ANNEXURE II- Procedures for the clinical specimen collection:

- **Nasopharyngeal and Oropharyngeal swabs in screw capped plain tube:**
  - Explain the procedure to the patient
  - Remove the polystyrene swab. Don't let anything touch the sterile swab on the end of the stick
  - Ask patient to open mouth and stick their tongue out
  - Use tongue spatula to press the tongue downward to the floor of the mouth.
  - Use sterile polystyrene swab to swab both of the tonsillar arches and the posterior nasopharynx, without touching the sides of the mouth.
  - Reach behind the uvula and swab: a. tonsillar fauces, b. the posterior pharynx, and c. any ulceration, exudate, lesion, or area of inflammation.
  - Don't let the sterile swab touch the patient's tongue, gums, or teeth as you gently remove it from his/her mouth
  - Place the swab into the screw-capped plain plastic tube [without any medium or VTM]
  - Similarly, tilt patient's head back 70 degrees. Gently and slowly insert a polystyrene swab with a flexible shaft through the nostril parallel to the palate until resistance is encountered.
  - The distance is equivalent to that from the nostril to the ear of the patient, indicating contact with the nasopharynx
  - Gently rub and roll the swab, leaving it in place for several seconds to absorb secretions. If a deviated septum or blockage creates difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril
  - Slowly remove swab while rotating it. Specimens can be collected from both nostrils
  - Place the swab into the same screw-capped plain plastic tube [without any medium or VTM] in which the OPS swab was kept
  - Break the excess stick and recap the tube tightly.
  - Keep the tube in an upright position in the stand.
  - Surface decontaminate the tube using 2% Lysol or 0.5-1% Sodium hypochlorite wipes

- **Venous Blood Collection in SSGT and EDTA tube:**
  - Explain the procedure to the patient
  - Check the patient's fore-arm/median cubital fossa for a prominent vein of good size. Use the median cubital vein wherever feasible.
  - Apply the tourniquet 4-5 fingerbreadths above the site of venipuncture.
  - Perform hand hygiene by using an alcohol-based hand rub on the outer pair of gloves.

- Disinfect the skin site using a wipe containing 70% alcohol, in a circular motion, from the centre to periphery. Allow the skin to dry.
- Do not re-touch the site of puncture again. In case of accidental touch, repeat the skin disinfection as above.
- Anchor the vein by holding your thumb below the puncture site.
- Ask the patient to make a fist so as to make the veins prominent.
- Insert the needle (vacutainer or syringe needle) into the vein, bevel side up, at an angle of about 30º and advance the needle into the vein.
- Collect 5 mL of blood (into the syringe or into tubes) and aliquot 2 ml in EDTA (Purple top) 3 ml in SSGT (Yellow top) for serum separation.
- Release the tourniquet.
- Withdraw the needle gently and apply a piece of sterile gauze to the puncture site
- Ask the patient to gently press down on the gauze on the puncture site, keeping the arm folded
- If a syringe and needle were used for collection, transfer the blood inside the tube by piercing the stopper of the tubes placed firmly on a rack.
- Invert EDTA tubes gently 4-5 times to ensure proper mixing of the additives
- Keep the tubes in an upright position in the stand.
- Discard the syringe and needle into the sharp's container
- Surface decontaminate the SSGT and the EDTA tube using 2% Lysol or 0.5-1% Sodium hypochlorite wipes

- **Urine sample collection in the screw capped sterile urine container:**
  - Explain the procedure to the patient
  - Provide privacy to the patient
  - First ask patient to wipe/clean the genitals
  - Ask patient to urinate a small amount into the toilet bowl, and then stop the flow of urine.
  - Then collect a sample of urine into the clean or sterile container provided
  - Ask the patient to collect about 3 to 5 mL of mid-stream urine sample into the collection tube provided, taking care not to contaminate the outside of the container
  - Ask patient to finish urinating into the toilet bowl
  - Close the lid carefully and keep the container standing position
  - Surface decontaminate the SSGT and the EDTA tube using 2% Lysol or 0.5-1% Sodium hypochlorite wipes

- **Lesion roof, base scrapping, fluid and crust/scab collection [collect from multiple sites]:**
  - Explain the procedure to the patient
  - Sanitize the skin covered with lesion with 80% alcohol wipes to start the collection
  - Remove the lesion roof using sterile scalpel or plastic scrapper
  - Place the roof in the screw cap plain tube.
  - Similarly, use 1ml intradermal syringe to collect the pustule/ vesicular fluids from multiple lesions and collect in fresh screw cap plain tube
  - Use the polystyrene swab to collect the scrapings from the base of the lesions by gentle scrapping and put it in fresh screw cap plain tube
  - The scab/crust if formed using polystyrene swab, should also be collected in fresh screw cap plain tube

Procedures for the transport of the clinical specimens:

- Keep the samples immediately in +4 degree Celsius as soon as they are collected
- After collection of samples, appropriately labelled sample tubes need to be sealed with parafilm
- Centrifuge the serum tube before shipment
- Tubes need to be wrapped with the absorbent tissue paper/cotton and paced in Zip lock bags/Secondary receptacle

Samples should be transported in dry ice as per the instruction provided in Annexure- 3 (adapted from the WHO Guidance on regulations for the transport of infectious substances 2021-2022) along with the case record form provided with this document