

6.0 SPECIMEN COLLECTION AND PROCESSING

6.1 General Guidelines

1. Practice proper universal and COVID precautions throughout specimen collection and handling.
2. We suggest that all COVID biospecimen processing, including centrifuging and aliquoting, should be performed in a BSL2 safety hood, but we defer to your local institutional guidelines regarding safe biospecimen handling for COVID patients.
3. All efforts should be made to collect biospecimens in conjunction with clinical care biosampling.
4. Obtain the Specimen Collection Kit for the correct visit and time-point.
5. Verify the expiration date on the Specimen Collection Kit. If the expiration date has not expired, open the Specimen Collection Kit.

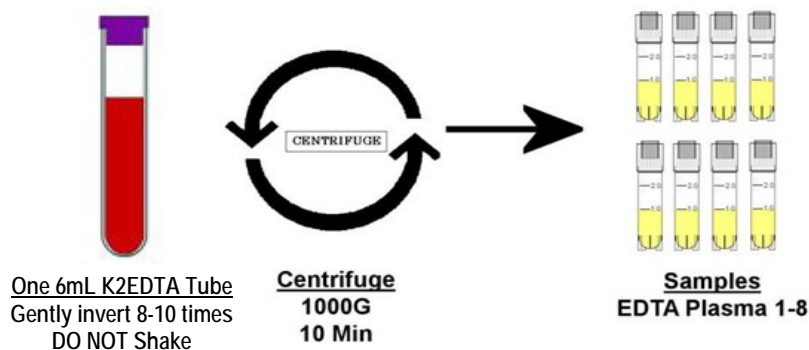
6.2 EDTA Plasma-All Visits

Collection:

1. Fill the provided 6 mL EDTA blood tube with blood from patient via arterial line, venous line, or by venipuncture. Use largest needle routinely used in your ICU for phlebotomy (up to 18g) for venipuncture and when instilling blood into the EDTA vacutainer to prevent hemolysis of the specimen. Hemolyzed samples should be redrawn if possible.
2. Gently invert the vacutainer 8-10 times to mix. Do NOT shake.
3. Place on ice if anticipated time to processing is greater than **30 minutes**.

Processing:

1. Centrifuge for 10 minutes at approximately 1000 G (using an approved centrifuge for COVID biospecimen processing).
2. Aliquoting of plasma:
 - Withdraw plasma (do not remove buffy coat) using a pipette and fill pre-labeled cryovial tubes with plasma.
 - Fill all gray-top aliquots with 500 mcL of plasma. NOTE: If you do not have enough plasma to fill all 8 aliquot vials, distribute so that those vials filled have at least 250 mcL of plasma (may not be able to fill all vials). Please note this in the comments field of the Lab Requisition Form. Place on ice if anticipated time to freezing is greater than 30 minutes.
3. Store promptly in a -70 or -80°C freezer.



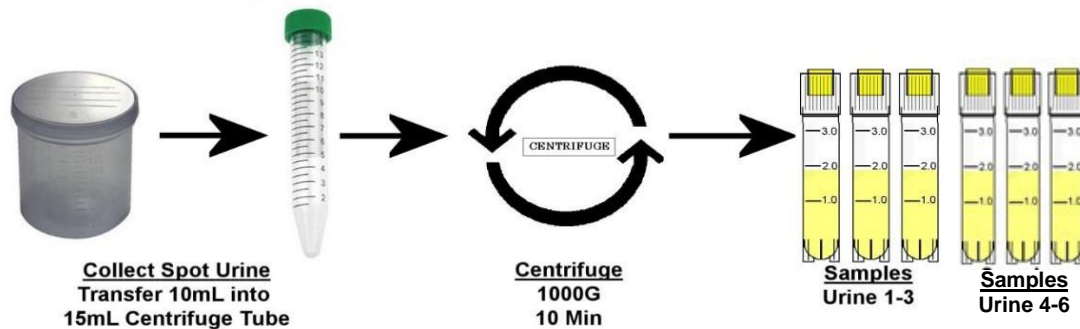
6.3 Urine

Collection:

Collect 10 mL of freshly voided urine from patients with a Foley catheter in place; obtain from the catheter tubing port.

Processing:

1. Place in 15 ml tube provided and centrifuge for 10 minutes at approximately 1000 G (using an approved centrifuge for COVID biospecimens).
2. Use provided labeled cryovials.
3. Place 1 ml of urine supernatant in each of the 6 vials.
4. Store promptly (within 30 minutes) in a -70 or -80°C freezer.
5. Place on ice if anticipated time to freezing is greater than 30 minutes.



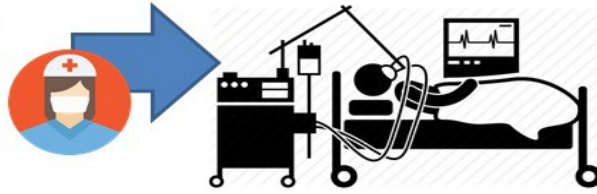
6.4 Tracheal Aspirate and BAL Specimens

Collection:

1. Hand the sputum trap and tube of Zymo (DNA/RNA) shield (containing 3 mL of DNA/RNA shield) to the nurse or respiratory therapist prior to their entry into the room for suctioning (or for a BAL procedure). Request that no more than 3 mL of sputum or BAL fluid be collected into the sputum trap.
2. After collection of the tracheal aspirate (or BAL) into the sputum trap, estimate the volume of the sputum or BAL sample collected in the sputum trap, and in the patient's negative pressure room add an equal amount of Zymo shield to the tracheal aspirate (or BAL) (e.g., approximately 1 mL of Zymo shield mixed with 1 mL of tracheal aspirate or BAL fluid) for a maximum of 3 mL of tracheal aspirate or BAL fluid mixed with 3 mL of Zymo shield (6 mls total). Close the container tightly.
3. Invert tracheal aspirate or BAL fluid mixed with Zymo shield 7-10X in the patient's negative pressure room. This will inactivate the virus, and the sample can be transported with blood and urine specimens for processing per the site's COVID biospecimen processing protocol.



Hand sputum trap & vial of Zymo shield to the Nurse or RT



After collection of the tracheal aspirate (or BAL), RN/RT should combine an equal amount (1:1 ratio) of tracheal aspirate (or BAL) and Zymo shield together (6 mls total max).



Invert tracheal aspirate or BAL fluid mixed with zymo shield 7-10X in the room. This will inactivate the virus, and the sample can be transported with blood and urine specimens for processing.

Processing:

1. Use provided labeled cryovials.
2. Place 1 mL of tracheal aspirate or BAL mixed with Zymo shield in up to as many as 6 vials.
3. Store promptly (within 30 minutes) in a -70 or -80°C freezer.

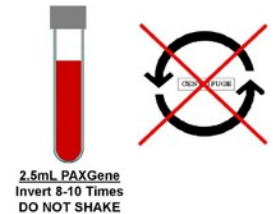
6.5 DNA and RNA Collection (one DNA PaxGENE tube and one RNA PaxGENE tube)

Collection:

1. Each PaxGENE tube contains a special preservative, and every effort should be made to prevent backflow of the preservative. *The PaxGENE tubes should always be the last tubes filled, and the tubes should be filled upright in a vertical position to avoid contact between the preservative and stopper, if possible.*
2. The provided 2.5 mL blood tubes (one for DNA and one for RNA) can be filled with blood from patient via arterial line, venous line, or by venipuncture.
 - a. For venipuncture, a butterfly needle should be used for venipuncture to prevent tube additive from coming in contact with patient bloodstream (alternatively collection into a syringe for transfer into vials including PaxGENE can be done with any needle)
 - i. Place the donor's arm in a downward position
 - ii. Hold tube in a vertical position, below the donor's arm during blood collection
 - iii. Release tourniquet as soon as blood starts flowing into the tube
 - iv. Make sure the tube additives do not touch the stopper or the end of the needle.

3. Gently **invert the vacutainer 8-10 times to mix**. Do NOT shake. The blood will turn much darker, which is expected.

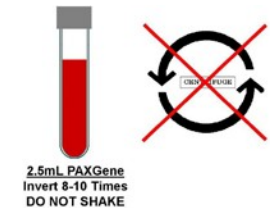
DNA PaxGENE Tube



4. Cure both the DNA and RNA PaxGENE tubes (i.e., let it sit at room temperature) for 24 hours (target). *Allowable range: 2 to 72 hours.*

5. **Store tubes upright in a -70 or -80°C freezer [of note this can take up substantial space in the freezer].**

RNA PaxGENE Tube



IMPORTANT:

- *The quality and yield of the sample is dependent upon immediate and proper mixing!*
- *The sample cannot be used unless the **cure time** (hours at room temp) is documented*

CORAL Lab Requisition Form

1 x 2.5mL PAXGene
Gently Invert 8-10 Times

**Do Not
Centrifuge**

1 x 2.5mL PAXGene Tube
Gently Invert 8-10 Times

**Do Not
Centrifuge**

How long was the RNA sample held at room temperature prior to freezing?

Hours
Target 24hrs
(Range 2-72hrs)

RNA Sample

DNA Sample