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Version 2

Sinai SCENT TMC - Peripheral Blood Collection V.2

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Cellular Senescence Net...



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Protocol status: Working

We use this protocol and it's working

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Abstract

This Standard Operating Procedure is to provide guidance toresearch team members who are involved in the retrieval, processing, and storing of peripheral blood samples obtained from research participants.

The research team shall use this SOP within the scope of an IRB-approved protocol.

Materials

Blood Collection

- 2x Yellow Top Tubes (PBMC) - Acid citric dextrose additive (ACD)
- 1x Red Top Tube (Serum) – No additive
- 3x Purple Top (Plasma) - Ethylenediaminetetraacetic Acid (EDTA)
- 1x PaxGene Tube
- Blood Collection Supplies: Gloves, Butterfly needle, vacutainer, tourniquet, gauze, alcohol pads, Band-Aids
- Biohazard bag
- Transport container (check with lab manager for appropriate transport container)

Processing

- Microtubes (5× 0.2mL, 1-2× 1.5mL)
- PBS
- Histopaque – 1077 (Sigma, 10771)
- 10% bleach solution (to discard used tips, tubes, etc.)

Scope

- 1 This document outlines the process of collecting, processing, and storing peripheral blood from research participants enrolled in SCENT U54.

- 2 **CRITERIA 1 - Collecting blood samples from colonoscopy and bronchoscopy**

dx.doi.org/10.17504/protocols.io.bp2l628yrgqe/v3

dx.doi.org/10.17504/protocols.io.rm7vzjz4xlx1/v1

CRITERIA 2 - Collecting blood samples (banked, FAMRI)

Inclusion criteria: a) age 65 or older, b) persons who agreed to be contacted for the EDOPeCC study, c) persons who provide a smoking history and agree to be contacted for the current study.

Exclusion criteria: a) immunocompromised adults (HIV, malignancy diagnosis in past 5 years, systemic chemotherapy, end-stage renal failure), as these factors may influence the expression of TLRs; b) adults with other obstructive lung disease (history of asthma, cystic fibrosis, bronchiectasis, bronchiolitis obliterans, or vocal cord dysfunction); c) adults who cannot give informed consent due to language, cognitive, or other barriers; d) adults who cannot perform spirometry due to cognitive, health or other reasons.

Materials

- 3 **Blood Collection**

- 2x Yellow Top Tubes (PBMC) - Acid citric dextrose additive (ACD)
- 1x Red Top Tube (Serum) – No additive
- 3x Purple Top (Plasma) - Ethylenediaminetetraacetic Acid (EDTA)
- 1x PaxGene Tube
- Blood Collection Supplies: Gloves, Butterfly needle, vacutainer, tourniquet, gauze, alcohol pads, Band-Aids
- Biohazard bag
- Transport container (check with lab manager for appropriate transport container)

- 4 **Processing**

- Microtubes (5× 0.2mL, 1-2× 1.5mL)
- PBS (Phosphate Buffered Saline)
- Histopaque – 1077 (Sigma, 10771)
- 10% bleach solution (to discard used tips, tubes, etc.)

Procedures - Blood collection and Transport

- 5 **Sample retrieval:**



Peripheral blood shall be collected during visit 2, immediately before the bronchoscopy procedure.

6 **Order of blood draw:**

Red → Yellow → Purple → PaxGene

7 **Inversion times**

Once blood has stopped flowing in the tube, remove and gently invert (Avoid vigorous shaking or mixing):

- Red – No inversion necessary
- Yellow – 8 times
- Purple – 8 times
- PaxGene – 8 times. Do not force blood into the tube, which can affect RNA quality.

8 **Sample Transport:**

Samples should be transported to the lab and processed 30-60 minutes after collection.

Transport should occur at Room temperature



Procedures - Sample Processing

10m

9 **Serum (Red top) and Plasma (Purple top):**

10m

1. Label all tubes with Study ID and 'S' for serum, and 'P' for plasma.
2. Centrifuge tubes 2200 rpm, 00:10:00 , Break #9, #9
3. After spinning, aliquot supernatant to 5 × 0.2mL microtubes, and the remaining supernatant into 1.5mL tubes (label top of 1.5mL w/ volume (e.g. '300cc'))
4. Store in -80 °C freezer
5. Update sample inventory

10 **PaxGene:**

1. Store the PAXgene Blood RNA Tube upright Room temperature (18°C–25°C) for a minimum of 2 hours and a maximum of 72 hours before processing or transferring to the refrigerator (2–8°C) or freezer (–20°C).
2. Update sample inventory

11 **PBMC Isolation and Cryopreservation:**

See PBMC isolation protocol in LRT (Lee Research Team) shared drive



Protocol references

PAXGene: https://static.bdbiosciences.com/documents/PAXgene-RNA-Tube-Blood-Collection-Guide.pdf?_ga=2.64819439.1950632283.1619385573-1365518261.1618432443&_gl=1*1ma0ioc*_ga*NjU1NDQ5NjU5LjE3MDQ0NzkzOTg.*_ga_LL7XK30S8W*MTcwNDQ3OTM5Ny4xLjAuMTcwNDQ3OTQwMS41Ni4wLjA.