation and maintenance of the equipment. The initial cost of an instrument may seem reasonable, but it may be expensive to repair. Also, consider savings that could be negotiated if the laboratory needs more than one piece of equipment.

Key points to consider before purchasing equipment

- Wiring diagrams, computer and software information, a list of parts needed, printers and an operator's manual must be provided
- The manufacturer must install the equipment, train staff (covering travel expenses as necessary) and include installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) of equipment within the purchase price
- The warranty should include a trial period to allow verification that the instrument performs as expected
- Maintenance by the manufacturer may be included in the contract; if so, this should specify whether maintenance is provided on a regular basis

10.4 Equipment inventory

A laboratory must keep an inventory log of all equipment in the laboratory. For each piece of equipment, the equipment inventory log should have a record of:

- The instrument type, make and model number, and serial number, so that any problems can be discussed with the manufacturer
- The date the equipment was purchased, and whether it was purchased new, used or reconditioned
- Manufacturer/vendor contact information
- Presence or absence of documentation, spare parts and maintenance contract
- Expiration date of warranty

10.5 Installation

During installation, laboratories must take into account the factors shown in Figure 3.

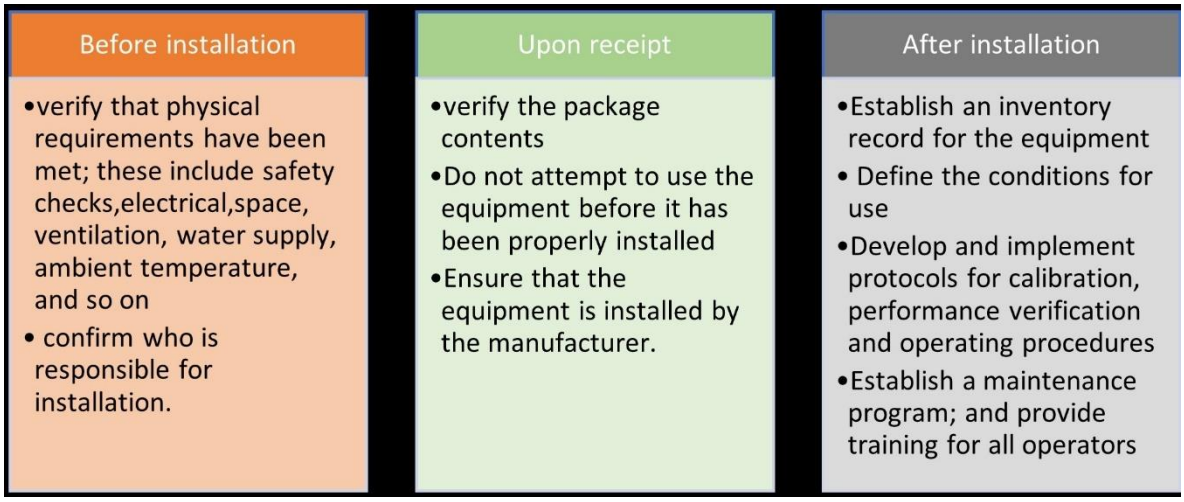

Figure 3: Factors to consider during equipment installation

10.6 Calibration and validation

The performance of any new equipment must be validated; the equipment must also be calibrated before use by:

- Testing known samples and analyzing the data
- Establishing stability or uniformity in temperature-controlled equipment
- For pipettes, checking their accuracy and precision
- Checking the speed (in revolutions per minute, rpm) of a centrifuge

10.7 Maintenance

Maintenance involves systematic and routine cleaning, as well as adjustment or replacement of instrument and equipment parts. Some of the initial steps in equipment maintenance include:

- Assigning responsibility for providing oversight
- Developing written policies and procedures for maintaining equipment, including routine maintenance plans for each piece of equipment that specify the frequency with which all maintenance tasks should be performed
- Developing the format for records, creating logs and forms, and establishing the processes to maintain records
- Training staff in the use and maintenance of the equipment and ensuring that all staff understand their specific responsibilities

10.8 Troubleshooting

Occasionally, equipment fails to operate. It is important that operators are trained to troubleshoot any equipment problems so that they can quickly get the equipment functioning again and resume testing as rapidly as possible. There are a number of points to consider, as outlined below.

- Problems with equipment may present in many ways. The operator may notice subtle changes such as drift in quality control or calibrator values, or obvious flaws in equipment function.
- Manufacturers will often provide a flowchart that can help determine the source of any problems.
- It is helpful to have access to backup instruments.
- Faulty instrument should never be used. ‘Do not use’ label shall be noted on such instruments. Support from technical experts shall be taken.

10.9 Service and repair

Manufacturers often provide service and repair assistance for equipment that is purchased from them. Points to consider include:

- A procedure for scheduling services shall be made available and that must be periodically performed by the manufacturer.
- When instruments need repairing, remember that some warranties require that repairs be handled only by the manufacturer.
- AMC/CMC of the equipment shall be taken regularly.
- The Biomedical Equipment Management and Maintenance Programme is an initiative by the Ministry of Health and Family Welfare to provide comprehensive support to state governments to outsource the maintenance of medical equipment for all facilities, to help improve the functionality and lifetime of equipment, simultaneously improving healthcare services in public health facilities, reducing the cost of care and improving the quality of care.

10.9.1 Condemnation and disposal of equipment

Condemnation and disposal of equipment involves evaluating laboratory equipment to determine whether it should be removed from service and sent for disposal. The following points should be noted.

- The list of laboratory equipment that needs to be condemned must be placed before the condemnation committee. This committee will then examine parameters such as asset life, reparability, and cost of repair.
- It is very important to have a policy and procedure for retiring older laboratory equipment. This will usually be necessary when it is clear that an instrument is not functioning and is not repairable, or when it is outmoded and should be replaced with new equipment.
- Once a piece of equipment is fully retired and it has been determined that it has no further use, it should be disposed of in an appropriate manner.
- This last step is often neglected in laboratories and old equipment accumulates, taking up valuable space and sometimes creating a hazard.

10.9.2 Equipment maintenance documentation

Equipment documents and records are an essential part of a quality management system.

- All policies and procedures for maintenance should be defined in appropriate documents; keeping good equipment records will allow for thorough evaluation of any problems that may arise.
- Each major piece of equipment must have its own equipment maintenance document.
- Smaller, commonly used equipment, such as centrifuges and pipettes, may be managed with an equipment maintenance document or manual that deals with all such equipment in the laboratory.
- An equipment maintenance document should include:

    - Step-by-step instructions for routine maintenance, including frequency of performance and how to keep records of maintenance
    - Instructions for carrying out function checks, frequency of performance, and how to record the results
    - Directions for calibrating the instrument
    - Guide for troubleshooting
    - Any service and repair required by the manufacturer
    - A list of any specific items needed for use and maintenance, such as spare parts

Tools that are helpful for keeping records of equipment management include:

- Charts
- Logs
- Checklists
- Graphs
- Service reports

Chapter 10 References

1. WHO. Guidance for countries on the specifications for managing TB laboratory equipments and supplies 2011 [Available from: http://apps.who.int/iris/bitstream/handle/10665/44798/9789241503068_eng.pdf?sequence=1.
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Chapter 11

COVID-19 Laboratory Assessment Tools Audit and Accreditation

Chapter 11: COVID-19 Laboratory Assessment Tools - Audit and Accreditation

11.1 Introduction

A COVID-19 laboratory is likely to be audited by a variety of organizations, including regulatory agencies, accreditation bodies international/funding agencies etc. The overall goal is to ensure that the COVID-19 testing being performed is correct, reliable, consistent and completed within accepted timelines. A laboratory audit ensures that the laboratory has quality systems in place, follows good laboratory practices, and generates data of integrity and quality. Audits in a COVID-19 testing laboratory shall be made for the common and fundamental requirements for all medical laboratories as per ISO 15189:2012 (NABL 112 evaluates this international standard as per the Indian setting) and also for specific requirements as per national/state guidelines. If there is a conflict or disagreement between two sets of guidelines, local regulatory requirements supersede the others.[1 2]

11.1.1 Audit mechanism

An audit works on the principle of "what is not documented was not done". For example, missing standard operating procedures (SOPs)/work instructions/records during an audit is generally considered to be a "major non-conformity". The audit mechanism involves the following main procedures:

- Review of documents, including policies, SOPs, manuals, workflow charts, and displays.
- Interviews with staff, including technical staff, transport staff and data entry operators.
- Witness of processes, including examinations, packing and transporting.
- Observe resources, including equipment, space, biosafety practices.
- Review of data and analysis, including records, reports, information management system.

11.2 Critical points for audit preparation

For ease of understanding, a quality system essentials approach has been considered here. The same elements also satisfy ISO15189 accreditation requirements; only the sequence and numbering are different. All components of a laboratory's quality system essentials are audited. The purpose of each component is to ensure management and technical quality, measure performance, monitor and improve consistently. The components are described below.

11.2.1 Organization

- Does the laboratory have a legal identity and permission to conduct the specified tests (COVID RT-PCT/NAAT)?
- Is there an organizational structure that is responsible for the processes at different levels?
- Does the organization work within a set of defined policies and procedures?
- Has the management identified and expressed its goals and responsibilities?
- Does the laboratory function as a seamless unit?
- Is there a certificate of registration under CEA/gazette notification for the institution or a letter from the government/ICMR granting permission to perform COVID-19 RT-PCR?
- Is there an organogram of the laboratory showing its relationship to a parent institution, if any? This should include key personnel and their deputies.
- Are there documented policies and procedures, quality policy and quality objectives in the Quality Manual, as per ISO 15189:2012[1] or ICMR-GCLP guidelines?[2]
- Is there a policy about how ethics is addressed in the organization? Staff training and undertaking records should be kept.

11.2.2 Personnel

- Are there designated individuals at various levels of expertise?
- Are there individuals with the required knowledge and skills?
- Do staff have relevant training in their area of work?
- Are these staff now competent to carry out their assigned work?
- Are the staff oriented to the laboratory's policies and safety practices?
- Is annual training of staff on quality management system (QMS) policies and safety procedures conducted, including fire drills and emergency preparedness?
- Is training followed by post-training evaluation?
- Are competency assessments conducted yearly, or whenever a new testing responsibility is assigned or following any non-conformity observed related to an individual?
- Do competence assessments include split-sample testing, equipment operation, maintenance, work records reviews, results from external quality assurance (EQA), and troubleshooting?
- Did the competency assessment identify training needs or a requirement for refresher training?
- Was this exercise conducted in a positive and motivating manner?
- Have staff been vaccinated according to the specific risks to which they could be exposed, such as COVID-19, hepatitis B, influenza, tetanus and meningococcal infection?

11.2.3 Equipment

- Did the laboratory purchase the correct equipment of the intended design, with no conflicts of interest?
- Is all of the equipment correctly installed, in proper space and conditions considering electrical and environmental safety?
- Does all of the equipment have calibration and validation certificates from accredited, calibrated laboratories?
- Are the equipment maintenance records and daily usage logs well maintained?
- Is there a register or other document that records downtime and other adverse events?
- Does each piece of equipment have specific work/operating instructions?
- Apart from documentation, are staff educated in the use of each piece of equipment, troubleshooting the equipment, and able to demonstrate the correct operation of the equipment?
- Are staff educated in safety practices to be used prior to the use of any equipment, including appropriate PPE to wear, disinfection, cleaning, maintenance, and completion of usage logs?
- Do equipment records include:
    - Unique ID, model, serial no.
    - Manufacturer/service person details.
    - Installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) documents with complete information,
    - Annual calibration certificates from an accredited laboratory.
    - Preventive/regular maintenance records.
    - Repair and service details, with details of downtime.
- Calibration requirements for specific equipment include:
    - Biosafety cabinet: airflow velocity, differential pressure, particle count, HEPA filter, and leak test.
    - Centrifuge-speed, timer and temperature (for refrigerated centrifuge).
    - Micropipette: precision and accuracy, as per ISO 8655.
    - Autoclave temperature and pressure.
    - PCR cycler: temperature, laser.
    - Refrigerator, incubator, deep freezers: thermometer calibration along with checks in various positions.

11.2.4 Reagents and kits

- Is every reagent available? Have there been any periods of non-availability?
- Are the reagents stored in the correct conditions to prevent deterioration?
- Is every kit labelled with the date of receipt, and each new lot verification record maintained?

- Does the laboratory have documented procedures for the selection, reception and inventorying of reagents?
- Is there an SOP for the preparation of reagents, buffers and disinfectants?
- Are all suppliers authorized, and do they have storage facilities as required prior to dispatch of supplies to the laboratory?

11.2.5 Processes

- Is there a work process flow chart for the laboratory?
- Are there SOPs for all procedures performed in the laboratory?
- Are there workbooks and registers in each area, covering all tasks sequentially from the test request? This should include sample collection until report generation and issue, disposal of waste, storage of RNA extracts and sample aliquots, which kits/reagents were used, which staff processed samples at each stage, with dates and times.
- Does each test/method have validation records with data?

11.2.6 Process control

- Are all SOPs/kit inserts, stored PCR graphs, and records in place?
- Is the laboratory ready to demonstrate the processes to the assessor? For example, sample collection, packing and transport, testing of both freshly collected and retained samples, reproducibility of testing with minimal variation.
- Are the staff ready for an interview about the work processes of the laboratory?
- Does the laboratory have an internal quality control plan? For example, details on the frequency, source, type of controls, expected results, Ct values, troubleshooting plan etc.
- Is there an EQA plan, including procedures, frequency, and analyses?
- Are all quality control records maintained and readily available?

11.2.7 Information management

- Is there an SOP for electronic laboratory information systems (LIS) that store patient and test information, including patient details, PCR run graphs, reports etc?
- Is patient test information protected from unauthorized access? Are patient data stored securely, ensuring confidentiality, safety and retrievability?
- Is there authorization of staff to perform different levels of activities, such as registration, data entry, archiving etc?
- Is there a format for sharing reports (e.g. line listings, positivity rates, indicators) with the authorities?

11.2.8 Occurrence management

- Is there a documented procedure to receive complaints, perform root-cause analysis, take corrective action, prevent and monitor recurrence?
- Are records of complaints, corrective and preventative actions (CAPA) available?
- Are any laboratory accidents, such as needle-stick injuries etc., reported and monitored?

11.2.9 Internal assessment:

- Is the laboratory periodically checking its own functioning, in a thorough, systematic manner, using trained personnel?
- Does the laboratory take actions based on the findings of these audits?
- Do internal audits include the procedure, audit plan, non-conformity reports, audit checklists used, audit summary reports, and discussion in a Management Review Meeting (MRM)?

11.2.10 Facilities and safety

- Does the laboratory have adequate space, bench areas, light and ventilation?
- Are the work areas clean and routinely disinfected?
- Is the room temperature and humidity acceptable for functioning and are these parameters routinely monitored?
- Are infectious and non-infectious areas segregated? Within the molecular testing laboratory, are there separate rooms with unidirectional workflow?
- Are there waste segregation bins, with sharps correctly disposed of?
- Are there any electrical wires hanging? Are extension boards used in the laboratory?
- Are fire extinguishers present and fire exit routes clearly displayed?
- Are there dedicated freezers for the storage of samples, nucleic acid extracts and reagents/kits?
- Does the laboratory have sufficient stocks of handwash, hand sanitizer, spill kits, and hazardous materials storage facilities?
- Are there staff facilities and storerooms available? Is entry into the laboratory access-controlled?
- Is there a separate autoclave for waste disposal? Is the record/log of its usage maintained?

11.2.11 Process improvement

- Does the laboratory periodically evaluate its performance?

- Are there any improvements that can be made, either in terms of technical or quality improvements?
- Are quality indicators evaluated for pre-analytical, analytical and post-analytical phases?
- Does the laboratory have a means of periodically reviewing any non-conformities, complaints, feedback or incidents?
- Does the laboratory carry out risk assessments and take preventive actions based on risk severity scores?

11.2.12 Customer service

- Is the laboratory satisfying the needs of its users, such as patients and referring doctors)?
- Are there records of user feedback surveys and any actions taken based on the feedback?
- Are the records of complaints and corrective actions maintained?

Once a decision has been made to be audit-ready, the laboratory management team should create a definite plan of action and nominate individuals to coordinate all activities related to the audit. Based on the checklist of audits, all documents/records/registers must be kept updated and reviewed under the supervision of the laboratory supervisor. The guiding audit checklist for point-of-care (POC) test and RT-PCR laboratories can be used for laboratory preparation (see Annexures 15 to 17).

11.3 Documents required during an audit

Quality Management System documentation

- Laboratory quality policy and quality objectives document
- Quality manual document
- Organization and management structure
- Service agreement document
- Roles and responsibilities of laboratory management
- Personnel records
- Training and competency assessment
- Internal audit file
- Management review file
- Complaint and feedback register
- Corrective and preventive action document
- Incident register
- SOP for control of records

Laboratory equipment, reagents and consumables

- Documented procedure for selection and purchase of equipment
- Equipment acceptance testing document
- Equipment calibration and metrological traceability
- Equipment records
- Equipment maintenance and repair document
- Equipment adverse incident report file
- Documented procedure for reception, storage and acceptance testing
- Inventory management of reagents and consumables
- Reagents and consumables – records, instructions for use
- Reagents and consumables – adverse incident reporting

Pre-examination processes

- Information document on laboratory services to patients/users
- Request form for collecting patient details
- SOP for sample collection and transport

Examination processes

- SOP for sample registration and report management
- SOP for sample processing
- SOP for CBNAAT
- SOP for Truenat
- SOP for RNA isolation
- SOP for RT-PCR
- SOP for immunoassays
- SOP for rapid antigen testing

Safety and waste disposal

- Biosafety manual
- SOP for biomedical waste management
- SOP for spill management
- SOP for use of PPE
- SOP for handwashing
- SOP for disinfection and decontamination

Quality control processes

- Quality control document
- Record of internal quality control procedures
- External quality assessment report
- Interlaboratory comparison report

Post-examination processes

- Report format
- SOP for result reporting
- Report storage, retention and disposal document

11.4 Laboratory accreditation

Laboratory accreditation is a procedure by which an authoritative body gives formal recognition of technical competence for specific tests/measurements based on a third-party assessment and following international standards. Laboratory accreditation provides many advantages:

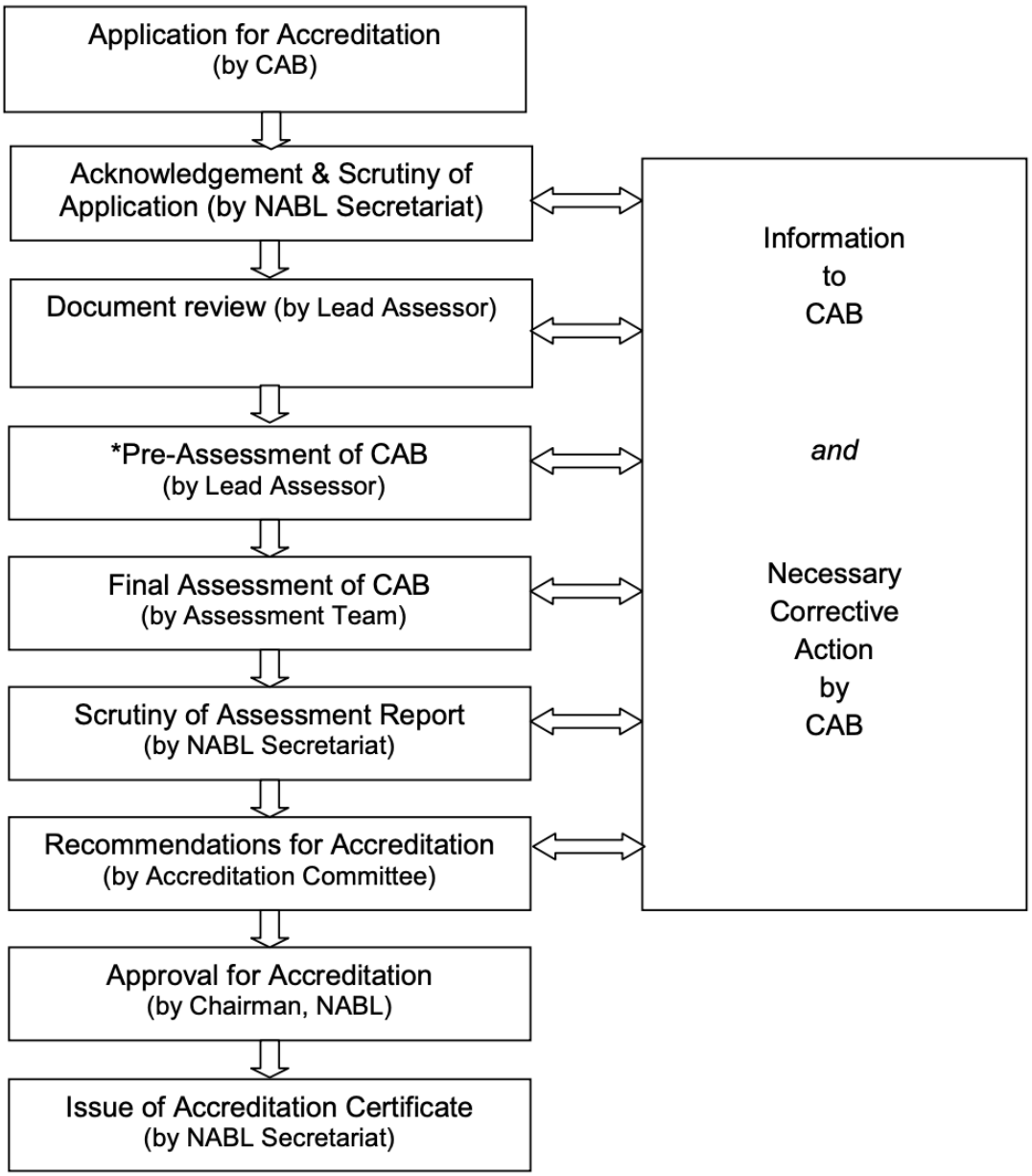
- Aids customers/patients to identify and select reliable testing services
- Increased confidence in testing or calibration reports issued by the testing laboratory
- Better control of laboratory operations and feedback to laboratories
- Potential increase in customer/patient confidence and satisfaction
- Savings in terms of time and money due to reductions in the need for re-testing

11.4.1 NABL accreditation

The National Accreditation Board for Testing and Calibration Laboratories (NABL) grants accreditation to medical testing laboratories in accordance with NABL 112 as per International Standard ISO 15189 “Medical laboratories – Requirements for quality and competence”. A COVID-19 testing laboratory falls within the discipline of “Microbiology & infectious disease serology” as per NABL. Any laboratory that performs testing, including a medical laboratory, having decided to undergo NABL accreditation is referred to as a Conformity Assessment Body (CAB) (Figure 1).

The process of accreditation can take weeks to months, depending on the readiness of the CAB. However, NABL has established expedited approval mechanisms for COVID-19 testing laboratories, with fast-track approvals being granted within 7 days. As per ICMR guidelines, it is mandatory for all private COVID-19 testing laboratories, including those using the Truenat/CBNAAT platform, to obtain NABL accreditation. ICMR will provide expedited approval for Truenat/CBNAAT subject to NABL approval, which can be submitted within a maximum time span of four weeks from the date of approval.

- Once accredited, the laboratory shall conform to the requirements of ISO 15189:2012 and NABL policies.
- The NABL accreditation certificate is valid for a period of 2 years.
- For newly accredited CABs, NABL will conduct on-site surveillance within 12 months from the date of accreditation.
- The accredited CAB is subjected to re-assessment every 2 years, for which the CAB must apply 6 months before the expiry of accreditation.
- From the second cycle onwards, the CAB is subjected to desktop surveillance within 2 months of each re-accreditation.
- A uniform fee structure is maintained for all CABs for application, enhancement of scope, change in authorized signatory, change of certificate, annual accreditation fee, overhead charges and assessment charges.



* *Preassessment of CAB is optional and depends on the willingness of the CAB to undertake*

Figure 1: Flow diagram showing the accreditation process

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	
Qualification and contact details of the responding person/s	
Level of laboratory	
Affiliation/ type of laboratory (several answers possible)	

Public Health / Hospital / Health Centre / Environment / Food Control / Veterinary / Private / University / Research / Other?

Affiliated Ministry (if applicable)	

Health / Agriculture / Trade, Commerce / Education / Defense / Other?

Estimated population covered by this laboratory	

Describe participation in international programmes/networks (if applicable)	

Polio, FluNet, INFOSAN, Global Foodborne Infections Network, etc.

Laboratory testing

Disciplines addressed in the laboratory undertaking SARS-CoV-2 testing (eg. bacteriology, virology, hematology, environmental testing, veterinary testing, etc.)	
Average number of specimens tested per day	
Average number of PCR or RT-PCR tests run per week	
Average number of specimens tested in serology per week	

Comments:

1. Organization and Management:

Service hours

1.1	*What are the days and hours of operation of routine service?*			
1.2	*If relevant, what are the days and hours of operation of emergency service? and number of shifts?*			

External communication

	Is the laboratory equipped with:			
1.3	Telephone?			
1.4	Fax?			
1.5	Computer with Internet access?			

Internal communication and structure

1.6	Is there an organizational structure defining the lines of authorities and responsibilities for key laboratory staff?	X		

Budget

1.7	Is the budget for staff salaries adequate for the need?			
1.8	Is there an adequate budget assigned for consumable and reagent purchase?			
1.9	Is there an adequate budget assigned for equipment purchase/maintenance?			
1.10	Is there an adequate budget assigned for surveillance and/or overall public health activities?			

Licensing/Supervision/Accreditation

1.11	Has the laboratory undergone an audit or assessment by a third party within the last two years and implemented recommendations where relevant?	X		
1.12	Has the laboratory received a certification or ISO accreditation through a national or international body?	X		

Comments:

1. **Documents:**

Document control

2.1	Is a system in place to organize the management of laboratory documents and records?	X		
2.2	Does the laboratory have an archive system?	X		
2.3	Are the archived documents retrievable?			

Quality procedures

2.4	Is there a quality manual describing the quality system of the laboratory?	X		
2.5	Are specimen handling and testing procedures (RNA extraction, RT-PCR, serology, etc.) readily available to staff, as relevant?	X		
2.6	Are current versions of published standards and other similar documents in use in the laboratory available (e.g. norms, guidelines, instrument manuals, test kit inserts etc.)?	X		
2.7	Is there a procedure for the storage of primary specimens once analysed?	X		
2.8	Are there procedures for the validation and verification of methods and equipment as relevant?	X		
2.9	Are procedures in place to record incidents or complaints?	X		

Biosafety procedures

2.10	Has a risk assessment related to the procedures undertaken in the laboratory been performed and documented?	X		
2.11	Are written biosafety procedures available?	X		
	If yes or partial, are the following subjects addressed:			
2.12	- Handwashing?	X		
2.13	- Personnal Protective Equipment (PPE)	X		
2.14	- Disinfection of contaminated materials?	X		
2.15	- Sterilisation?	X		
2.16	- Glassware and equipment washing?	X		
2.17	- Waste disposal?	X		
2.18	- Laboratory cleaning?	X		
2.19	- Storage and destroy hazard sample?	X		
2.20	- Spillage?	X		
2.21	- Laboratory related injury?	X		
2.22	- Fire emergency?	X		

Comments:

3.Specimen collection, Handling and Transport:

Specimen collection

3.1	Are collection procedures documented and available to relevant personnel?	X		
3.2	Do these include minimum patient identification details?			
3.3	Is a standard specimen request form available for those requesting tests?	X		
3.4	Are specimens recorded in a book, worksheet, computer or other comparable system?			
3.5	Are specimen aliquots traceable to the original primary sample (identification number, etc.)?			

Specimen handling

3.6	Does the laboratory experience problems with specimens from outside the facility due to inadequate request form, specimen identification, container, etc. ? (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)			
3.7	Are there any criteria for acceptance or rejection of primary specimens (including potential caution if non-conforming specimens are accepted)?			
3.8	Are primary specimens adequately stored if not immediately examined (fridge, -20C freezer, -70C freezer, or other recommended storage conditions)?			
3.9	Is there a procedure for the storage of primary specimens once analysed?			

Specimen referral / transport

3.10	Does the laboratory have appropriate packaging for referring specimens (triple package if air transport, or any package in conformity with local regulations or recommendations)?			
3.11	Is a transportation system for sample referal (bus, ambulance, national postal service, etc.) already set-up?			
3.12	Is/are the person/s in charge of shipments trained for the transport of infectious substances?			
3.13	If yes or partial:			
3.14	- Is he/she trained for local or national regulations or recommendations?			
3.15	- Is he/she trained in international regulations?			

Comments:

4.Data and Information management:

Test results and reports

4.1	Are all original observations/results of the laboratory recorded in a worksheet or electronic database?			
4.2	Are the results reviewed and authorized before the results are released?			

4.3	When samples need to be referred further to another laboratory, is there a procedure to define how report is then issued and by which laboratory?			

4.4	Is there an immediate notification of physicians when results are critical for patient care?			
4.5	Is there an immediate notification of relevant ministry/surveillance network when results are critical?			

Data analysis and statistics

4.6	Can the laboratory provide basic statistical data from its activities (e.g. number of tests ordered, aggregated qualitative/quantitative data, etc.)?			

Data security – Confidentiality

4.7	Are access and modification of patient data protected (for paper-based and/or electronic system)?			
4.8	Is efficient back-up in place to prevent loss of patient result data in case of theft or other incident for the above system(s)?			

IT and Laboratory Information Management System (LIMS)

4.9	*What are the softwares/applications used in the laboratory:*			

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.)?			

Use

5.6	Is the date of opening clearly written on reagents/ kits?			
5.7	Are new reagents (new product, new lot, including home-made reagents) verified against old reagents or reference materials before use?	X		
5.8	Are expired reagents used (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)?			
5.9	Are disposable supplies (e.g. tips, plastic pipettes, gloves) reused (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)?			

5.10	Is there a system for accurately forecasting needs for consumables and reagents?	X		

Comments:

6. Equipment Management:

Equipment inventory

6.1	Is there an equipment inventory?	X		
	If yes or partial, does it include:			
6.2	Name of the equipment?			
6.3	Name and contact details of manufacturer (or local supplier)?			
6.4	Condition (i.e. new, used)?			
6.5	Maintenance activities?			
6.6	Is the laboratory's equipment appropriate to perform testing undertaking the following?			
6.7	- Nucleic acids extraction			
6.8	- Serology			
6.9	- RT-PCR			
6.10	- Other (please specify in 'comments')			

Equipment maintenance, calibration and monitoring

6.11	Does the laboratory have a dedicated person in charge of the equipment (maintenance management, etc.)?			
6.12	Is the equipment maintained in a safe working condition (including electrical safety)?			
6.13	Is there daily monitoring and recording of temperatures for temperature-dependent equipment?			
6.14	Is there daily monitoring and recording of airflow in biosafety cabinets?			
6.15	Is a preventive maintenance programme in place?			
6.16	Is there a defined protocol and time period for pipette calibration?			
6.17	Is the staff duly trained and authorized before first using equipment?			

	List of FUNCTIONING and USABLE equipment (necessary for SARS-CoV-2 testing) *	**Number**	**Is it registered?**	**Is it maintained (including calibration if applicable)?**	**Is it certified?**
6.18	Autoclave (clean)				
6.19	Autoclave (dirty)				
6.20	Biosafety Cabinet class II				
6.21	Centrifuge, simple				
6.22	Computer for laboratory work				
6.23	Nucleic acid automated extractor				
6.24	ELISA equipment (Washer/Incubator/Reader)				
6.25	Extraction manifold				
6.26	Freezer -20°C				
6.27	Freezer -70°C				
6.28	Microfuge				
6.29	Micropipette 20 µl				
6.30	Micropipette 0.5 - 10 µl				
6.31	Micropipette 10 - 100 µl				
6.32	Micropipette 20- 200 µl				
6.33	Micropipette 100-1000 µl				
6.34	Multichannel pipette				
6.35	PCR working station				
6.36	Plexiglass screen				
6.37	Printer for laboratory work				
6.38	Refrigerator				

6.39	Thermal cycler (Thermocycler, PCR Machine or DNA Amplifier), Real Time with 4 chanels				
6.40	Vacuum pump				
6.41	Vortex				
6.42	Water distiller				
	* *List additional equipment as appropriate*				

Comments:

7. Facilities:

Infrastructure

7.1	What is the general condition of laboratory building and infrastructure? For the following questions, choose one of the following answers: 1.Good; 2.Medium; 3.Bad; 4.Non applicable			
7.2	Walls, floors and roofs?			
7.3	Windows and doors?			
7.4	Benches?			
7.5	Heating / air conditioner / ventilation?			
7.6	Lighting?			
7.7	Waste disposal equipment?			

Work conditions

7.8	Does the laboratory face electricity interruption (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)?			
7.9	If applicable, do you have an emergency electric generator or other backup power source?			
7.10	Is key/sensitive equipment protected by a UPS (Uninterruptable Power Supply)?			
7.11	Does the laboratory face water shortages (1.Never; 2.Sometimes; 3.Regularly; 4.Non applicable)?			

Space (provide floor plan or sketch of laboratories if possible)

7.12	Total m2 available:			
7.13	Number of rooms:			
7.14	Is the laboratory size adequate for all the activities that are undertaken?			
7.15	Specifically, is the facility and lab space adequate to perform testing undertaking the following:			
7.16	- Nucleic acids extraction			
7.17	- Serology			
7.18	- RT-PCR			
7.19	- Other (please specify in 'comments')			
7.20	Does the laboratory space provide physically separate rooms for the different steps of nucleic acid amplification testing (if PCR procedures performed):			
7.21	- Dedicated clean room for the preparation of reagents (including dispensing of master mix)?			
7.22	- Room for extraction of nucleic acids and for the addition of nucleic acids to master mix prior to amplification?			
7.23	- Dedicated contained room for nucleic acids amplification and product detection?			

Comments:

8. Human resources:

Staff number

8.1	Number of:			
8.2	*Managers/senior staff (postgraduate degree)*			
8.3	*Laboratory technologists or technicians (performing tests)*			
8.4	*Laboratory assistants/medical aides (not doing tests)*			
8.5	*Support/administrative staff*			
8.6	*Other staff*			
8.7	*For other staff, please specify:*			
8.8	*Total number of persons working in the laboratory*			
8.9	Is the staff number adequate to undertake the required work?			
8.10	Is there a plan for surge capacity?			
8.11	If yes, is the laboratory already in surge capacity?			

Qualifications and skills

8.12	Is laboratory staff available and competent to perform testing undertaking the following?			
8.13	- Nucleic acids extraction			
8.14	- Serology			
8.15	- RT-PCR			
8.16	- Other (please specify in 'comments')			
8.17	Are there any type of periodical competency assessment of the personnel?			
8.18	Has a quality manager been designated?			

8.19	Has a biosafety officer been designated?			
8.20	Are the personnel trained in quality management?			
8.21	Are the personnel trained in biosafety?			
8.22	If yes, or partial:			
8.23	- in conducting a risk assessment			
8.24	- in biosafety while sampling			
8.25	- in biosafety while manipulating laboratory samples			
8.26	- in using disinfectants and procedures in disinfections			
8.27	- in proper waste management (handling hazardous and non hazardous wastes)			
8.28	- in biological spill clean up			
8.29	Are the personnel trained in molecular biology?			
8.3	If yes, or partial:			
8.31	- in nucleic acid extraction			
8.32	- in conventionnal PCR and gel electrophoresis			
8.33	- in real-time PCR			
8.34	- in primer design			
8.35	- in PCR assay optimization and validation			
8.36	- in assay data analysis			
8.37	- in multiple sequence alignment for PCR performance troubleshooting			
8.38	- in phylogenetic analyses			
8.39	Are the personnel trained in conducting serology assay optimization?			

Comments:

9.Biorisk Management:

The biosafety level of your lab is:

9.1	BSL 2			
9.2	BSL 3			
9.3	Other (please provide details below)			

Disinfection and waste management

9.4	Are disinfection and decontamination procedures implemented?	X		
9.5	Is there enough disinfectants available for use at any time?			
9.6	Are waste management procedures implemented effectively?	X		
9.7	Is there adequate separate disposals for infectious and non-infectious wastes?			
9.8	Is there dedicated waste for used solvents?			

Safety conditions

9.9	Do rooms have different sinks for handwashing only? (one per unit)			
9.10	Do you use biosafety cabinets to manipulate samples producing potential dangerous aerosols?			
9.11	Has the biosafety cabinet been checked to see if it is installed, calibrated and functions correctly?	X		
9.12	Do you have a biohazard sign indicated on the doors of the rooms where microorganisms are handled?			

9.13	Are there door entry locks with security measures that prevent non relevant staff or visitors to enter the laboratory?			
9.14	Are accident/incident and nonconformities related to biorisk correctly managed (i.e. reported, recorded, investigated, and leading to preventive or corrective actions)?	X		
9.15	Where are labcoats and laboratory linens washed? (1 outside laundry service, 2 lab, 3 home)			
9.16	Are there locks on the freezers where primary specimens and aliquots are stored?			

Use of safety equipment (PPE) and Biosafety behaviour

9.17	Are PPE available in enough quantities for the work load and number of laboratory personnel?			
9.18	Does the laboratory staff use adequate PPE while working in the laboratory?			
9.19	Do staff adhere to basic biosafety behaviours (PPE not worn outside lab areas, no eating or drinking within lab, no open-toed footwear, etc.)			
9.20	Are there dedicated PPE for each PCR area (extraction, mastermix, and amplification)?			

Staff health services

9.21	Does your staff have access to occupational health services?			
9.22	Does your staff follow a regular testing for SARS-CoV-2 as health workers	X		

Comments:

10. Public Health Function:

	Type of laboratory:		**1 (Yes) / 2 (No)**	Provide here the answer to the open question/s and/or insert any additional information
10.1	SARS-CoV-2 national reference laboratory			
10.2	Other laboratory			
		Documents to be collected	1; 2; 3; 4	Provide here the answer to the open question/s and/or insert any additional information
	Surveillance and response			
10.3	If non reference laboratory assessed, does the laboratory know the designated national reference laboratories for SARS-CoV-2 testing?			
10.4	If national reference laboratory assessed, does the laboratory know the designated international WHO COVID-19 reference laboratories?			
10.5	Is the laboratory part of a national surveillance network ?			
10.6	If national reference laboratory assessed, is the laboratory part of an international surveillance network?			

10.7	Has the laboratory defined responsibilities in national preparedness and response to public health emergencies like (but not limited to) COVID-19 outbreak?			
	Specimens			
10.8	Does the laboratory receive specimens from the field during the investigation of public health events or public health surveys?			
10.9	Does the laboratory give advice on specimen collection and transport practices from the field during the investigation of public health emergencies?			
10.10	Does the laboratory have outreach arrangements with clinical health care facilities for specimen collection and transport practices from the field during the investigation of public health emergencies?			
10.11	Does the laboratory have a stock of emergency laboratory sampling kits (personal protective equipment, sample collection material, transport media, sample transport packaging)?			
10.12	If non reference laboratory assessed, does the laboratory refer specimens or isolates to a national reference laboratory for public health purpose (e.g. routine surveillance, outbreak investigation)?			
10.13	Does the lab receive clinical specimens from local laboratories for confirmation and other tests?			
10.14	If national reference laboratory assessed, does the laboratory refer specimens or isolates to an international reference			

	laboratory for public health purposes (e.g. routine surveillance, outbreak investigation)?			
	Reporting			
10.15	Is COVID-19-related reporting to public health authorities established and implemented?			
10.16	If yes, is there a standardized form/document to report notifiable diseases or other events?			

Comments:

11. SARS-COV2 Testing capacity and capabilities:

	Laboratory capacity for SARS-CoV-2 testing	Documents to be collected	1; 2; 3; 4	Provide here the answer to the open question/s and/or insert any additional information
11.1	Are COVID-19 specific collection procedures documented and available to relevant personnel?	X		
11.2	Are specimen handling and SARS-CoV-2 testing procedures (RNA extraction, RT-PCR, serology, etc.) written and readily available to staff, as relevant?	X		
11.3	Has the testing procedure for SARS-CoV-2 been verified before starting regular testing?	X		

11.4	Are current versions of published standards and other similar documents in use in the laboratory for SARS-CoV-2 testing available (e.g. norms, guidelines, instrument manuals, test kit inserts etc.)?	X		
11.5	Are procedures in place to report SARS-CoV-2 testing results?	X		
11.6	Has a risk assessment related to the procedures undertaken for SARS-CoV-2 testing in the laboratory been performed and documented?	X		
11.7	Are written biosafety procedures related to the handling and management of specimens tested for SARS-CoV-2 available?	X		
11.8	Are appropriate PPE for the handling and testing of specimens for SARS-CoV-2 available?			
11.9	Are personnel trained in running SARS-CoV-2 testing?			
11.10	If yes, with which technique and on which platform?			
11.11	Has laboratory staff been assessed for competency before testing for SARS-CoV-2?			
11.12	Is equipment used for SARS-CoV-2 testing adequately maintained?	X		
11.13	Is a validated biological safety cabinet (BSC) available to perform the initial processing of the specimens (before virus inactivation)?			
11.14	Are the necessary reagents for SARS-CoV-2 testing available?			
11.15	Are the reagents required for SARS-CoV-2 testing in-date?			
11.16	Are the necessary consumables for SARS-CoV-2 testing available?			

11.17	Are there dedicated equipment for each PCR room (pipettes and pipette tips, microfuge, vortex, etc)?			
	Testing capability for SARS-CoV-2			
11.18	Are internal quality controls (IQC) specimens included when performing SARS-CoV-2 testing?	X		
11.19	Is there a procedure for recording and reporting IQC results for corrective action if not working properly?	X		
11.20	Are corrective actions implemented if IQC results are not acceptable?	X		
11.21	Does the laboratory participate in EQA for the SARS-CoV-2 test?	X		
11.22	Is there a system in place to record and assess EQA results?	X		
11.23	Are corrective actions implemented if EQA results are not acceptable?	X		
11.24	Does the laboratory have arrangements with the SARS-CoV-2 national reference laboratory for referring specimens for confirmation of results, or with a WHO COVID-19 reference laboratory as applicable?			
11.25	Are personnel trained in troubleshooting SARS-CoV-2 PCR results and a change in assay performance?			

Comments:

Annexure 16: Assessment Sheet for RT-PCR Facilities

Name of the laboratory/institute:

Date of assessment:

Number and model of RT-PCR machine:

Number and model of automated extractor (if applicable):

Number and model of centrifuge:

S. No.	Assessment parameter	Observations	Remarks
A. **Specimen receiving, transport and storage**			
1.	Is there a dedicated counter for receiving samples?		
2.	Does the person receiving the samples have adequate PPE?		
3.	Is the sample secured adequately after receiving it?		
4.	Is the specimen accession process adequate? Please share the document of the same.		
5.	Is the sample storage appropriate in case of delay of sample processing		
6.	Do the laboratory personnel check proper packing of the sample, and cold chain maintenance before starting the test?		

7.	Does the laboratory have defined sample rejection criteria? Please share the rejection criteria		
8.	Are the sample rejection criteria appropriately implemented?		
B. **Laboratory design and biosafety**			
1.	Does the laboratory have a dedicated donning and doffing area?		
2.	Is the doffing area located nearby to the laboratory and workers do not have to travel a considerable distance for doffing?		
3.	Are biomedical waste bins/bags in the doffing area adequate?		
4.	Is the ventilation of the doffing area appropriate?		
5.	Is the poster for the donning sequence displayed inside donning area?		
6.	Is the poster for the doffing sequence displayed inside the doffing area?		
7.	Is the doffed PPE pre-treated before moving out of the department?		

8.	Is there a spill kit and posted spill response instructions in every laboratory? Please share the picture of the spill kit and that of the displayed spill management protocol.		
9.	Is a first aid kit available in the department? Please share the picture showing content		
10.	Is the ventilation system of the laboratory appropriate?		
11.	How many BSC II A2s are available in the laboratory?		
12.	Is the BSC Class II A2 functional?		
13.	Is the available BSC calibrated? Please share the date of last calibration.		
14.	Is a maintenance label present on the equipment? Please share the next due date of calibration.		
15.	Do the staff check airflow inside the biosafety cabinet daily? Smoke test record to be shared		
16.	Is there traceability of results from accession to the final dispatch of the report?		
17.	Are log books signed daily by qualified personnel preferably doctors?		

18.	Do the test results reporting mechanisms ensure timely and safe dissemination of reports?		
19.	Does the laboratory have a mechanism to ensure timely logging of reports on the ICMR portal? Is TAT being monitored		
20.	Is there a mechanism to perform a risk assessment of laboratory personnel? Share risk assessment format.		
21.	Are the mechanisms for labelling and storage of aliquoted specimens safe and adequate?		
22.	Does the laboratory have adequate internet connectivity?		
23.	Is the cleaning protocol for the laboratory documented and implemented?		
C. **Kits, reagents and consumables**			
1.	Are adequate consumables for performing the test available? Mention current stock status		
2.	Are the kits and consumables stored at the appropriate temperatures?		
3.	Are adequate consumables for BMW management available? Mention current stock status		

D. **Biomedical waste management**			
1.	Is biomedical waste (BMW) generated in the laboratory stored in appropriately coloured BMW bags?		
2.	Is BMW transported in double bags as per BMW guidelines?		
3.	Are BMW bags appropriately labelled as per BMW guidelines?		
4.	Is all BMW from the laboratory pre-treated before moving out of the facility?		
5.	Is all biomedical waste stored safely with appropriate signage before handing it over to CBMWTF?		
6.	Does the laboratory/ institute have authorization from DPCC for generation, collection, storage, transportation and disposal of BIO medical waste? Mention validity		
E. **Training and education**			
1.	Is the staff performing the tests sensitized in GLP and biosafety?		
2.	Is the staff trained for procedures and safe use of equipment?		
F. **Other information**			

1.	Date of the last calibration of equipment/s		
2.	Does the laboratory maintain equipment-downtime logs?		
3.	Is the institution using RT-PCR application for sample processing? If not, please mention the reason?		
4.	Are reports uploaded to the ICMR without any delay?		
G. **Final comments**			
H. **Recommendation**			

Annexure 17: Assessment sheet for point-of-care testing (POCT) facilities

Name of the laboratory/institute:

Date of assessment:

Number and model of POCT machine (CBNAAT/Truenat):

S. No.	Assessment parameter	Observations	Remarks
A. **Specimen receiving, transport and storage**			
1.	Is there a dedicated counter for receiving samples?		
2.	Does the person receiving the samples have adequate PPE?		
3.	Is the sample secured adequately after receiving it?		
4.	Is the specimen accession process adequate? Please share the document of the same.		
5.	Is the sample storage appropriate in case of delay of sample processing		
6.	Do the laboratory personnel check proper packing of the Sample, and cold chain maintenance before starting the test?		

7.	Does the laboratory have defined sample rejection criteria? Please share the rejection criteria		
8.	Are the sample rejection criteria appropriately implemented?		
B. **Laboratory design and biosafety**			
1.	Does the laboratory have a dedicated donning and doffing area?		
2.	Is the doffing area located nearby to the laboratory and workers do not have to travel a considerable distance for doffing?		
3.	Are biomedical waste bins/bags in the doffing area adequate?		
4.	Is the ventilation of the doffing area appropriate?		
5.	Is the poster for the donning sequence displayed inside donning area?		
6.	Is the poster for the doffing sequence displayed inside the doffing area?		

7.	Is the doffed PPE pre-treated before moving out of the department?		

8.	Is a spill kit and posted spill response instructions in every laboratory? Please share a picture of the spill kit and that of the displayed spill management protocol.		
9.	Is a first aid kit available in the department? Please share a picture showing content		
10.	Is the ventilation system of the laboratory appropriate?		
11.	How many BSC II A2s are available in the laboratory?		
12.	Is the BSC Class II A2 functional?		
13.	Is the available BSC calibrated? Please share the date of last calibration.		
14.	Is a maintenance label present on the equipment? Please share the next due date of calibration.		
15.	Do the staff check airflow inside the biosafety cabinet daily? Smoke test record to be shared		
16.	Is there traceability of results from accession to the final dispatch of the report?		

17.	Are log books signed daily by qualified personnel, preferably doctors?		
18.	Do the test results reporting mechanisms ensure timely and safe dissemination of reports?		
19.	Does the laboratory have the mechanism to ensure timely logging of reports on the ICMR portal? Is TAT being monitored		
20.	Is there a mechanism to perform a risk assessment of laboratory personnel? Share risk assessment format		
21.	Are the mechanisms for labelling and storage of aliquoted specimens safe and adequate?		
22.	Does the laboratory have adequate internet connectivity?		
23.	Is the cleaning protocol for the laboratory documented and implemented?		
C. **Kits, reagents and consumables**			
1.	Are adequate consumables for performing the tests available? Mention current stock status		
2.	Are the kits and consumables stored at the appropriate temperatures?		

3.	Are adequate consumables for BMW management available? Mention current stock status		
D. **Biomedical waste management**			
1.	Is biomedical waste (BMW) generated in the laboratory stored in appropriately-coloured BMW bags?		
2.	Is BMW transported in double bags as per BMW guidelines?		
3.	Are BMW bags appropriately labelled as per BMW guidelines?		
4.	Is all BMW from the laboratory pre-treated before moving out of the facility?		
5.	Is all biomedical waste stored safely with appropriate signage before handing it over to CBMWTF?		
6.	Does the laboratory/ institute have authorization from DPCC for generation, collection, storage, transportation and disposal of BMW? Mention validity		
E. **Training and education**			
1.	Is the staff performing the tests sensitized in GLP and biosafety?		

2.	Is the staff trained for procedures and safe use of equipment?		
F. **Other information**			
1.	Has the engineer paid an inspection visit for testing the functioning of the CBNAAT machine? Are record of the visit maintained?		
2.	Is the institution using an RT-PCR application for sample processing? If not, please mention the reason?		
3.	Are reports uploaded to the ICMR without any delay?		
G. **Final comments**			
H. **Recommendation**			

Chapter 11 References

1. NABL. Assessment Forms and Checklist (based on ISO 15189:2012) 2021 [Available from: https://nabl-india.org/nabl/file_download.php?filename=202102191216-NABL-217-doc.docx.
2. ICMR. ICMR Guidelines for Good Clinical Laboratory Practices (GCLP) 2021 2021 [Available from: https://ethics.ncdirindia.org/asset/pdf/GCLP_Guidelines_2020_Final.pdf.
3. WHO. Laboratory assessment tool for laboratories implementing SARS-CoV-2 testing 2020 [Available from: https://www.who.int/publications/i/item/laboratory-assessment-tool-for-laboratories-implementing-covid-19-virus-testing.

Annexure 18: Standard operating procedure for sample collection

FIND Because diagnosis matters	**LABORATORY STANDARD OPERATING PROCEDURES**			
Country and Lab Demo Site	India	**DOCUMENT NUMBER**	**VERSION**	**EFFECTIVE DATE**
		SOP-001	1.0	
PROCEDURE	**Specimen collection and transport of clinical specimens**			

Approved By	Name, Title		Signature	Date
SOP Annual Review	Name, Title		Signature	Date
Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)	
Distributed Copies to	Name (or location)	# of copies	Name (or location)	# of copies

I acknowledge that I have read, understand and agree to follow this SOP.

Name (print)	**Signature**	**Designation**	**Date**

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1. Purpose

To describe the procedure of collection of clinical specimens and the transport from the sample collection centres 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 particular 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

3.3 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

4. 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 2-8ºC.

4.1 Patient preparation

The first step in the 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:

4.1.1 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.

4.1.2 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.

4.1.3 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 paediatric patients, they may be accompanied by their parents during the collection. Where appropriate, sensitivity to cultural and social issues, as well as concern for modesty, is important in providing psychological support.

4.1.4 Instructions regarding pretesting preparations

Instructions should be given 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.

4.1.5 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.

4.2 Sample collection

4.2.1 Nasopharyngeal swab

- Tilt patient’s head back 70 degrees
- Insert swab (sterile dacron/nylon flocked) into nostril (the 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

4.2.2 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

4.2.3 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)
- The 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

4.2.4 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

4.2.5 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.

4.3 Labelling

Each sample should be clearly labelled with:

- The patient's first and last name, with consent
- A unique barcode generated by the hospital for sample labelling or UHID number
- The time and date of collection

- The initials of the person-in-charge

4.4 Specimen storage:

The storage of specimens is to be done as per Table 2.

Table 2: Specimen collection

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[1]

4.5 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.

4.5.1 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 contents from outside influences such as physical damage and water while in transit.

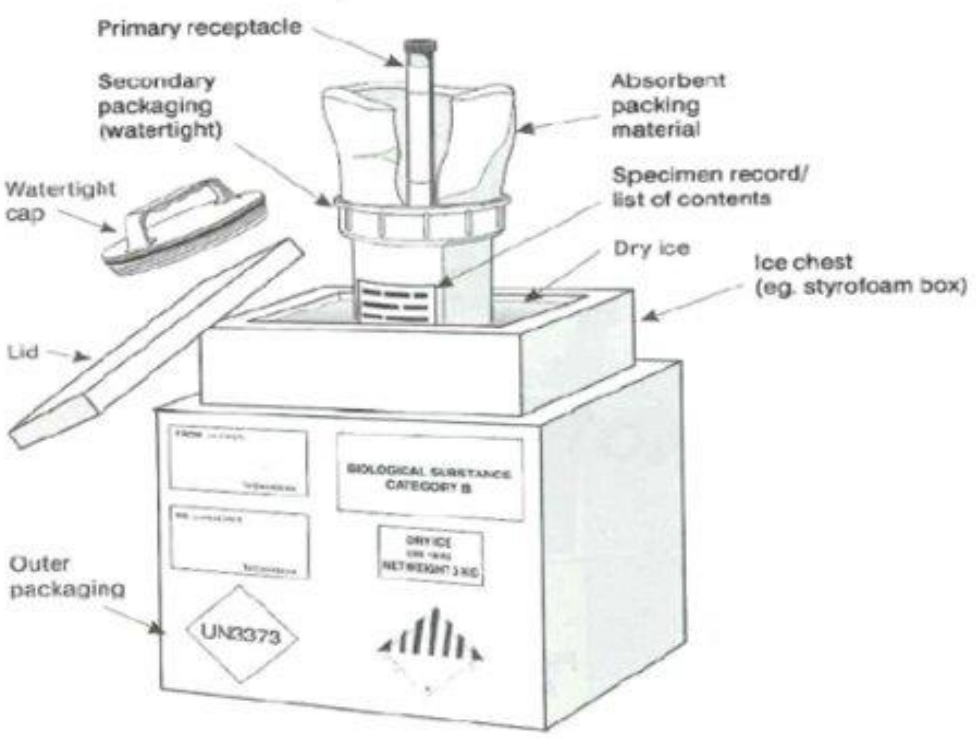

Figure 1: Triple layer packaging

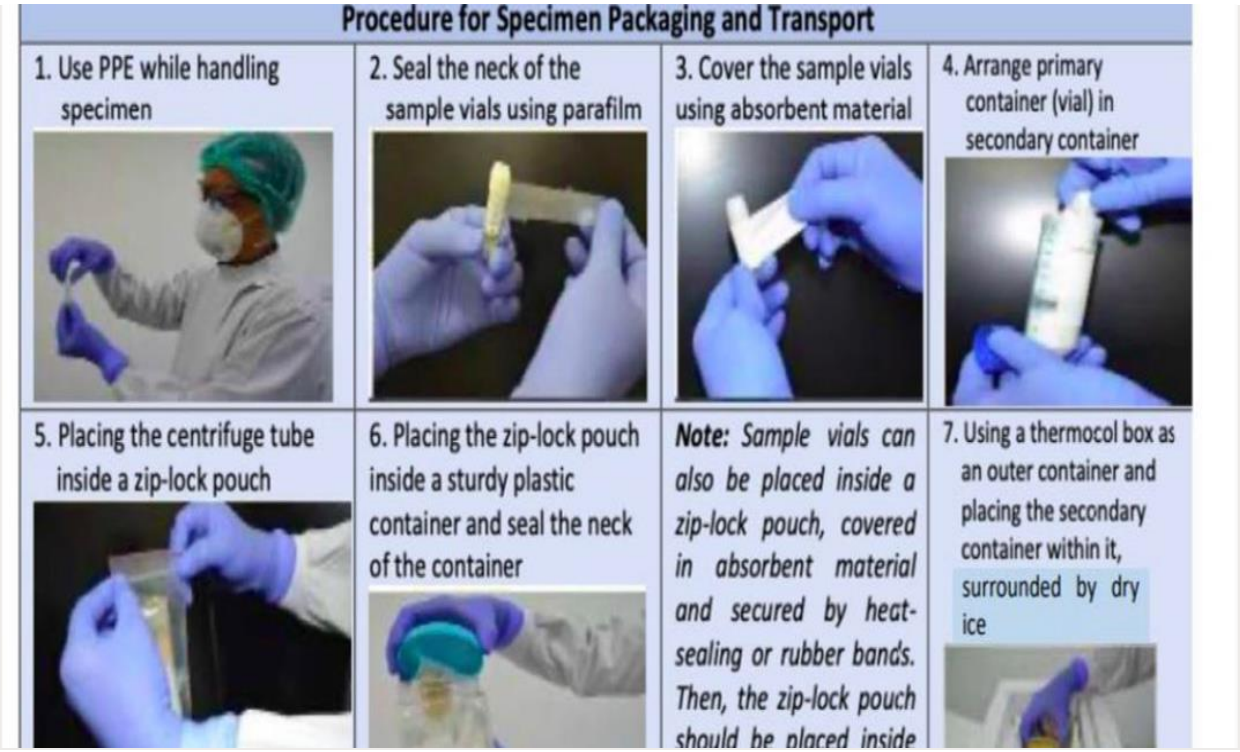

Figure 2: Procedure for specimen packaging and transport – part 1

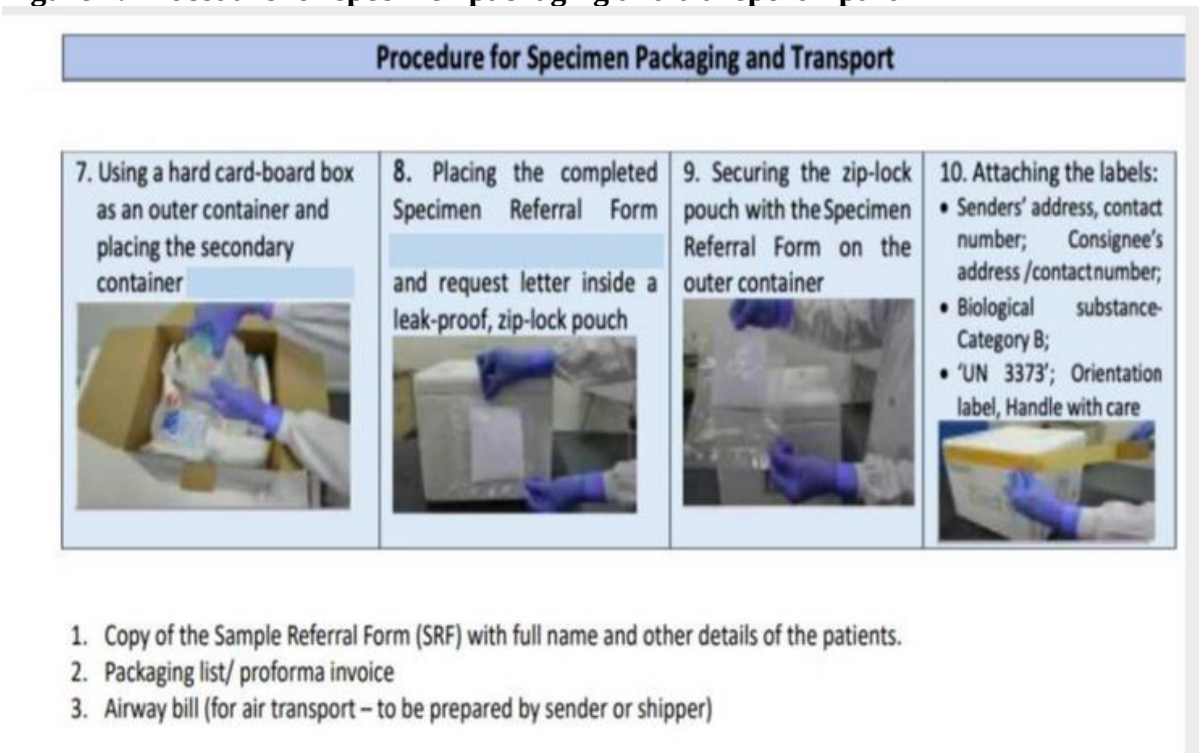

Figure 3: Procedure for specimen packaging and transport – part 2

Packing and transport of specimens for whole genome sequencing (to the Regional Genome Sequencing Laboratory (RGSL))

- Only those samples that 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°C), which are RT-PCR positive (Ct value <30), should be transported in VTM with a cool pack (4-8°C) or in ice.
- Alternatively, *remaining RNA samples may be stored and aliquoted in 1.5-mL micro-centrifuge tubes followed by proper labelling and sealing with parafilm (stored at -70°C).*
- *RNA samples should be placed together in a plastic/cardboard cryobox and packed in a thermocol box with dry ice and be shipped to the respective RGSL for sequencing*.
- Samples should be packaged and transported in standard triple packaging with all biosafety precautions and should be accompanied with a line list and details of samples, including the Ct values of all target genes detected.
- The line list with the required details that must accompany the samples or RNA that are sent to the RGSL is shown in Figure 4.

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)

Figure 4: Line list of details to be sent to the WGS laboratory

4.6 Spill management

Refer to the chapter on spill management

4.7 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 to chapter on Biomedical Waste Management.

Reference

1. ICMR-NIV SOP for specimen transport: https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20%202019-nCoV.pdf

Annexure 19: Standard operating procedure for CBNAAT

FIND Because diagnosis matters	**LABORATORY STANDARD OPERATING PROCEDURES**			
Country and Lab Demo Site	India	**DOCUMENT NUMBER**	**VERSION**	**EFFECTIVE DATE**
		SOP-002	01	
PROCEDURE	**Detection of SARS-CoV-2 using CBNAAT**			

Approved By	Name, Title		Signature	Date
SOP Annual Review	Name, Title		Signature	Date
Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)	
Distributed Copies to	Name (or location)	# of copies	Name (or location)	# of copies

I acknowledge that I have read, understand and agree to follow this SOP.

Name (print)	**Signature**	**Designation**	**Date**

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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 the 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 SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/aspirate specimens collected from individuals suspected of having 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, probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory tract 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.[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 immunosuppressed individuals away from the high-risk areas of the COVID laboratory should be considered.
- If 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 droplet and 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 and 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 the laboratory
- Procedures to be performed
- Waste handling
- Importance of laboratory results for individual patient and COVID pandemic management

4. Responsibilities

All staff members trained to use the GeneXpert System working in the laboratory are responsible for the implementation of this Standard Operating Procedure.

Laboratory Technicians or Consultants who are trained, certified and designated by the laboratory to collect and process infectious specimens.

5. Pre-analytic procedure

Refer to the following procedures

1. Specimen collection and transport
2. Specimen accessioning, aliquoting and long-term storage

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

5.1 Equipment

GeneXpert instrument - Cepheid GeneXpert GX-IV

Xpert Xpress SARS-CoV-2 1X10 tests kit per box

Computer and barcode scanner - Operator manual

5.2 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

5.3 Storage and handling

- Store 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.

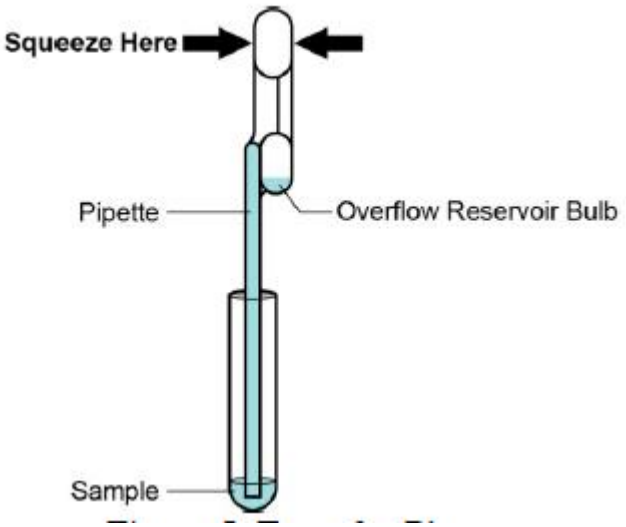
5.4 Calibration procedures (metrological traceability)

- Equipment calibration: Once per year

6. Analytic procedures

6.1 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 five times. Open the 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 the pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (as shown in the above diagram). 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 (sample chamber) in the cartridge shown in Figure 6. Dispose of the used pipette.

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.

- 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.

6.2 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 username 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.

6.3 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.

6.4 Quality control procedures

6.4.1 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. 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.

6.4.2 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
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*; all or one of the probe check results fail *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

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, LoD)

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 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 (%)	Hit Rate (%)	Mean Ct	Mean Ct
			N2 Target	E Target	N2 Target	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 individually mapping primers and probes in the Xpert Xpress SARS-CoV-2 test to 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 chapter on spill management for the handling of infectious spills.

11. 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 a biosafety cabinet. Filled biohazard bags should be tied inside the biosafety cabinet with a 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 a 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 the 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: chapter on Biomedical Waste Management for detailed protocols.

Reference

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

Annexure 20: Standard operating procedure for Truenat

FIND Because diagnosis matters	**LABORATORY STANDARD OPERATING PROCEDURES**			
Country and Lab Demo Site	India	**DOCUMENT NUMBER**	**VERSION**	**EFFECTIVE DATE**
		SOP-003	01	
PROCEDURE	**Detection of SARS-CoV-2 using Truenat**			

Approved By	Name, Title		Signature	Date
SOP Annual Review	Name, Title		Signature	Date
Revision History	Version # [0.0]	Revision Date [dd/mm/yy]	Description (notes)	
Distributed Copies to	Name (or location)	# of copies	Name (or location)	# of copies

I acknowledge that I have read, understand and agree to follow this SOP.

Name (print)	**Signature**	**Designation**	**Date**

Table of contents

1. Purpose

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

2. Introduction

Truenat SARS-CoV-2 testing kits work on the same principle as real-time reverse transcription polymerase chain reaction (RT-PCR) based on TaqMan chemistry.[1] Truenat is highly sensitive and has a very low limit of detection. The procedure is carried out after viral RNA has been extracted from ICMR-approved specimen types, ideally in a BSL-2 equivalent facility. Adherence to good laboratory practices is critical for quality test results.

3. 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 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

4. Knowledge & responsibilities

Knowledge

The laboratory 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 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. 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)

6. Equipment and materials

6.1 Truenat equipment

- 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.

- 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

6.2 General equipment

- Powder free disposable gloves,
- Waste disposal container with lid
- Autoclave
- Biosafety cabinet (optional)
- Refrigerators
    - -70/ -80°C, -20°C freezers
    - 4°C refrigerators
    - Benchtop minifuge

6.3 Reagents and disinfectants

- RNAse Zap
- Truenat Covid -19 RT- PCR Kit that contains
    - Trueprep Auto Universal Extraction Kit
    - Truenat Covid 19 duplex Detection Kit
    - Universal Control Kit Panel 1
    - Should be stored at the temperature recommended by the manufacturer and always protected from light. The reagents should be aliquoted to avoid repeated freeze–thawing.
- Nuclease-free water
- Reagent-grade ethanol and isopropanol
- Absolute alcohol
- Sodium hypochlorite
- Biohazard bags (red and yellow)

6.4 Personal protective equipment (PPE)

- Laboratory gowns
- Surgical/medical masks/N95 masks
- Gloves
- Shoe covers
- Hair covers

7. Analytic procedure

7.1 Principle

Truenat SARS-CoV-2 testing kits work on the same principle as real-time reverse transcription polymerase chain reaction (RT-PCR) based on Taqman chemistry. The RNA from the patient sample is first extracted using the 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 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.

7.2 Sample processing protocol on Trueprep® AUTO (Figure 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

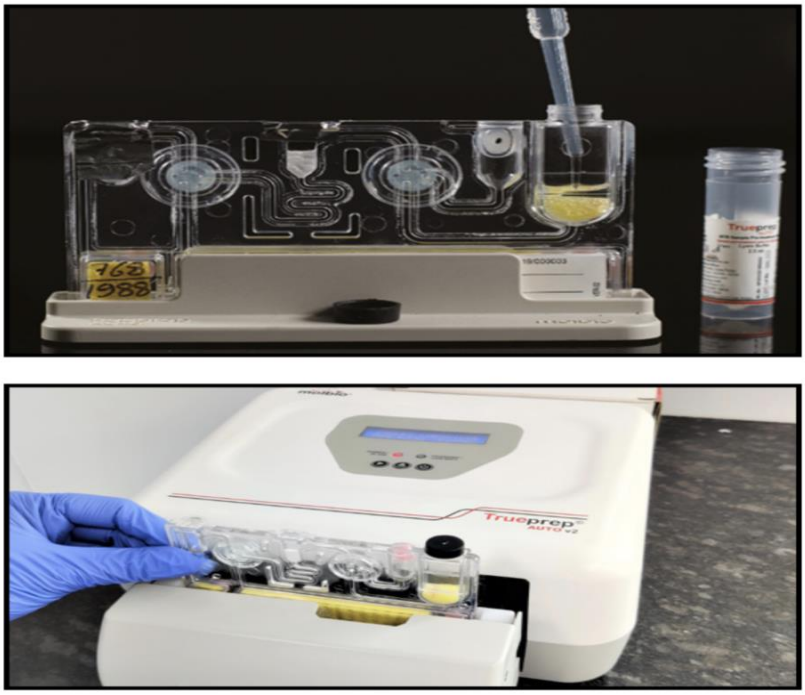
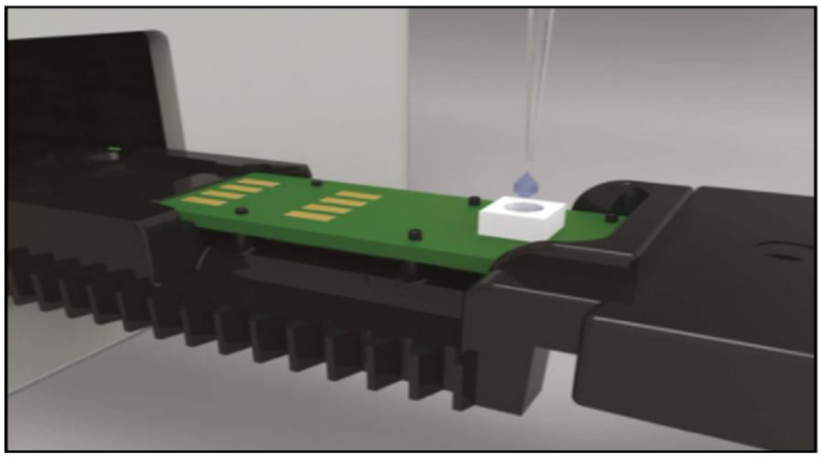


Figure 1: Trueprep® AUTO

7.3 Testing

1. Switch on the Truelab™Analyzer
2. Select User and enter password.
3. Select the test profile for "H1N1" to be run from the TM Profiles Screen on the Analyzer screen.
4. Enter the patient details i.e. Patient Name, Patient ID and Age in the Truelab™Analyzer screen.
5. Press Start Reaction.
6. Press the eject button to open the chip tray.
7. Open a pouch of Truenat™ Covid 19 and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Label the tube with the patient ID using a marker pen on the microtube.

9. Place the Truenat™ Covid 19 chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly (Figure 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.
11. Note:- Do not mix it by tapping, shaking or by reverse pipetting.
12. Using the same filter barrier tip, pipette out 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 of the microtip in 1% hypochlorite solution.
13. Slide the chip tray containing the Truenat™ Covid 19 chip™ based Real Time PCR test loaded with the sample into the TruelabAnalyzer™. Press Done on the "Please Load Sample" Alert message
14. Read the result from the screen.
15. After the reaction is completed, for Truelab Uno Dx, push the Eject button to TM eject the chip tray.
16. Take out the Truenat™ Covid 19 chip-based Real Time PCR test at end of the test and dispose of it in 1% hypochlorite.
17. Turn on Truelab™ micro PCR printer and select print on the screen for printing out a hard copy of the results. Test results are automatically stored and can be retrieved at any time later.
18. Switch off the Truelab™Analyzer or repeat steps 3 to 16 to run another sample.



Transferring the solution to a Truenat chip

Figure 2: Loading the Truelab Analyzer

7.4 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.

7.5 Data collection

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

7.6 Results and 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 must 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 32 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

The Dos and Don'ts of Truenat testing should be followed appropriately to produce quality results (Annexure 2).

8. 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 is to be used as control material.

Run one positive and one 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.

9. Waste management and contamination control

9.1 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.

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

i. Always properly clean work areas after completion of tasks (Annexure 1)
ii. Establish regular (e.g. weekly) and thorough laboratory cleaning protocols (floors, doors, walls)

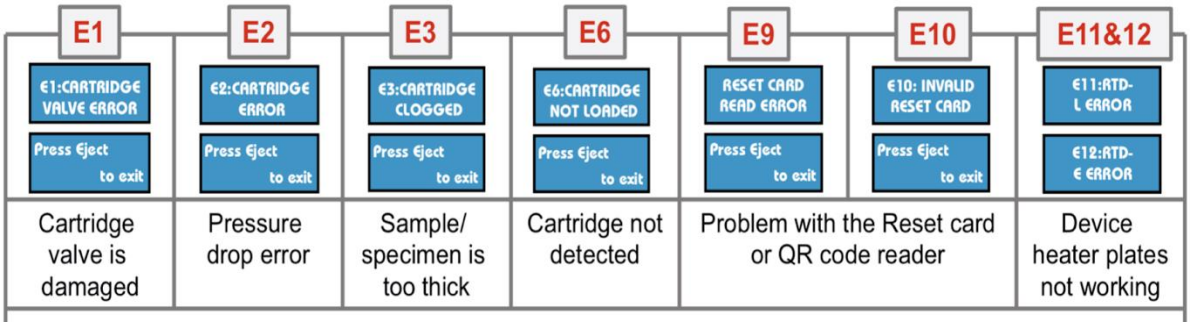
9.2 Contamination Control

i. 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 (a 10-times dilution of 5% sodium hypochlorite (household bleach)) before continuing work.

ii. 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 of as potentially biohazardous waste e.g. in a biohazard waste container.

10. Troubleshooting

Trueprep Auto v2 Universal Cartridge based Sample Prep Device[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 elute 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.

11. Limitations

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 probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.

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.

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.

References

1. Truenat™ Operating Manual
   https://www.molbiodiagnostics.com/uploads/product_download/20211115.173628~Truenat-COVID-19-packinsert-VER-04.pdf
2. Practical Guide to Implementation of Truenat™ Tests
   http://stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf

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:

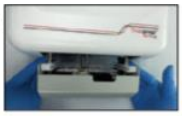
Indicate completion of an activity by writing your initials in the corresponding date box.

- 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

Dos and Don'ts of Truenat testing

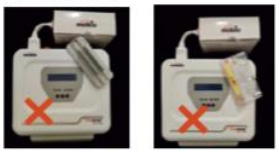
Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

- ✓ 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

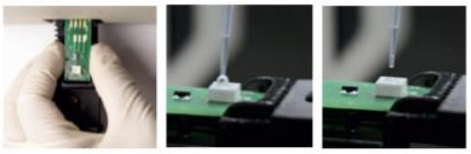


- ✗ Do not tilt or shake the device while connecting charger, during extraction or while removing cartridge from device
- ✗ Do not touch the nozzle of the liquefaction buffer bottle to the lysis buffer tube during sample preparation

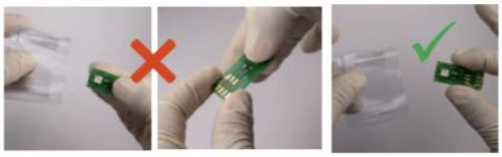
Truelab® Uno Dx/Duo/Quattro Real Time Quantitative micro PCR Analyzer

- ✓ 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