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1. PURPOSE

To describe the procedure of collection of clinical specimens and the transport from the sample collection centers to the diagnostic laboratory and Referral Labs.

2. INTRODUCTION

Detection of viral RNA from clinical specimens is the key to the diagnosis of SARS-CoV-2 infection. Recommended upper and lower respiratory specimens obtained from suspected SARS-CoV-2 infected individuals are collected in suitable transport medium and transported to the diagnostic laboratories in a triple layer packing at 2-8ºC. Specimens are stored at 4ºC till tested. The specimen storage recommendations by ICMR are shown in the image below:

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

3. PERSONNEL QUALIFICATIONS

3.1 Medical fitness

All personnel involved in sample receiving should be tested for COVID-19 beforehand.

3.2 Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Hygiene requirements
- Donning and doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials
- Workflow in the laboratory
- Waste handling
- Importance of laboratory results for patient management

3.3 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.

4. EQUIPMENT & MATERIALS

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

The Figure (Fig 1) below illustrates the required materials for collection and transport

5. PROCEDURE

PPE to be used: Lab coat, N95 mask, and gloves and goggles

Detailed Instructions:

Ensure the availability of requisite materials such as specimen forms, specimen registers, marker pens, disinfectants and biohazards bags in the specimen receiving room. Hand sanitizer should be available.

5.1 Sample Significance:

The sample collected from the patient should contain viable microorganism with minimal contamination.

Appropriate sample collection ensures quality laboratory test

The sample must be transported to the laboratory promptly.

5.2 **Patient preparation:**

The first step in laboratory or diagnostic procedure is patient preparation or patient teaching before the performance of the procedure.

This pretesting explanation to the patient or caregiver follows essentially the same pattern for all sites and types of studies and includes the following:

5.2.1 Statement of the purpose of the study. The level of detail provided to patients about the test purpose depends on numerous factors and should be individualized appropriately in each particular setting.

5.2.2 Description of the procedure, including site and method. It is a good idea to explain to the patient that you will be wearing gloves throughout the procedure. The explanation should help the patient understand that the use of gloves is standard practice established for his or her protection as well as yours. The collection procedure may require hand washing at the beginning and end of each specimen collection.

5.2.3 Description of the sensations, including discomfort and pain, that the patient may experience during the specimen collection procedure. Address concerns about pain related to the procedure and suggest breathing or visualization techniques to promote relaxation. For pediatric patients, the parents may be accompanied during the collection. Where appropriate, Sensitivity to cultural and social issues, as well as concern for modesty, is important in providing psychological support.

5.2.4 Instruction regarding pretesting preparations related to diet, liquids, medications, and activity as well as any restrictions regarding diet, liquids, medications, activity, known allergies, therapies, or other procedures that might affect test results. To increase patient compliance, the instructions should include an explanation of why strict adherence to the instructions is required.

5.2.5 Recognition of anxiety related to test results. Provide a compassionate, reassuring environment. Be prepared to educate the patient regarding access to the appropriate counselling services. Encourage the patient to ask questions and verbalize his or her concerns.

5.3 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 labeling or UHID number
- The time and date of collection
- The initials of the phlebotomist

5.4 Sample Collection:

5.4.1 **Nasopharyngeal wash/aspirate or nasal wash/aspirate (performed by a trained healthcare provider)**

- Attach catheter to suction apparatus.
- Tilt patient's head back 70 degrees.
- Instill 1 mL-1.5 mL of non-bacteriostatic saline (pH 7.0) into one nostril.
- Insert the tubing into the nostril parallel to the palate (not upwards). Catheter should reach depth equal to distance from nostrils to outer opening of ear.
- Begin gentle suction/aspiration and remove catheter while rotating it gently.
- Place specimen in a sterile viral transport media tube.

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

- Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.
- Due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/or fatal cases.

5.4.3 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.
- 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 universal, viral or Amies transport medium. If transport medium is not available, use sterile saline

5.5 Specimen storage and transport:

The storage of the specimens are to be done as per the table below

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

6. 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 2, 3 and 4.

6.1 Triple Packaging Procedure:

The packaging consists of three layers as follows.

- **Primary receptacle:** A labelled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.
- **Secondary receptacle:** A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.
- **Outer shipping package**: The secondary receptacle is placed in an outer shipping package which protects it and its content from outside influences such as physical damage and water while in transit.
  Fig 2:

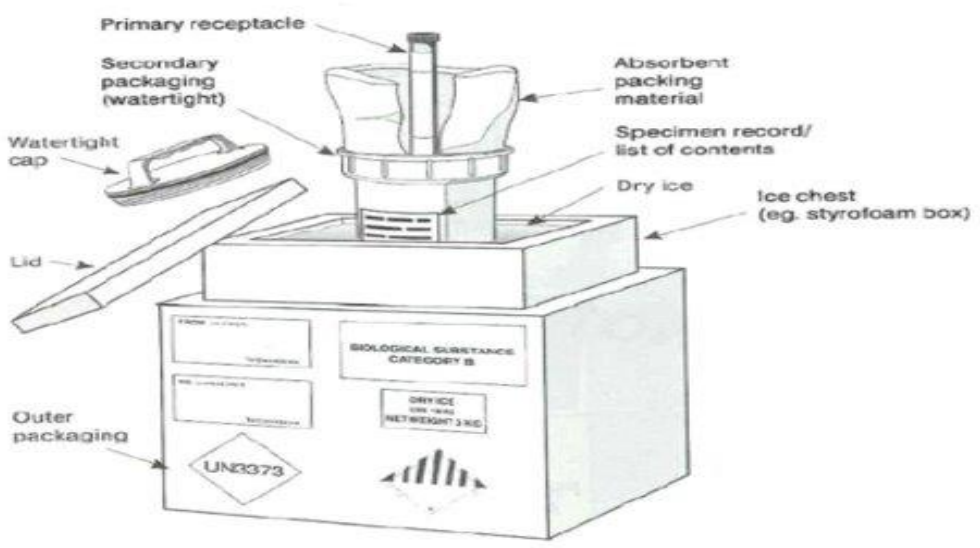


Fig 3: Procedure for Specimen Packaging and Transport

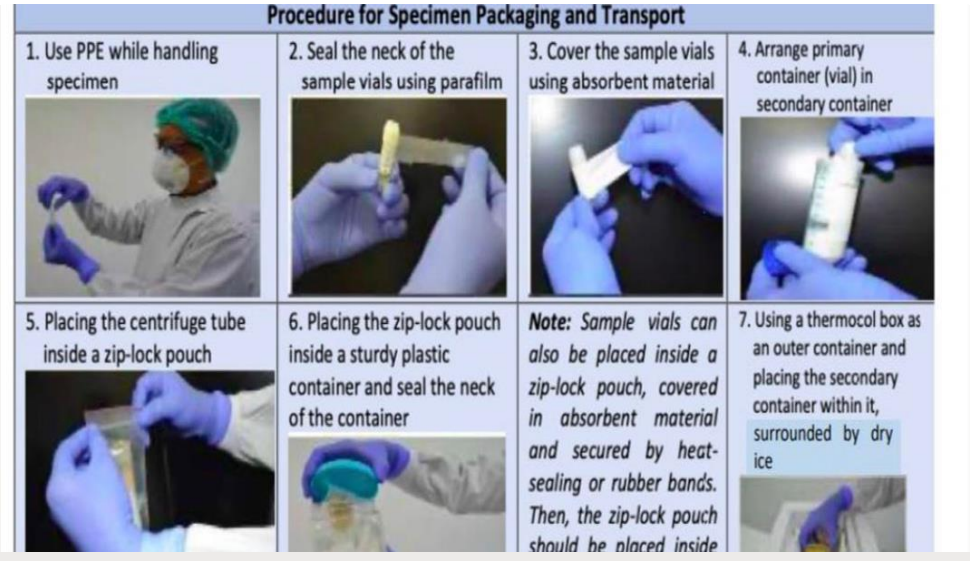
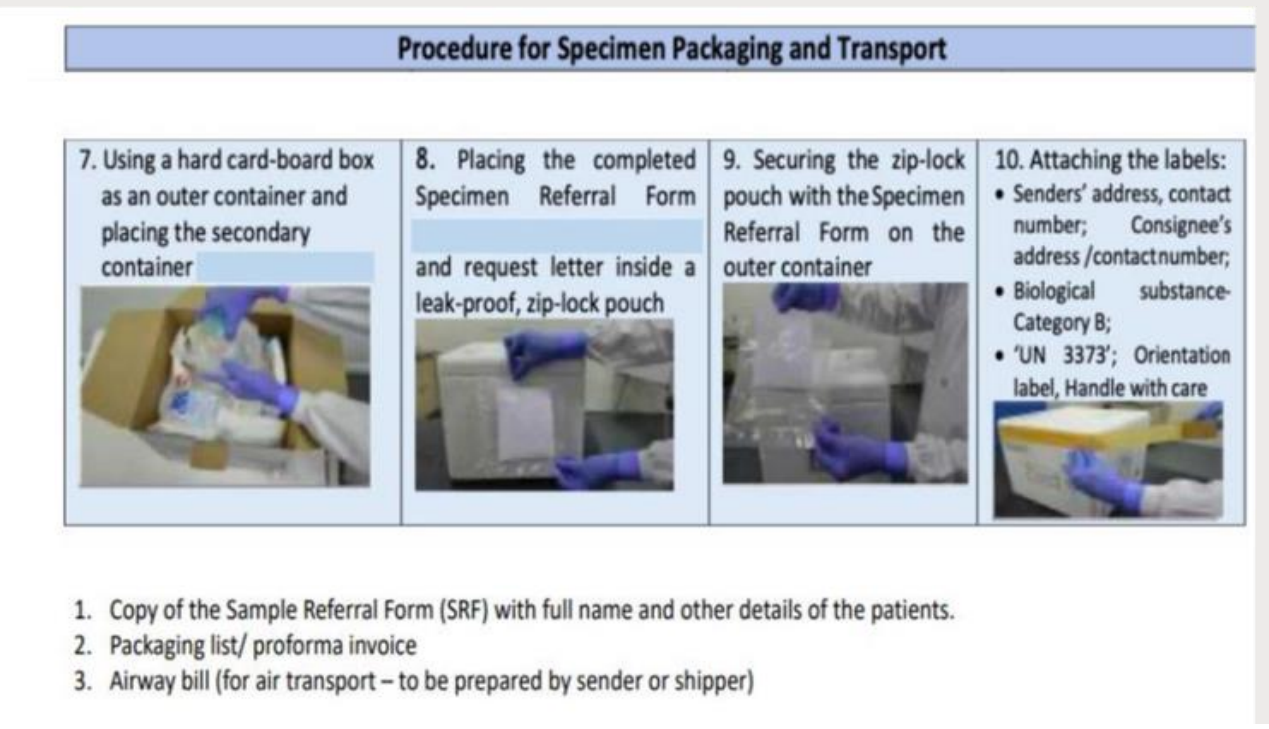


Fig 4: Procedure for Specimen Packaging and Transport



7. Guidelines for Specimens for Whole Genome Sequencing (Regional Genome Sequencing Laboratory(RGSL).

- Only those samples which are *positive for SARS-CoV-2 by RT PCR preferably with a Ct value of 25 or less* should be packaged & transported.
- After carrying out the RT-PCR test the remaining samples (within 72 hours of collection, stored at 2-8°), which are RT-PCR positive (Ct value <30), will be transported in VTM with cool pack (4-8 degree) or in ice.

- Alternatively, *remaining RNA samples may be stored and aliquoted in the 1.5 ml micro-centrifuge tubes followed by proper labelling and sealing with the parafilm (stored at -70degree).*
- *RNA placed together in plastic/ cardboard cryobox and packed in the thermocol box with dry ice should be shipped to the respective RGSL for sequencing*.
- Samples should be packaged and transported with all biosafety precautions and should be accompanied with line list and details of samples including the Ct values of all the target genes detected in standard triple packaging.
- Line list excel with the below details has to be accompanied along with the samples or RNA that are sent t RGSL

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

8. SPILL MANAGEMENT

Refer to the SOP #004 on spill management

9. WASTE MANAGEMENT

- All uninfected solid waste collected in the reception room should be discarded only in labelled discard bins.
- Removed PPE should be discarded in marked designated bins lined with biohazard bags.
- Biohazard bags containing soiled PPEs should be handed over to the Autoclave Team
- Refer SOP # 005 on Biomedical Waste Management.

10. REFERENCES:

1. Diagnostic testing for SARS-CoV-2: interim guidance, 11 September 2020: https://apps.who.int/iris/handle/10665/334254
2. ICMR-NIV SOP for specimen transport: https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20%202019-nCoV.pdf
3. ICMR Guidelines for Testing Strategy https://www.icmr.gov.in/pdf/covid/labs/Govt_labs_sample_retention_advisory_25062020.pdf

SOP CHANGE HISTORY:

New Version # (Date)	New Version # (Date)	No of Changes	Description of Changes	Source of Change Request