

## **Isolation of Plasma from EDTA Whole Blood**

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1. Blood samples are collected from patient in lavender top (EDTA) tubes wrapped in biohazard bags with deidentified study labels.
2. SARS-CoV-2 positive blood is processed in a BSL2+ facility in adherence to the SOP, safety precautions and PPE requirements for COVID-19 (isolation gown, face shield, mask and double gloves).
3. The Biosafety cabinet (BSC) surface is disinfected with Quatricide, and a small autoclavable biohazard bag is placed for solid waste collection (includes everything that comes into the BSC). Do not dispose anything into the red biohazard bin.
4. A plastic bin with appropriate dilution of Quatricide is placed along with a strainer to collect used tips and blood collection tubes for disinfection before disposing into the solid waste collection bag.
5. The biohazard bag containing blood collection tubes is wiped with Quatricide before moving it into the biosafety cabinet and the blood collection tubes are removed from the biohazard bag.
6. 1-2 mL of whole blood is aliquoted into 0.5 mL aliquots in individual cryovials using a filter tip 1 mL micropipette, used pipette tips are disposed into the strainer in the disinfectant liquid and the aliquoted cryovials are stored at -80°C.
7. Centrifuge buckets are removed from the tabletop centrifuge along with the adapter and containment lid and disinfected before moving it into the BSC.
8. Remaining blood in the lavender top collection tubes is transferred to the centrifuge bucket after decontamination and the bucket containing the samples is moved back to the tabletop centrifuge after decontamination.
9. The blood is spun down at 200g for 10 minutes in the lavender top collection tubes.
10. Centrifuge buckets containing the samples are removed from the tabletop centrifuge are disinfected and moved into the BSC.
11. The tubes are carefully removed from the centrifuge bucket, the clear layer above the whole blood layer is the plasma.
12. Plasma is carefully removed but slightly tilting the tube and aspirating the plasma using a filter tip 1 mL micropipette tip to not disturb the whole blood layer at the bottom. The blood collection tubes with the remaining blood is disposed into the strainer for disinfection.

\* Originally adapted from *Stanford Human Immune Monitoring Center* protocol for plasma isolation

13. The plasma is transferred to a 15 mL conical tube, the tube is decontaminated and transferred to the centrifuge bucket.
14. The centrifuge bucket with the samples is decontaminated before inserting into the tabletop centrifuge and the samples are spun down at 1,000g for 10 minutes.
15. After centrifugation, centrifuge buckets containing the samples are removed from the tabletop centrifuge are disinfected and moved into the BSC.
16. The tubes are carefully removed from the centrifuge bucket and disinfected.
17. The isolated plasma supernatant is aliquoted into cryotubes into 0.5-1 mL aliquots into cryovials using filter tip 1 mL micropipette, the tips are disposed into the strainer for disinfection and the aliquoted plasma is frozen at -80°C until use.
18. The centrifuge bucket, adapter and containment lid are thoroughly wiped down and inserted back into the tabletop centrifuge after decontaminating the rotor and centrifuge chamber walls.
19. In the BSC, the micropipettes, tip box and markers are disinfected. The cabinet surface is wiped down and all used paper towels are disposed in the solid waste collection bag.
20. The contents in the strainer (used tips, collection tubes and caps) with disinfectant is transferred to the solid waste collection bag carefully to not transfer any liquid into the solid waste collection bag.
21. The solid waste is double bagged and secured with a autoclave tape (do not twist the autoclave bag to allow steam escape).
22. Disinfect the biosafety cabinet again including the walls and interior of the glass stash.
23. Place another small biohazard bag and dispose used paper towels into that and perform glove hygiene by spraying outer glove with Quatricide.
24. Put on new outer gloves and secure biohazard bag before disposing into the autoclavable waste accumulation area.