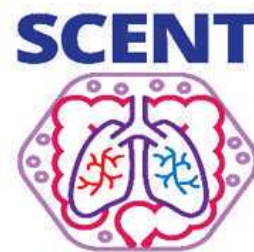


May 25, 2023

Version 1

U54 SCENT Normal Lung Autopsy Tissue Collection Procedure V.1



DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvr416zgmk/v1>

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Protocol Citation: Carolyn Glass, andrew.nixon , Mary Jordan* 2023. U54 SCENT Normal Lung Autopsy Tissue Collection Procedure. protocols.io <https://dx.doi.org/10.17504/protocols.io.6qpvr416zgmk/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 25, 2023

Last Modified: May 25, 2023

Protocol Integer ID: 82482

Keywords: u54 scent normal lung autopsy tissue collection procedure, collection protocol for normal lung specimen acquisition, normal lung specimen acquisition, autopsy at duke university hospital, department of pathology, precision pathology center, specimen, duke university hospital, pathology, tissue, collection protocol

Funders Acknowledgements:

NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director
Grant ID: U54AG075936

Abstract

This document outlines the required criteria and collection protocol for normal lung specimen acquisition from autopsy at Duke University Hospital through the Biorepository and Precision Pathology Center (BRPC) in the Department of Pathology.

Inclusion and Exclusion Criteria

1 Inclusion Criteria:

- >18 weeks fetal lung to age >85 years old from decedents with consented autopsy
- All sexes
- Grossly normal lung (reviewed and triaged by autopsy staff/faculty)

Exclusion Criteria:

- No primary lung disease (pulmonary embolism, COPD, primary lung cancer, pneumonia, interstitial lung disease, emphysema/heavy smoker, etc)
- If "pulmonary hypertension" stated in clinical notes, this tissue may still be taken and reviewed by histologic exam
- No metastatic cancer to lung
- No active infection (remote >2 weeks ago is acceptable, viral titers must be negative)
- Check blood culture for infection
- No recent treatment with chemotherapy (recent <1 year)
- Time of death <48 hours prior to collection

Collection Protocol

- 2 U54 Clinical Research Coordinator will access Microsoft Teams DUH Decedent Care - Labs Autopsy Team.
- 3 U54 Clinical Research Coordinator will screen the afternoon prior for upcoming autopsy collections that fit inclusion and exclusion criteria.
- 4 U54 Clinical Research Coordinator will call the morning of procedure day at to request for resident or PA for:
 - a. Lung tissue available from autopsy
 - b. Before and after pictures of lungs
- 5 Autopsy team will notify BRPC staff of available tissue.
- 6 Receive 1 fragment of normal lung tissue (total size collected will be 3×3×3 cm) from the RUL, RML, RLL, LUL, and LLL upon notification by the autopsy suite staff.
- 7 Process each lobe fragment into the following samples. Create a QC for each sample collected
 - a. FFPE (3×3×0.5 cm)

- b. Snap frozen: split in half
 - i. ½ snap freeze in cryovial
 - ii. ½ will be cut into "rice -size piece"
 1. Wash tissue with PBS to remove the blood and epithelial lining fluid.
 2. Using blade, mince tissue into approximately 20 small pieces (1-2mm).
 3. Evenly distribute the rice sized pieces into 3 cryopreserved tubes with 1mL freezing media each.
 4. Cells should be frozen slowly at 1°C/min. Put tubes in the freezing container (e.g., Mr. Frosty) with isopropyl alcohol and place them overnight in a -80'C freezer.
 5. Next day, transfer tubes to liquid nitrogen.
 - c. If tissue remaining: 3 additional cryovials of snap frozen tissue cut into rice sized pieces and OCT
- 8 Route FFPE samples to Research Histology Lab to:
- a. Create an H&E from each FFPE sample and route to pathologist for review.
 - b. After review, create 2 tissue scrolls from the tissue blocks and coordinate RNA and DNA extraction.
 - c. Once RNA and DNA extraction is complete, the frozen LN2 samples will be shipped to lab for scRNAseq.