

COVID-19 - Sample collection guidelines

Indian Council of Medical Research – National Institute of Epidemiology

Scope of this document:

To be used by hospitals involved in collecting samples for COVID-19 testing

Purpose:

This document gives information on sample collection, packing and transport to laboratory for COVID-19 testing

Responsibilities:

- Clinician:
 - To identify the appropriate patient for COVID-19 testing
 - To identify the appropriate specimen to be collected
- Lab technician
 - Should have received appropriate training for sample collection from suspected SARI/COVID-19 cases
 - To collect appropriate samples as decided by the treating doctor
 - To wear proper PPE as prescribed by NIV Pune
 - To strictly follow biosafety measures during collection and packing of samples and to follow the biomedical waste management guidelines for safe disposal of generated bio-waste.

Selection of patient for testing:

- Any Patient who satisfies the testing “Criteria for SARI/COVID-19” as prescribed by State or Central Government.
- Any patient identified by “As a suspected case of SARI/ COVID-19” the treating doctor

Details of sample collection materials and temperature during transport/ storage (till testing) of different samples from suspected SARI/ COVID cases.

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 flocced 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.*

Steps to follow-up during the sample collection:

1. Materials needed for the sample collection: (Nasal & Throat swab):

S.No.	Item	Description/Details (if any)	Cat no. (If any)
1	Viral transport medium (VTM) #	Hi-Media	AL167
2	Polyester tipped plastic shaft swab *	Fisher Brand	23-400-111
		Hi-Media	PW1180
3	Flocked Swab *	Hi-Media	PW1172-1X500NO
		Copan	Ref 519CS01
4	Tongue depressor	Disposable	
5	Face mask	N95	
6	Apron	Disposable apron	
7	Gloves	Disposable Nitrile Gloves	
8	Hand sanitizer	Alcohol based rub	
9	Discard bag	Appropriate Biohazard Discard bag	
10	Cool box	Thermocol box	
11	Gel packs	To be frozen at -20°C prior use	
12	Head cap	Disposable	
13	Goggle	Reusable lab goggle	
14	Brown tape	Sealing the sample box	
15	Surgical spirit	Disinfect sample collection area	
16	Cryo label	Sample Labelling	

- **# VTM Precautions:** Ready to use VTM to be used within the expiry date. Always check for turbidity or any growth in VTM prior to use for sample collection or before dispatch of VTM to surveillance site. If any turbidity or growth is found, please discard the whole batch of VTM.
- *Use of cotton-tipped or calcium alginate swabs or swabs with wooden shafts is **NOT RECOMMENDED**.

2. Steps to be followed for specimen collection using aseptic method:

1. Nasal swab and/or throat swab specimens is to be collected as per GOI guidelines.
2. Sample collection area should be kept clean and table or work surface should be properly disinfected with surgical spirit.
3. Collect required details from the patient and fill the sample collection form.
4. Ensure that patient is seated in a comfortable seating position.
5. Clearly explain the sample collection procedure to ensure full cooperation from the patient.
6. Wear appropriate personal protective equipment (PPE) such as gloves, apron, N95 mask, head cap, goggles before starting the procedure.
7. Label the specimen collection vial containing VTM with the unique participant/ sample ID.
8. Specimen should be collected under good illumination.

9. Throat/ oro-pharyngeal swab collection

- a. Gently tilt the patient's head back
- b. Steady the chin
- c. Ask the patient to open his/her mouth
- d. Use a disposable tongue depressor to hold the tongue well
- e. Insert a sterile swab
- f. Swab both the tonsils and the posterior pharynx vigorously with a rotating motion, till the patient starts to gag
- g. Remove the swab without touching the tongue.
- h. The swab is then placed in the labelled tube containing VTM
- i. The applicator stick is broken off at the indicated mark (if provided) or at below the level of the tube opening
- j. Close and tightly screw cap the tube.

10. Nasal swab collection

- a. Take a fresh sterile swab
 - b. Gently tilt the patient's head Backwards and steady the chin
 - c. Insert the swab into the nostril parallel (1-2 cm) to the palate until the resistance is met at turbinate
 - d. Hold the swab in that position for few seconds and then withdraw slowly in a firmly rotating motion (5 times clockwise and 5 times anticlockwise).
11. Appropriate precautions should be taken in collecting specimens since this may expose the lab technician/ sample collector to respiratory secretions from the patient.
12. Specimens from both nostrils are obtained with the same swab vigorously, irrespective of nasal congestion if any.
13. The nasal swab is placed in the same VTM tube containing throat swab. The applicator stick is broken off as done for throat swab and the tube is screw capped tightly.
14. The vial is then placed in the cool box containing tube rack in between the frozen gel packs.

3. Sample handling at collection site:

1. A unique specimen ID is written/ pasted on each VTM sample by the lab technician.
2. VTM containing samples are to be kept in cool box immediately after collection.
3. VTM tube IDs are cross checked with the details in the filled sample collection form.
4. If the specimens cannot be sent to the laboratory within specified time frame (refer table on page 1), they should be stored at or below -70°C in ultra-low freezer.
5. Repeated freezing and thawing must be avoided.

4. Transportation of samples from field site to testing laboratory:

1. Samples should be transported at $2-8^{\circ}\text{C}$ within specified time frame to the testing laboratory.

2. Samples should be kept in proper standing position in appropriate test tube rack.
3. The VTM containing part of the tube should be in direct contact with frozen gel packs.

5. Precautions:

1. Always use personal protective equipment (PPE) e.g. Laboratory apron/ gown, face mask, gloves and goggles.
2. Wipe gloves thoroughly with a disinfectant (e.g., surgical spirit) before and after taking the sample.

Current testing modalities for laboratory diagnosis of COVID-19

1. Real Time PCR testing is the recommended testing for COVID-19 diagnosis.
2. Viral RNA is extracted from the Nasal/ throat swab specimen in VTM.
3. Initial **Screening RT PCR** involves detection of 'E' gene (coding for SARS-CoV-2 viral envelope)
4. **Confirmation** of samples positive in screening PCR involves detection of one of the following two gene targets:
 - RdRp gene (coding for SARS-CoV-2 RNA dependent RNA Polymerase)
 - ORF gene (coding for SARS-CoV-2 Open Reading Frame)