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## 1. PURPOSE

To describe the method for receiving the specimen box containing clinical specimens, their aliquoting and storage.

## 2. INTRODUCTION

Detection of viral RNA from clinical specimens is key to the diagnosis of SARS-CoV-2 infection. Recommended upper and lower respiratory specimens obtained from suspected SARS-CoV-2 infected individuals are collected in suitable medium and transported to the diagnostic laboratory. Once received by the laboratory, the specimens must be evaluated for acceptance criteria and aliquoted for further use by the specimen processing team. Proper recording of the specimen receipt and appropriate storage of specimens till the completion test procedure, is the key for a successful test results.

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**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<br>>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<br>>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<br>>48 hours: –70 °C | Not applicable |
| Sputum | sterile container | 4 °C | ≤48 hours: 4 °C<br>>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<br>>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<br>>5 days: –70 °C | Collect paired samples:<br>• acute – first week of illness<br>• 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 (2)

## 3. PRINCIPLE

5.1 Specimens collected from suspected cases of SARS-CoV-2 infection are transported in a triple layer packing to the laboratory at 2-8°C (1). All specimens received in the laboratory should be considered as "potentially infectious" and handled appropriately. The specimen box is opened in a Bio Safety Level 2 (BSL-2) equivalent laboratory inside a certified biosafety cabinet (BSC) equipped with a Virus burnout Unit (Optional). The specimen must pass acceptance criteria in order to be eligible for receiving by the laboratory and for further processing. Specimen must be processed within the recommended time frame when stored at 2-8°C (As shown in the image above). They should be aliquoted and frozen at -70/ 80°C for long-term storage (2, 3).

### 3.1 Specimen acceptance criteria

3.1.1 Specimen is properly labeled with unique identification number and date

3.1.2 Specimen label matches (unique identification number and date) with the request form/ online details received

3.1.3 Specimen is received at recommended temperature (2-8°C)

### 3.2 Specimen rejection criteria

3.2.1 Viral transport media (VTM) tube content has leaked

3.2.2 The Specimen Referral Form is soiled with leaked content

3.2.3 At least one of the two unique patient identifiers missing/mismatching on specimen tube and Specimen Referral Form

3.2.4 Specimens not transported with ice packs or the ice packs not cold (2-8°C)

## 4. PERSONNEL QUALIFICATIONS

### 4.1 Medical fitness

- All personnel involved in specimen receipt and handling should be tested for COVID-19 beforehand. Only those who test negative should be involved in performing the test procedure.
- Options for reassignment of personnel with COVID-19 comorbid conditions, such as diabetes, chronic respiratory diseases, high blood pressure, or immuno-suppressed individuals away from the high-risk areas of the COVID laboratory should be considered.
- If the resources are limited, they should be made aware of the risk of experiencing severe symptoms of the disease.

### 4.2 Education and training

Education and training must be given on the following topics:

- Potential risks to health (symptoms of COVID-19 disease and transmission)
- Precautions to be taken to minimize droplets & aerosol formation and prevent exposure
- Hygiene requirements
- Donning and Doffing of PPE
- Laboratory biosafety, specifically handling of potentially infectious materials
- Laboratory design, including airflow conditions
- Use of biological safety cabinets (operation, identification of malfunctions and maintenance)
- Use of autoclaves, microcentrifuge, micropipettes & refrigerators, (operation, identification of malfunctions and maintenance)
- Prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical and fire hazards)
- Good laboratory practice and good microbiological techniques
- Workflow in laboratory
- Procedure to be performed
- Waste handling
- Importance of laboratory results for individual patient and COVID pandemic management

## 5. RESPONSIBILITIES

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

## 6. CROSS REFERENCE

SOP#001 Specimen Collection and Transport of Clinical Specimens

## 7. EQUIPMENT & MATERIALS

### 7.1 Equipment

7.1.1 Certified Biosafety Cabinet Class II Type A2

7.1.2 Vortex mixer

7.1.3 -70°C/ -80°C (with free space for sample storage)

7.1.4 2 – 8 °C Refrigerator

7.1.5 Tabletop refrigerated centrifuge

### 7.2 Consumables

7.2.1 Transfer pipettes sterile
7.2.2 Absorbent liner
7.2.3 Sterile tweezers /forceps
7.2.4 Cryovials
7.2.5 Biohazard bags
7.2.6 Markers
7.2.7 Twist tag (to tie the bags)
7.2.8 Personal Protecting Equipment
7.2.8.1 Coverall/ Gown
7.2.8.2 Powder free nitrile Gloves, N95 masks,
7.2.8.3 Goggles
7.2.8.4 Shoe covers
7.2.8.5 Head cover/Hair cover
7.2.8.6 Face shield

**7.3 Disinfectants**

7.3.1 Ethanol/Isopropyl alcohol
7.3.2 Sodium Hypochlorite stock solution

## 8. PROCEDURE

Detailed Instructions:

Specimen box containing specimens is opened inside a certified biosafety cabinet (BSC Class II Type A2) in a BSL2 equivalent laboratory. (Note: *Class II BSC to be used for handling of COVID-19 should be tested and certified by a trained professional prior to use.)*

8.1 Don the PPE (cover all/ Lab gown, N95 mask, head cover, goggles, outer shoe cover, and two pairs of gloves) required for the procedure.

8.2 Review the checklist and ensure the availability of required consumables, worksheets, disinfectants and biohazards bags in the sample processing room.

8.3 After donning the PPE, transfer the specimen box from cold room/ sample reception area on a trolley to the specimen processing area.

8.4 If the PASS BOX is available, labeled cryo vials*, specimen reception log and specimen box should be taken inside the negative pressure room through the PASS BOX. Switched on the PASS BOX UV for 15 minutes.

8.5 All tubes should be labelled correctly and legibly with a permanent marker. For e.g., virus_year_site_serial number (choose the serial number carefully). nCOV_20_SITE_0001(tubes should be labelled included the following details i.e._sample ID_sample type_date of receipt_2 patient identifiers). Two tubes per sample should be labeled)

8.6 Turn on the BSC, let it run for 10 min and ensure the functionality check.

8.7 Turn on the refrigerated centrifuge.

8.8 Wipe the BSC work area with freshly prepared 1% Sodium hypochlorite and give a contact time of 15 minutes. To remove hypochlorite residue that may corrode the BSC surfaces, thoroughly clean the surfaces with 70% ethanol. Turn on the UV for 15-20 min (optional).
(Note: Do not spray 1% sodium hypochlorite inside the BSC, instead soak the paper towel outside the BSC and use them to wipe the surfaces.

8.9 Spread the absorbent liner inside the biosafety cabinet and prepare the work area. Bring in the biohazard bags for solid waste and container for liquid waste. Bring in a container with appropriate amount of 1% sodium hypochlorite for rinsing the Pasteur pipettes and VTM tubes. Bring in the other required items on your checklist.

8.10 Arrange items in such a way that work area is divided into a clean area, central work area and dirty area.

8.11 Bring the specimen box to BSC.

8.12 Open the specimen box and take out all the VTM tubes containing the specimen and place on a stand.
8.13 Disinfect the inner side of the specimen box with freshly prepared 1% sodium hypochlorite solution and take it out of the BSC.
8.14 Carefully wipe the outer side of the VTM tubes with 1% sodium hypochlorite.
8.15 Arrange the VTM tubes as per the order in the specimen reception log.
8.16 Verify the specimen labels with the specimen reception log sheet.
8.17 Apply the specimen acceptance and rejection criteria as stated in Section 3.
8.18 Mark the RNA ID (Lab ID) of the specimen on top of the VTM tubes. Place the tubes on a tube rack in serial order.
8.19 Vortex the VTM tubes for 30 seconds to ensure release of specimen from the swab into the VTM.
8.20 Bring in the centrifuge safety cups inside the BSC and load the VTM tubes. Make sure the VTM tube caps are tightly secured. Wipe the centrifuge cups with disinfectant and take them out to the centrifuge for spin.
8.21 Spin the VTM tubes in refrigerated centrifuge at 1500g (~1000 rpm) for 10 minutes.
8.22 This step will ensure the settling down of cellular material in the VTM tube, to ensure cell free viral suspension.
8.23 Arrange the RNA ID labeled cryovials on the tube rack.
8.24 Bring in the centrifuge safety cups inside the BSC and arrange VTM tubes on tube rack. Wipe the safety cups with 1% sodium hypochlorite, followed by 70% alcohol and take them out of the BSC. Aliquot specimen (~1.0 ml) in pre-labelled cryo vials using a sterile pasteur pipette.
8.25 After aliquoting the specimen from a VTM tube, rinse the tube with 1% sodium hypochlorite using the same pasteur pipette. Discard the rinse in the liquid waste container. Close the cap of the VTM tube and discard both the pasteur pipette and VTM tube in the bio-hazard waste bag inside the BSC.
8.26 Store one set of vials at -70/ -80°C for later use. The other set can be stored at 4 °C for RNA extraction if to be used on the same day.
8.27 Remember the specimen should be processed as soon as possible on arrival. If this is not possible, follow WHO & ICMR guidelines for specimen storage at 4°C (1). Transfer the aliquots to -70/-80°C freezer for long-term storage.
8.28 Long-term storage of specimen is also useful for preparation of External Quality Controls (EQC), for ILC with designated referral lab and also for sending for Whole genome sequencing (WGS) to Regional Genome Sequencing Laboratory( RGSL). Refer to ICMR guidelines on the long term storage of specimens
8.29 After completing the work tie the biohazard bag inside the BSC and bring it out of the BSC. Place it in the biohazard bin. The waste should be handed over to the autoclave team.
8.30 Liquid waste should be discarded as per lab policy after appropriate contact time.
8.31 The BSC should be cleaned as above.
8.32 Leave the BSL-2 lab and doff the PPE.

## 9. QUALITY CONTROL

9.1 Only the specimen that pass the acceptation criteria should be considered for processing.
9.2 Specimen should always be stored at 2 – 8 °C upon arrival.
9.3 Specimen should be aliquoted and stored at –70/-80°C for long-term storage.

## 10. SPILL MANAGEMENT

Refer to SOP# 004 on spill management for handling the infectious spills.

## 11. BIOHAZARD WASTE DISPOSAL

11.1 All solid waste (tips, gloves, packaging, etc) collected in the specimen processing room should be discarded only in labelled biohazard bags (Labelled as COVID-19 WASTE) inside the biosafety cabinet. Filled Biohazard Bags should be tied inside the biosafety cabinet with tag.

11.2 Removed PPE should be discarded in marked designated bins. Bags should be tied and labelled.

11.3 Tied and labelled biohazard bags should be autoclaved at 121°C and 15 psi for 60 minutes (gravity flow) and 45 minutes in vacuum autoclave.

Note: Waste containing sodium hypochlorite should never be autoclaved.

11.4 Autoclaved waste should be weighed and clearly labeled as “COVID-19 waste” and handed over to Housekeeping Staff.

11.5 Housekeeping Staff should take the autoclaved waste to designated area for pickup and incineration

11.6 Any incidents including spills, mechanical breakdowns, failure in bio-containment or any other maintenance problem should be reported immediately to the biosafety officer.

11.7 Any incidence of exposure to personnel should be reported to the officer in charge.

11.8 Refer to Ref: SOP# 005 on Biomedical Waste Management.

## 12. REFERENCES

12.1 ICMR NIV Sample transportation SOP https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20%202019-nCoV.pdf

12.2 WHO Sample Storage Recommendations (Annexure I): https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

12.3 ICMR long term storage of specimen: https://www.icmr.gov.in/pdf/covid/labs/Govt_labs_sample_retention_advisory_25062020.pdf

12.4 Guidelines for Handling, Treatment and Disposal of Waste Generated during Treatment/Diagnosis/ Quarantine of COVID-19 Patients https://cpcb.nic.in/uploads/Projects/Bio-Medical-Waste/BMW-GUIDELINES-COVID_1.pdf

12.5 Biomedical Waste Guidelines https://cpcb.nic.in/uploads/Projects/Bio-Medical-Waste/Bio-medical_Waste_Management_Rules_2016.pdf

12.6 ICMR specimen referral form: https://www.icmr.gov.in/pdf/covid/labs/SRF_v12.pdf

12.7 QIAamp® Viral RNA Mini Handbook

## SOP CHANGE HISTORY

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

## ANNEXURES

## ANNEXURE – 1: Disinfectant- Sodium Hypochlorite

Formula for preparing working solution of Sodium Hypochlorite from the stock:

| Amount of stock solution required (ml) | Concentration of stock solution available | Working solution concentration required for use | Working solution volume required (ml) |
|---|---|---|---|
| 100 | 40 | 4 | 1000 |

Water Required = Working solution volume required − Amount of stock solution required
For 1L of 4% Sodium Hypochlorite solution= 100ml of stock (40%) +900ml of water

Preparation of different concentrations of Sodium Hypochlorite Solution.

| Required Strength (Available solution of chlorine) | Stock/commercially available Sodium Hypochlorite | | |
|---|---|---|---|
| | 4% (40g/L); dilute | 5% (50g/L); dilute | 6% (60g/L); dilute |
| 1% (10 g/L) | 1:3* | 1:4 | 1:5 |

**parts of stock solution: parts of water*

**1%** Working Solution from **4%** Stock Solution of **Sodium Hypochlorite**

| **Required Volume of Working Solution(ml)** | **Quantity of Stock Sodium Hypochlorite (ml)** | **Quantity of Water** |
|---|---|---|
| 250 ml | 62.5 ml | 187.5 ml |
| 500 ml | 125 ml | 375 ml |
| 1000 ml | 250 ml | 750 ml |
| 2000 ml | 500 ml | 1500 ml |

**NOTE:** Kindly see the concentration of commercially available Stock solution before Dilution

## ANNEXURE – 2: Disinfectant-70% Alcohol

Preparation of 70% Ethanol or 70% IPA (Isopropyl Alcohol)

1) Measure the required quantity of desired alcohol to be used using a clean measuring cylinder.
2) Use freshly collected distilled water for preparation of 70% alcohol solution.
3) Prepare solution in the proportion of 70:30, alcohol: water.
   Calculate the quantity of stock Ethanol solution required for preparation of desired amount of 70% Ethanol solution by using the formula given below:

$$\text{Volume (ml) of Ethanol (Stock)} = \frac{\text{Concentration of Ethanol required (70\%)} \times \text{Total Volume (ml)}}{\text{Concentration of Ethanol (Stock)}}$$

4) Transfer the prepared IPA to a sterilized glass bottle.
5) Affix the label on the bottle with following information.

Name of regent
Strength
Date of preparation
Use before date
Prepared by