CORONA VIRUSES

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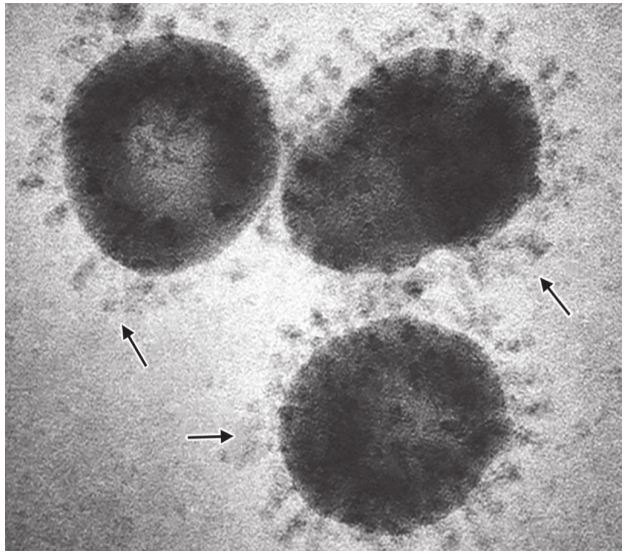
CORONA VIRUSES

Morphology-

- They are **enveloped**; carrying **petal or club-shaped** or **crown-like peplomer spikes** giving appearance of solar corona.
- **Large** (120–160 nm) **spherical** viruses having a **helical symmetry**.
- Possess linear, **positive-sense ssRNA** of 26 to 32 kbp size
- **Largest** among the non-segmented RNA viruses.

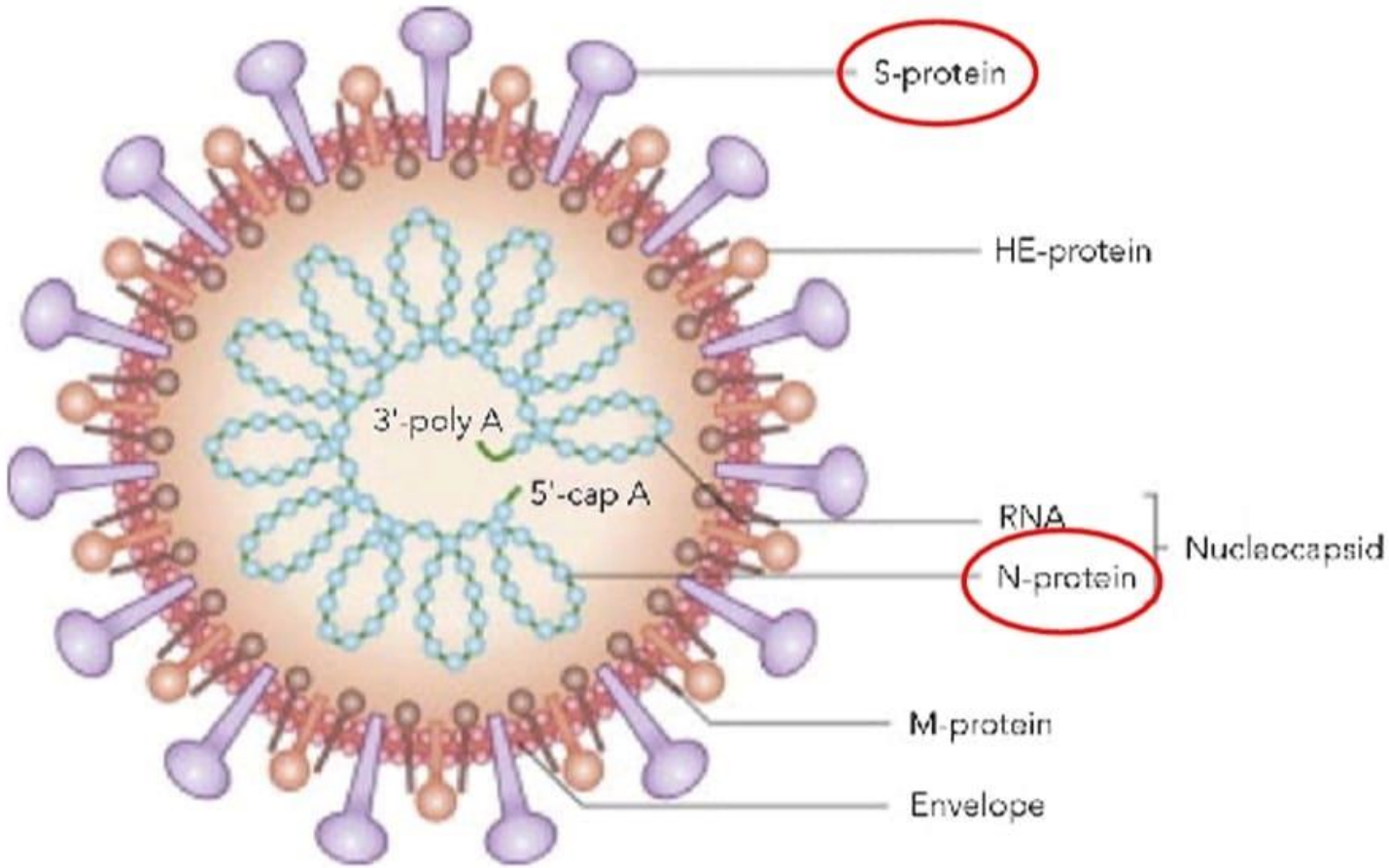


- There are **six** recognized coronaviruses that are known to cause **human infections:**
  - ✓Human Coronavirus 229E
  - ✓Human Coronavirus NL63 (New Haven Coronavirus)
  - ✓Human Coronavirus OC43
  - ✓Human Coronavirus HKU1
  - ✓SARS-CoV (Severe Acute respiratory syndrome Coronavirus)
  - ✓MERS-CoV (Middle East respiratory syndrome Coronavirus)
- Produce mild **upper respiratory tract infection** and occasional **diarrhea**.

- **Transmission-** Coughing and sneezing, and close personal contact. SARS-CoV can also spread via **droplets** and rarely spread through the air **(airborne spread)**

SARS-CoV-2 (The virus)

Structure



Giwa AL et al. *Emerg Med Pract*. 2020;22(5):1-28.

SARS-CoV (Severe Acute Respiratory Syndrome coronavirus)

- **History-** SARS was **first recognized** in **China** in **2003** by WHO physician
- **Epidemiology**- During 2003 outbreak, the SARS virus, spread from Asia to various regions of the world. However, India remained free from the infection. Since 2004, no case has been reported from anywhere in the world.
- **Source**- **Animals,** including monkeys, Himalayan palm civets, raccoon dogs, cats, dogs, and rodents.
- **Clinical manifestation** - severe **lower respiratory tract infection**, characterized by muscle pain, headache, sore throat and fever.

Laboratory diagnosis of coronavirus infections

- **Antigen Detection**: In the respiratory secretions detected by **ELISA** using specific monoclonal antibody
- **Electron microscopy** can be used to detect enteric coronaviruses from **stool**
- **RNA Detection**- **Reverse transcriptase PCR assays** are useful to detect coronavirus RNA in respiratory secretions (targeting pan-coronavirus polymersae gene) &in stool samples

Laboratory diagnosis of coronavirus infections

- **Isolation** of human coronaviruses in **cell culture** has been **extremely difficult**. Isolated from respiratory specimens using **Vero cell line.**
- **Serum antibody detection**:
  - **ELISA and hemagglutination inhibition test** are available.
  - **Rising titer** of antibody between acute and convalescent sera can be used to confirm the diagnosis.

LABORATORY SURVEILLANCE

(INCLUDING SAMPLE COLLECTION, PACKAGING,TRANSPORT & LAB TESTING)

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Assistant Professor

Strategy for COVID19 testing in India

- ✓ All **symptomatic individuals** who have undertaken **international travel** in the **last 14 days**
- ✓ All **symptomatic contacts** of **laboratory confirmed cases**
- ✓ All symptomatic **health care workers**
- ✓ All patients with **Severe Acute Respiratory Illness** (fever AND cough and/or shortness of breath)
- ✓ **Asymptomatic direct and high-risk contacts** of a confirmed case should be tested once between day 5 and day 14 of coming in his/her contact

Samples to be collected

- Essential samples:
  - Throat swab (Oropharyngeal swab).
  - Nasal swab (Nasopharyngeal swab)
- Other preferred samples:
  - Bronchoalveolar lavage
  - Tracheal aspirate
  - Sputum

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

Collection of OP and NP swabs

Optimal timing:

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

Collection of Oropharyngeal swab

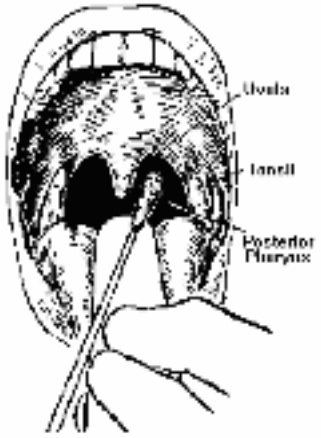
Materials:

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

Viral Transport Media (VTM)

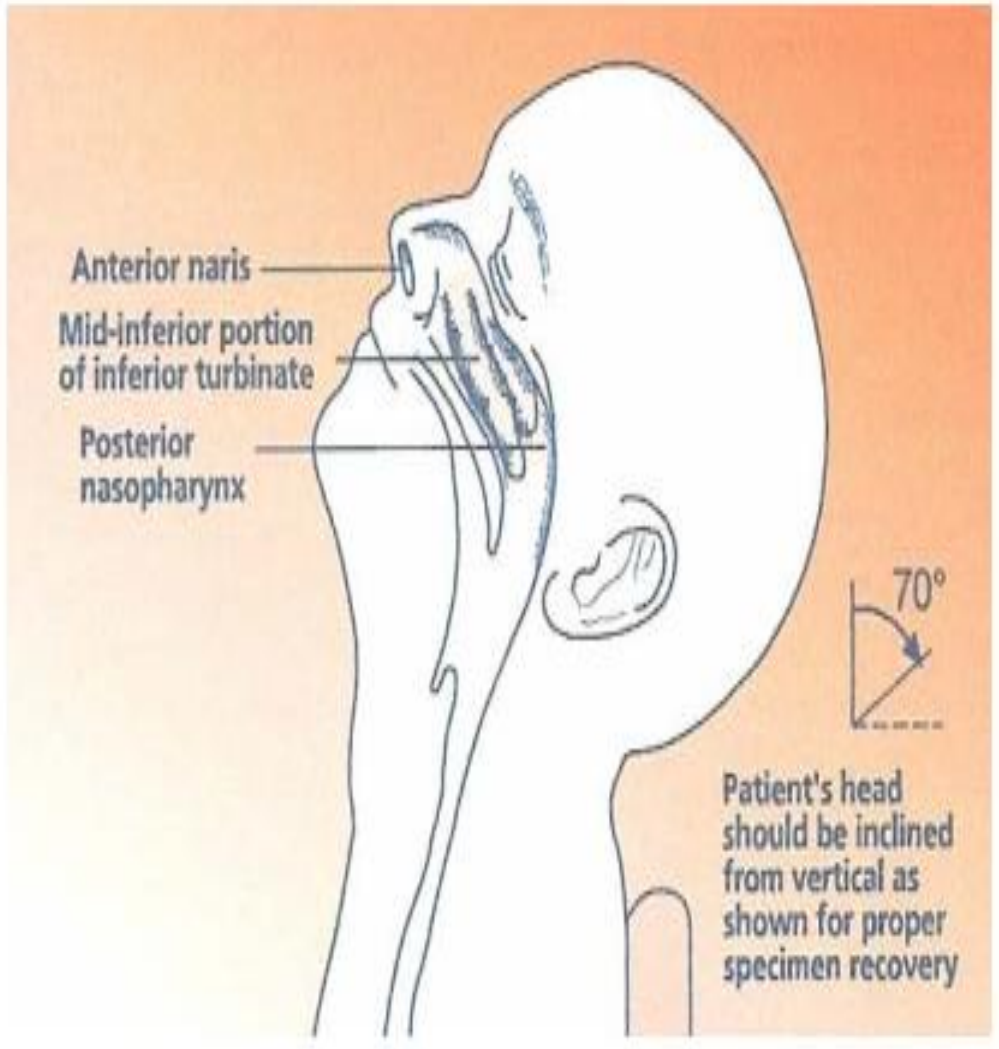
Procedure:

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



Collection of Nasopharyngeal swabs

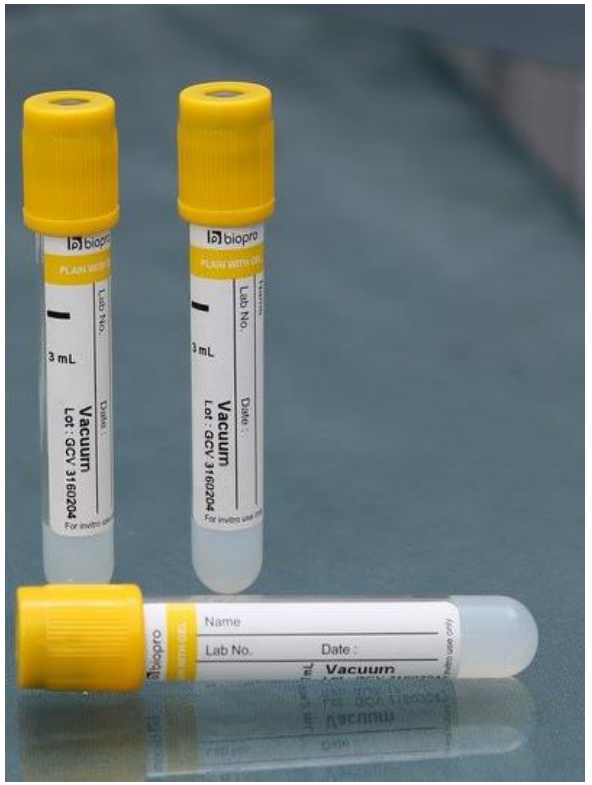
- Materials
  - Sterile **Dacron/Nylon** flocked swab
  - **Viral Transport Medium** (3 ml sterile VTM)
- Procedure
  - Tilt patient's head back **70 degrees**
  - Insert swab into nostril (Swab should reach depth to distance from nostrils to outer opening of the ear
  - Leave swab in place in place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




BED-24

Blood collection from positive cases

- Blood sample collection from all positive cases
- Plasma sample collection in EDTA vials
- Resin separator tubes for serum sample collection



Guidance for specimen Collection

- A **BSL2 containment level** is required to handle suspected samples.
- Consider **all specimens as POTENTIALLY HAZARDOUS / INFECTIOUS.**
- Handle all specimens with gloves in a secure manner.
- Place each specimen into a **separate container** labeled with the **patient's name and identification number**, the **collection site,** the **date & time of collection**.
- Do not contaminate the outside of the specimen container.
- Do not handle laboratory requisition forms with gloves.

Storage of Specimen

- Keep **refrigerated (2-8 °C)** if it is to be processed (or sent to a reference laboratory) **within 48 hours.**
- Keep **frozen (-10 to -20 °C)** if it is to be processed after the first **48 hours or within 7 days.**
- Keep **frozen (-70 °C)** if it is to be processed after a week. The sample can be preserved for **extended periods**.

Guidelines followed for sample packaging & transport

- **WHO Guidelines for Transport of Infectious Substances:**

  - Guidance on regulations for the Transport of Infectious Substances 2009–2010.

https://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10.pdf

- **IATA guidelines**

Classification of Infectious Substances

- Category A: *An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.*

  - UN 2814 for Infectious substances which cause disease in humans or both in humans and animals.

  - UN 2900 for Infectious substances which cause disease only in animals

Classification of Infectious Substances

- **Category B**: ***An infectious substance which does not meet the criteria for inclusion in Category A.***

  - **Infectious substances in Category B shall be assigned to UN 3373**

SARS-CoV-2 virus infectious/potentially infectious material falls under category B

Packaging System

- The original samples should be **packed, labeled and marked, and documented as Category B.**
- **Standard triple packing** for Category B to be followed.
- Samples to be sent on **dry ice** (if possible). However using cold packs is acceptable.
- Sender should provide **prior intimation** about shipment of samples to the nearest certified laboratory.

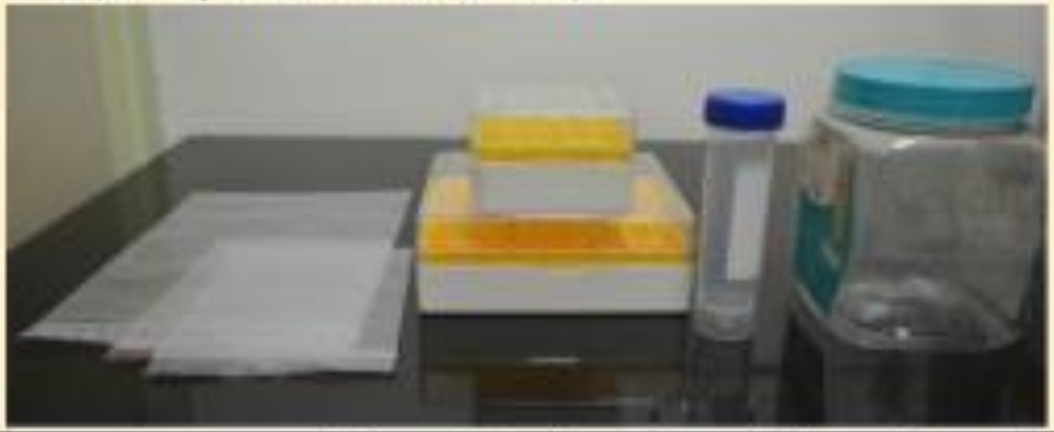
Specimen packaging

Requirements for Clinical Samples Collection, Packaging and Transport

1. Sample vials and Virus Transport Medium (VTM)

2. Adsorbent material (cotton, tissue paper), paraffin, seizer, cello tape

3. A leak-proof secondary container (e.g., ziplock pouch, cryobox, 50 mL centrifuge tube, plastic container)



4. Hard-frozen Gel Packs

5. A suitable outer container (e.g., thermocol box, ice-box, hard-board box) (minimum dimensions: 10 x 10 x 10 cm)

Procedure for Specimen Packaging and Transport

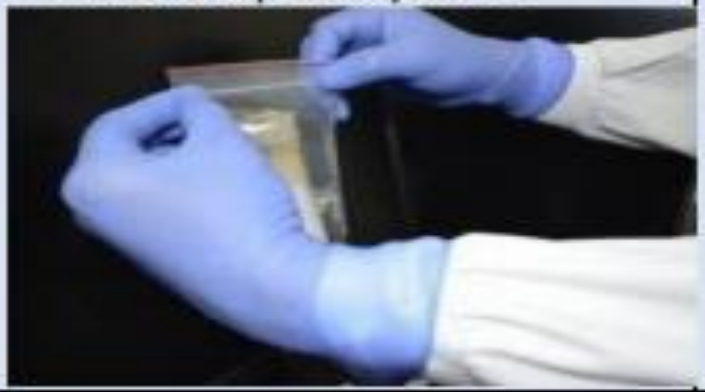
1. Use PPE while handling specimen

2. Seal the neck of the sample vials using parafilm

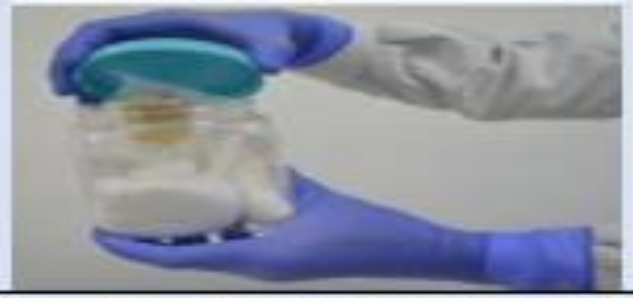
3. Cover the sample vials using absorbent material

4. Arrange primary container (vial) in secondary container

5. Placing the centrifuge tube inside a zip-lock pouch



6. Placing the zip-lock pouch inside a sturdy plastic container and seal the neck of the container



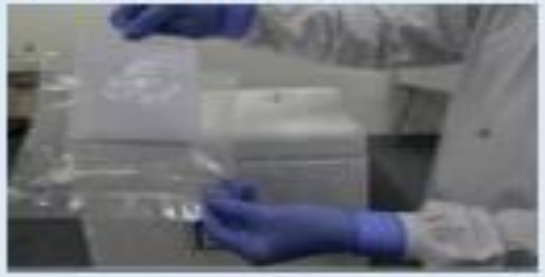
Note: *Sample vials can also be placed inside a zip-lock pouch, covered in absorbent material and secured by heat-sealing or rubber bands. Then, the zip-lock pouch should be placed inside another plastic pouch and secured*

7. Using a thermocol box as an outer container and placing the secondary container within it, surrounded by hard-frozen gel packs

7. Using a hard card-board box as an outer container and placing the secondary container and the gel packs

8. Placing the completed Specimen Referral Form (available on www.niv.co.in) and request letter inside a leak-proof, zip-lock pouch

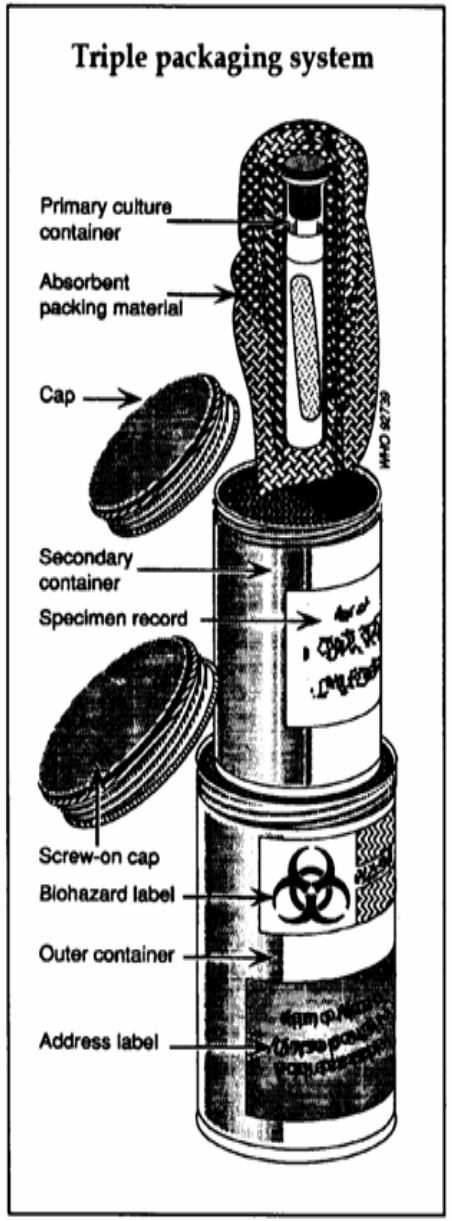
9. Securing the zip-lock pouch with the Specimen Referral Form on the outer container

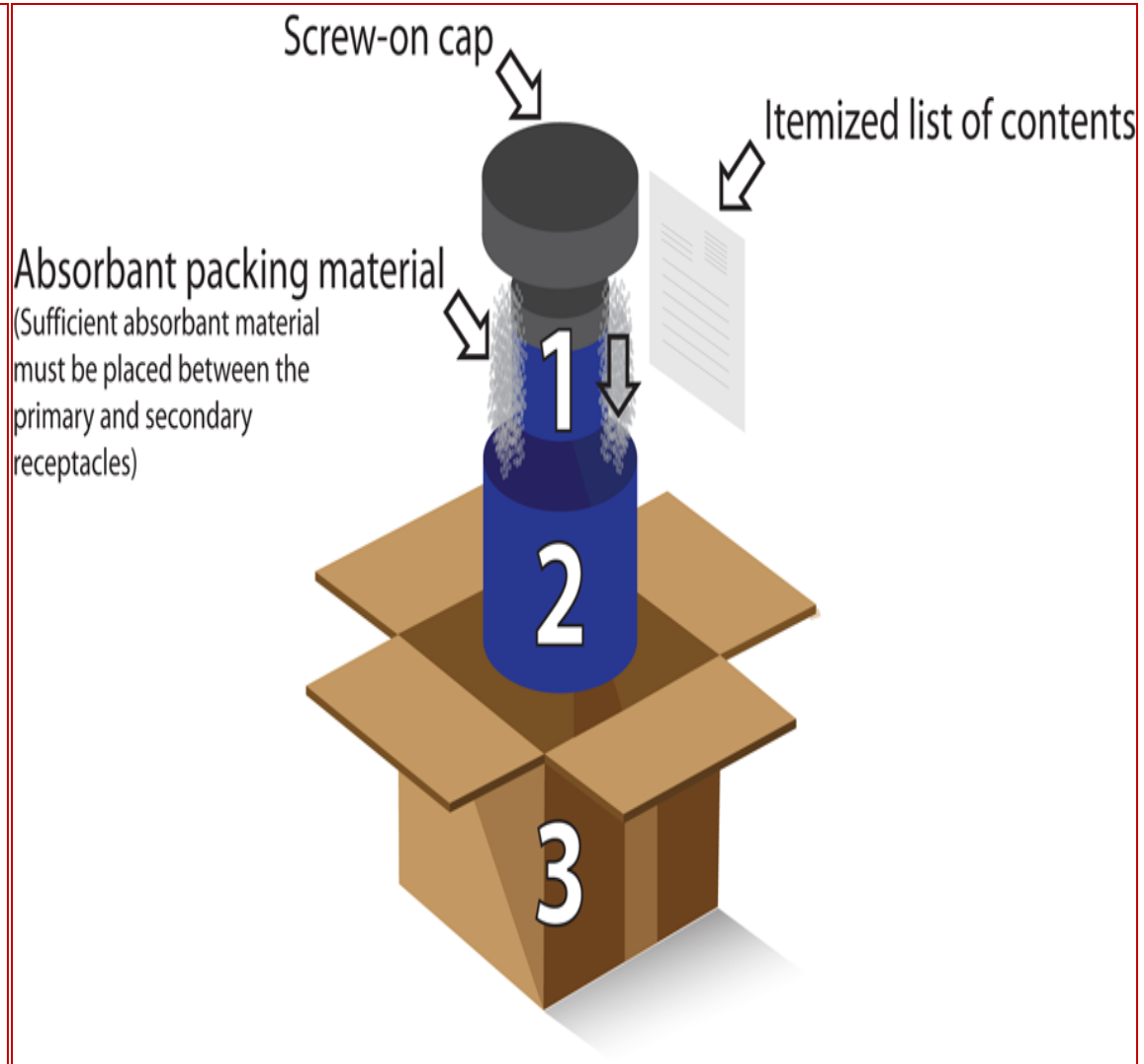


10. Attaching the labels:

- Senders' address, contact number; Consignee's address /contact number;
- Biological substance-Category B;
- 'UN 3373'; Orientation label, Handle with care

Documents to accompany:





1. Primary receptacle (leakproof, 95kPa)
2. Secondary receptacle (leakproof)
3. Outer container (w/list of itemized contents)

Transport Precautions

- Adequate **cushioning materials** inside the box to absorb shocks during transport
- Adequate **absorbing material** to absorb any spillage should it occur
- **Do not stick the request form on the specimen**
- Specimen request forms should be put into a **separate plastic bag**
- The outer container, secondary containers and specimen racks for transport should be **thoroughly cleansed and disinfected periodically** (i.e. **at least daily**) and when contaminated.

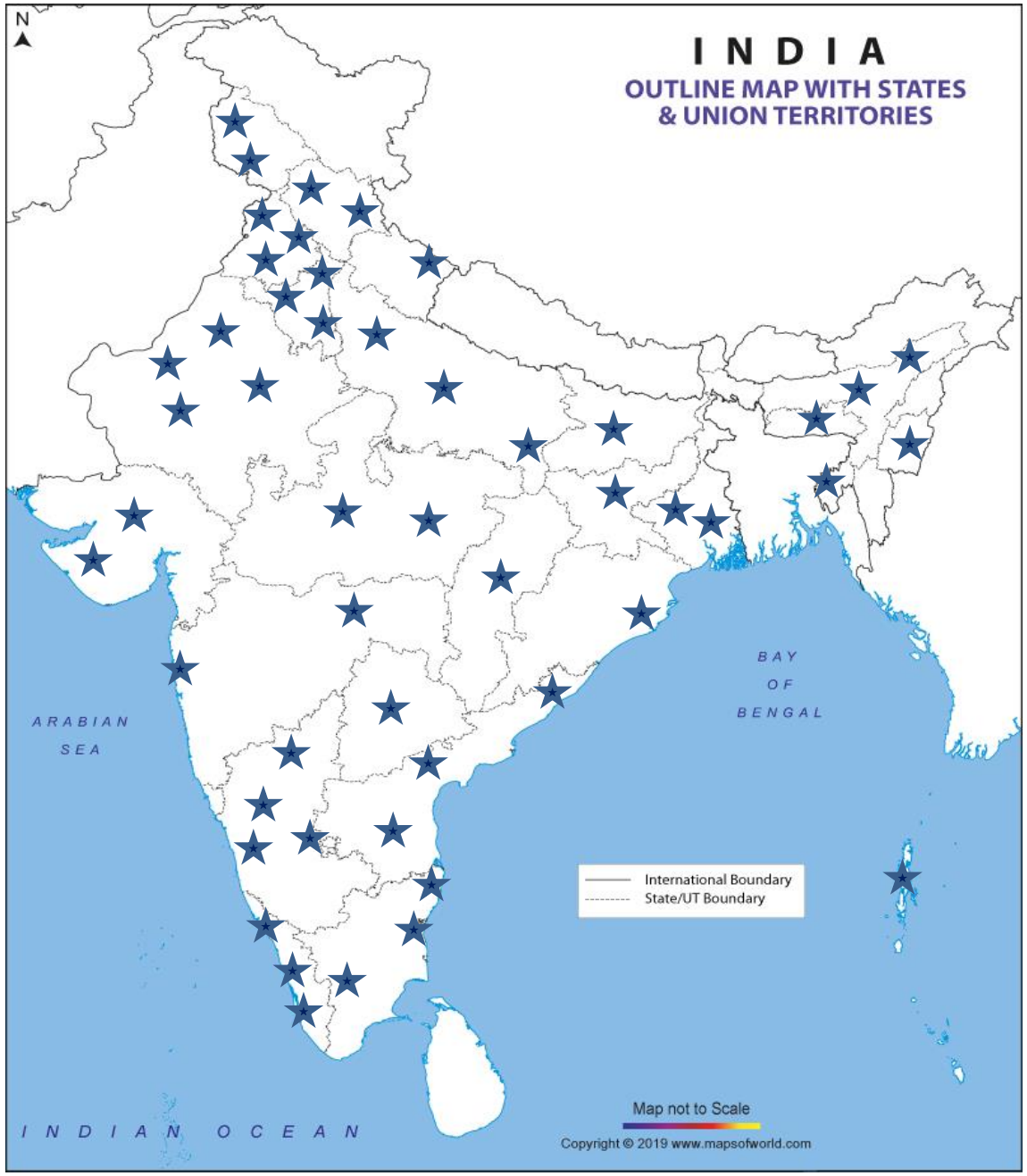
Labeling of Package

- **Sender's, name, address and telephone number**
- Whom to contact in case of emergency with telephone number
- **Receiver's name, address and telephone number**
- **Proper shipping name (e.g. "BIOLOGICAL SUBSTANCE, CATEGORY B")**
- **UN number e.g. 3373**
- Temperature storage requirements
- Quantity of dry ice inside the container
- **Arrow mark to indicate upright direction**

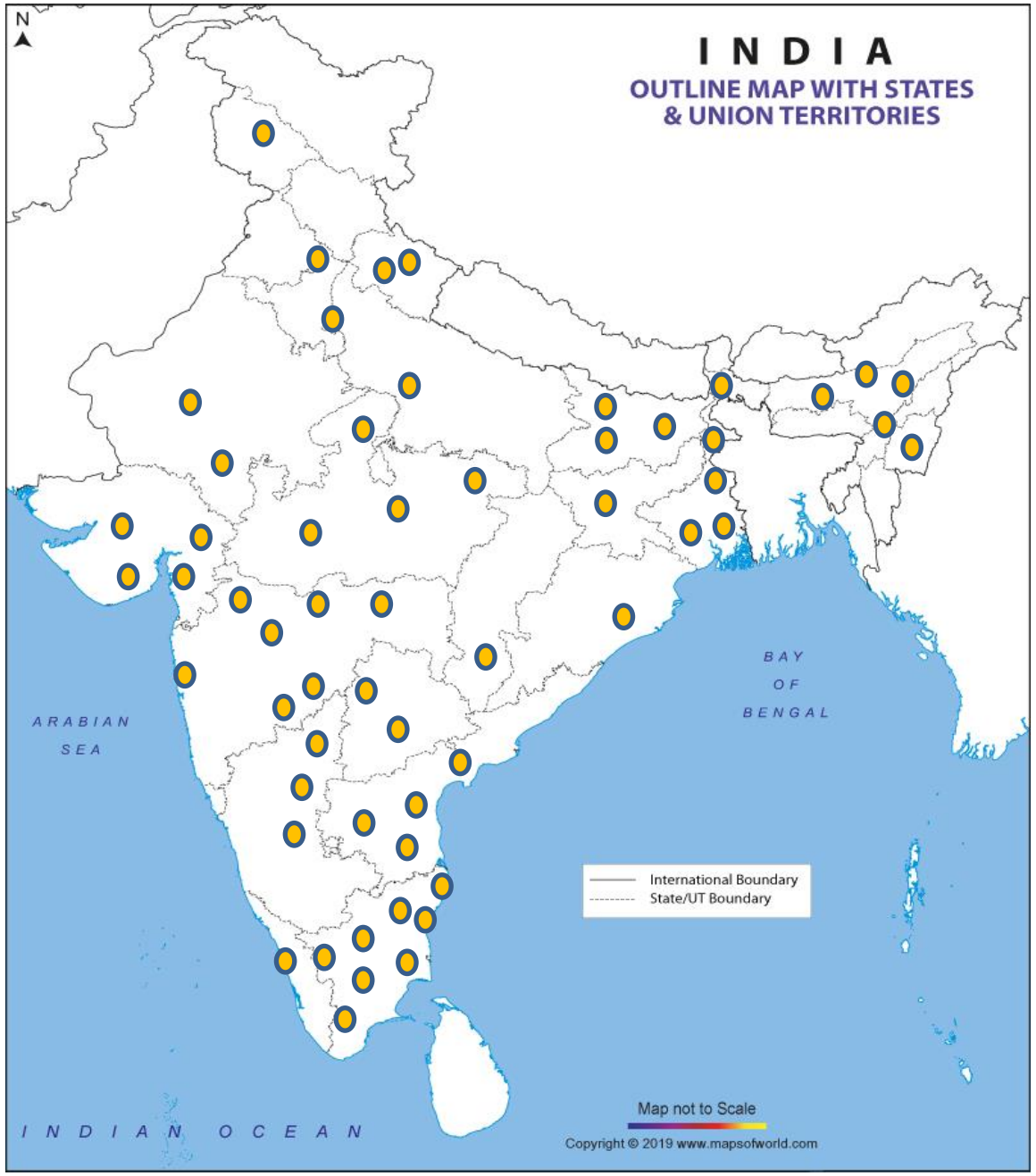
Responsibility of Receiver

- **Acknowledge receipt of specimen**
- Verify the **integrity of packaging**
- Box to be opened by personnel **wearing adequate PPE**.
- **Open within Biosafety cabinet**
- **Check the specimens with the data sent**
- **Apply acceptance and rejection criteria**

51 VRDLs doing SARS-CoV-2 testing



56 VRDLs as collection sites



Tests for SARS-CoV-2

- **No validated serological tests** are available.
- **Only Molecular tests** available.
- Laboratory protocols designed on the basis of **WHO guidance**
- First line **screening** assay: **E gene**.
- **Confirmatory** assays: **RdRp and ORF 1b**.
- **SoPs and testing protocol shared** with all testing laboratories.

Advisory for Rapid antibody test for COVID-19

- Strategy for areas reporting **clusters (containment zone**) and in large **migration gatherings/evacuees centres**

- Cases of Influenza Like Illness **(ILI)** to be monitored in **health facilities.**

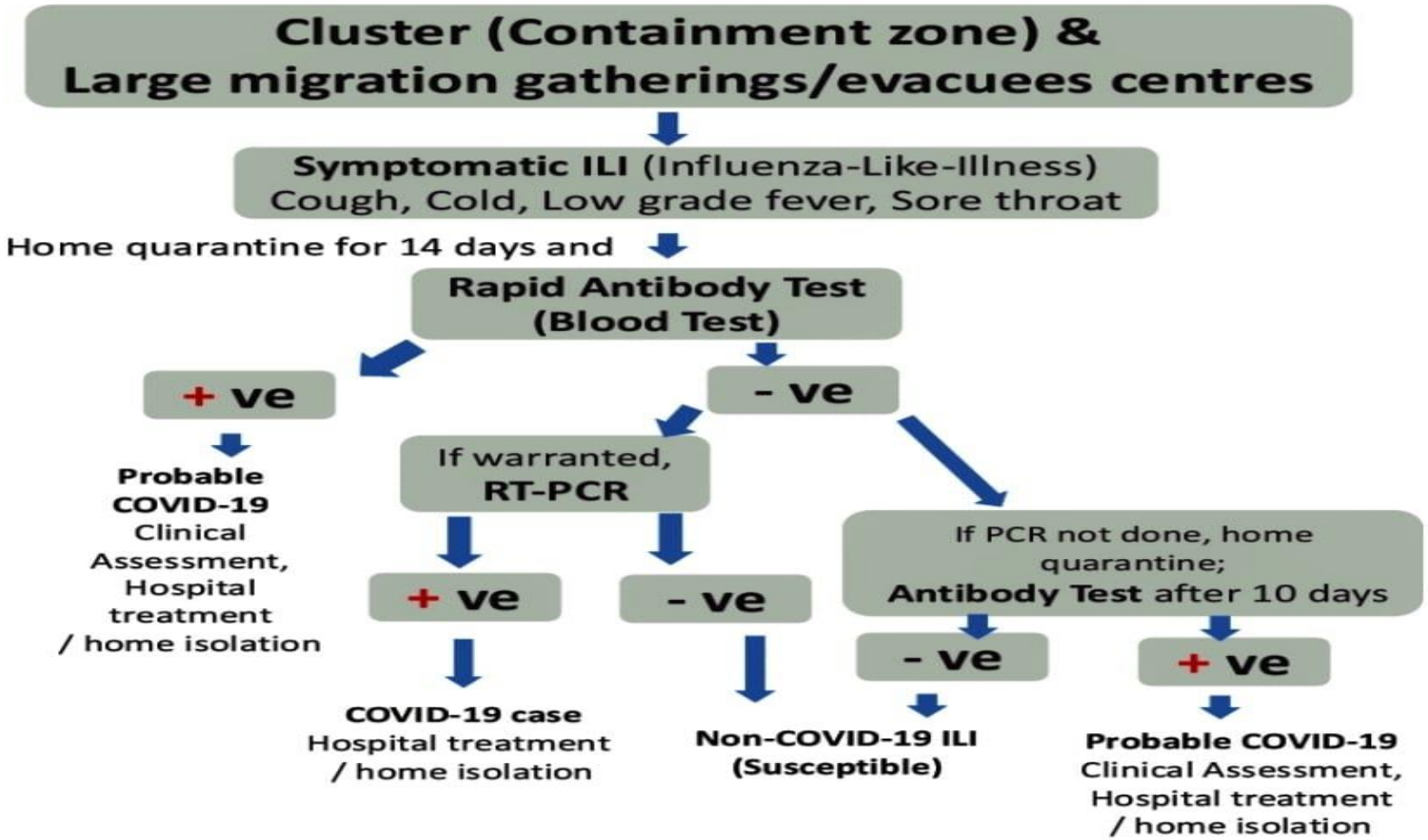
- At **facility level**,symptomatic ILI individuals to be tested using **Rapid antibody tests**

- ✓ **Gold standard frontline test** for diagnosis is **Real time PCR**
- ✓ The rapid antibody test **cannot replace** the frontline test.
- ✓ It will only be of utility **after a minimum of 7 days** of onset of symptoms.
- ✓ Data about these rapid tests is emerging and understanding of their utility for diagnosis is still evolving.
- ✓ The rapid tests are useful for **epidemiological studies** and **surveillance purpose**

The enclosed ICMR advisory is for Hot spots.

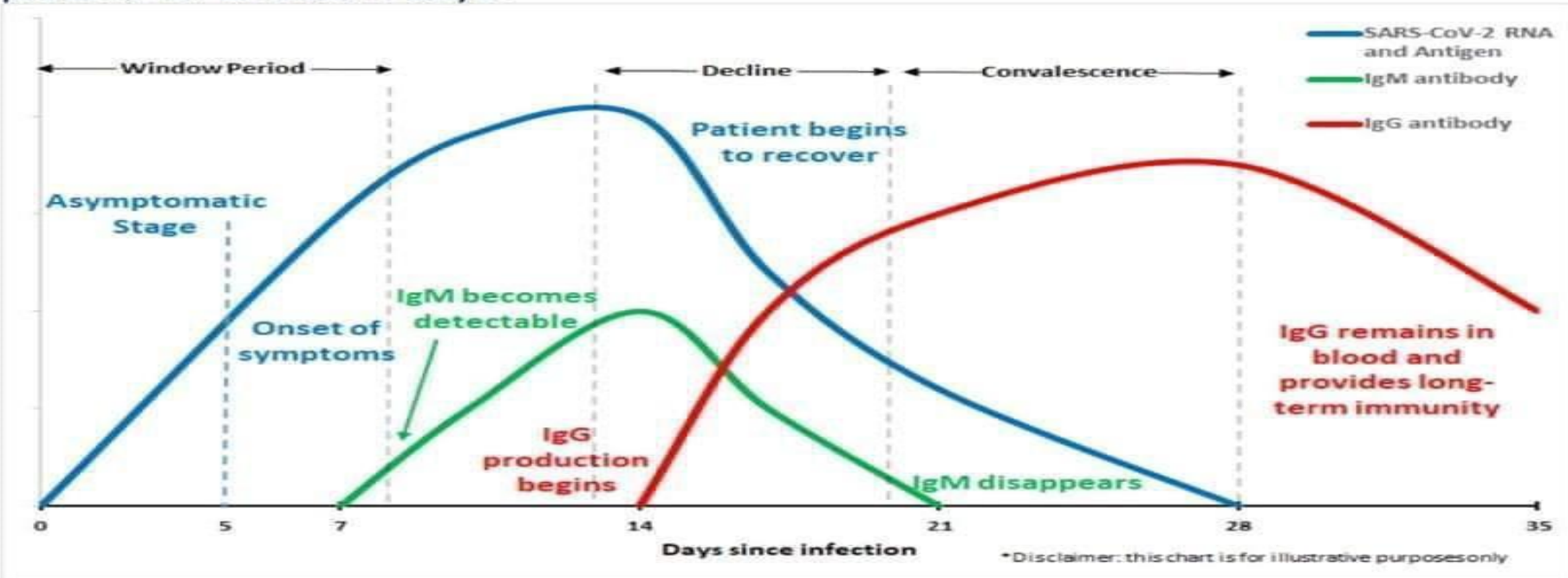
STRATEGY FOR USE OF RAPID ANTIBODY BASED BLOOD TEST

(4 April, 2020)



If symptoms worsen, refer to designated COVID-19 hospitals

Therefore, this COVID-19 Rapid Test should not be used until symptoms have been present for at least 3 days.



Test results			Clinical Significance
PCR	IgM	IgG	
+	–	–	Patient may be in the window period of infection.
+	+	–	Patient may be in the early stage of infection.
+	+	+	Patient is in the active phase of infection.
+	–	+	Patient may be in the late or recurrent stage of infection.
–	+	–	Patient may be in the early stage of infection. PCR result may be false-negative.
–	–	+	Patient may have had a past infection, and has recovered.
–	+	+	Patient may be in the recovery stage of an infection, or the PCR result may be false-negative.


Sugentech
SGTI-flex
COVID-19 IgM/IgG
SGTI-flex
COVID-19 IgM/IgG
INTENDED USE
SUMMARY AND EXPLANATION
SGTI-flex COVID-19 IgM/IgG
CONTROL
SGTI-flex COVID-19 IgM/IgG
CONTROL
SUGENTECH, INC.

Advantages of rapid tests

- ✓ Result is available within **30 mins**
- ✓ **Easy** to perform,does not involve multiple steps
- ✓ **Expertise hands** are not required
- ✓ **Sophisticated instruments** are not required
- ✓ **Economical**

Disadvantages of Rapid tests

- ✓ Test comes positive **after 7-10 days** of infection
- ✓ **Not recommended for diagnosis** of COVID-19 infection
- ✓ **Negative test doesnot rule out COVID-19 infection**
- ✓ Value of test is **limited** in patients with **impaired immune function or receiving immunosuppressive therapy**

Molecular assay

- What is PCR & What are the steps involved in PCR
- What is RT-PCR & Real time PCR

What is the workflow in the PCR lab:

✓ **Sample preparation room-** for RNA extraction

✓ **Pre-PCR Room** –for setting PCR reaction i.e.**Master mix**

✓ **PCR Room**-for performing RT-PCR

✓ **Dedicated areas** to avoid cross contamination

COVID-19 Protocol

- ✓ Aliquoting
- ✓ Storage of Sample
- ✓ RNA Extraction
- ✓ Master Mix
- ✓ Real Time-PCR

- **<u>The present strategy advocated by ICMR :</u>**

✓ Using pooled samples for molecular testing

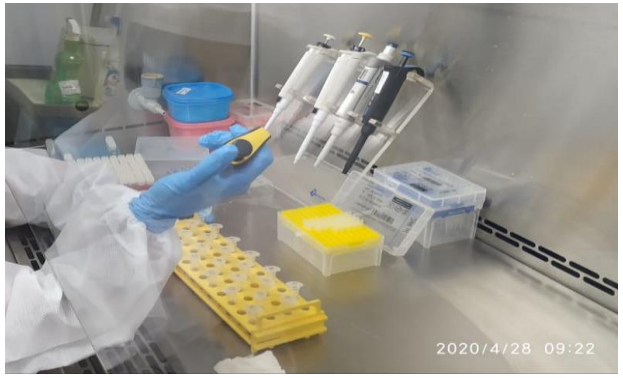
✓ Recommended for use in samples where prevalence is **less than 2%**

✓ Not recommended for areas where positivity is **more than 5%**

✓ **What are the Advantages & Disadvantages ?**

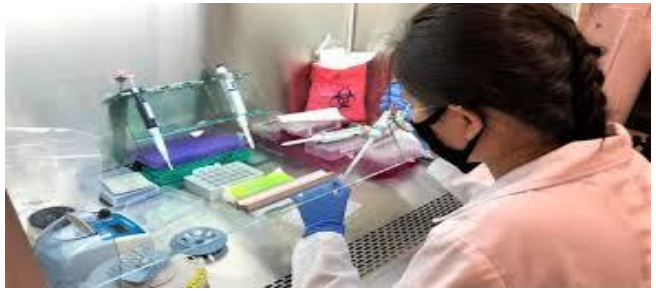
RT-PCR MACHINE

Sample Processing in BSL-II A2



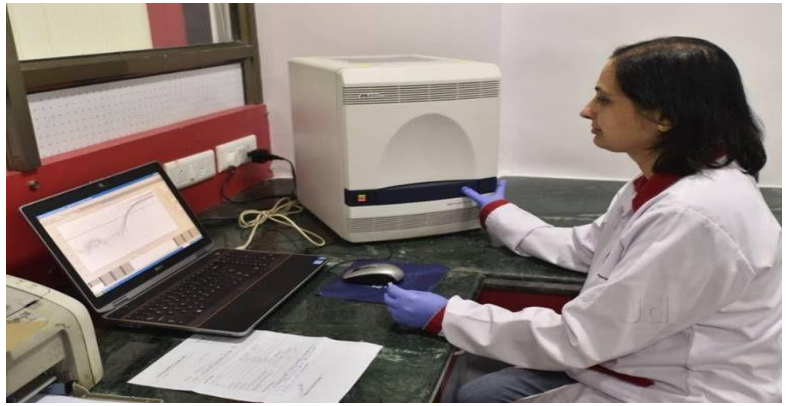
Aliquoting

Storage of Aliquotes in -80°C

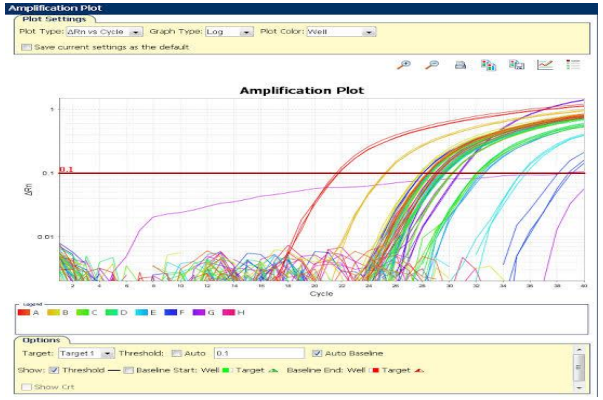


RNA Extraction

Master Mix Preparation



Real Time- PCR



Final Result by RT-PCR

Be INFORMED
Be PREPARED
Be SMART
Be SAFE

Be READY to fight
#COVID19

Thank You