ONLINE DATA SUPPLEMENT

Design of the SPIROMICS Study of Early COPD Progression (SOURCE)

Jeffrey L. Curtis, MD [1,2,3,* †], Lori A. Bateman, MS [4 *], Susan Murray ScD [5], David J. Couper, PhD [4], Wassim W. Labaki, MD, MS [1], Christine M. Freeman, PhD [1, 3, 6], Kelly B. Arnold, PhD [7,] Stephanie A. Christenson, MD, MAS [8], Neil E Alexis, PhD [9], Mehmet Kesimer, PhD [10], Richard C. Boucher, MD [10], Robert J. Kaner, MD [11], Igor Barjaktarevic, MD, PhD [12], Christopher B. Cooper, MD, PhD [12], Eric A. Hoffman, PhD [13], R. Graham Barr MD, DrPH [14], Eugene R. Bleecker, MD [15], Russell P. Bowler, MD, PhD [16], Alejandro Comellas, MD [17], Mark T Dransfield, MD [18], Michael B. Freeman, MD [19], Nadia N. Hansel MD, MPH [20], Jerry A. Krishnan, MD, PhD [19], Nathaniel Marchetti, DO [21], Deborah A. Meyers PhD [16], Jill Ohar, MD [22], Wanda K. O'Neal, PhD [10], Victor E. Ortega, MD, PhD [15], Robert Paine, III, MD [23], Stephen P. Peters MD, PhD [22], Benjamin M. Smith, MD, MS [14], Jadwiga A. Wedzicha, MD [24], J. Michael Wells, MD, MPH [17], Prescott G. Woodruff, MD, MPH [8], MeiLan K. Han, MD, MS [1 **], and Fernando J. Martinez, MD, MS [11 **], for the SOURCE Investigators

[1] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

[2] Medical Service, VA Ann Arbor Healthcare System, Ann Arbor, MI, USA

[3] Graduate Program in Immunology, University of Michigan, Ann Arbor, MI, USA

[4] Collaborative Studies Coordinating Center, Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[5] Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI, USA

[6] Research Service, VA Ann Arbor Healthcare System, Ann Arbor, MI, USA

[7] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

[8] Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of California San Francisco, San Francisco, CA, USA

[9] Division of Allergy Immunology and Infectious Disease, Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[10] Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[11] Weill Cornell Medicine, New York-Presbyterian Hospital, New York, NY, USA

[12] Division of Pulmonary, Critical Care and Sleep Medicine, University of California Los Angeles, Los Angeles, CA, USA

[13] Department of Radiology, University of Iowa, Iowa City, IA, USA

[14] Department of Medicine and Department of Epidemiology, Columbia University Medical Center, New York City, NY, USA

[15] Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Scottsdale, AZ, USA

[16] Division of Pulmonary, Critical Care & Sleep Medicine, National Jewish Health, Denver, CO, USA

[17] Department of Medicine, University of Iowa, Iowa City, IA, USA

[18] Division of Pulmonary, Allergy and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

[19] Division of Pulmonary, Critical Care, Sleep and Allergy, University of Illinois at Chicago, Chicago, IL, USA

[20] Department of Medicine, Johns Hopkins University, Baltimore, MD, USA

[21] Department of Thoracic Medicine and Surgery, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

[22] Section of Pulmonary, Critical Care, Allergy and Immunologic Diseases, Wake Forest University, Winston-Salem, NC, USA

[23] Division of Respiratory, Critical Care, and Occupational Pulmonary Medicine, University of Utah Health Sciences Center, Salt Lake City, UT, USA

[24] National Heart and Lung Institute, Imperial College, London, UK

*, co-first authors

**, co-senior authors

†, Corresponding author

SUPPLEMENTAL METHODS

Questionnaire data collection

Using the British Medical Research Council modified 1946 birth cohort early life exposure questionnaire[1], SOURCE is collecting extensive data on early life exposures, via self-report of: premature birth ("before 9 months or 37 weeks"); birth weight ("low, normal, high"), smoking during pregnancy (maternal or other household members); breastfeeding; childhood respiratory infections and symptoms; and childhood home exposures to gas- or wood-burning stoves, indoor tobacco smoking, and pets.

Several of the other questionnaires employed by SOURCE merit elaboration. The Chronic Airway Assessment Test (CAAT), a variation of the COPD Assessment Test (CAT) designed for use in those without established chronic obstructive pulmonary disease (COPD), was developed by an interdisciplinary group of international experts with support from GSK. The CAAT consists of nine questions regarding cough and other chest symptoms, activity limitation , sleep soundness, and energy, also self-assessed on a scale of 0-5. CAT, COPD Assessment Test, CAAT, Chronic Airway Assessment Test, and the CAT logo are trademarks of the GSK group of companies.

The COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk was developed for use in the CAPTURE study (ClinicalTrials.gov NCT03581227). This simple (five question) test was found in a recent US cohort of primary care patients (n=4325) to have low sensitivity but high specificity for identifying clinically significant COPD defined by presence of airflow obstruction that is of moderate severity or accompanied by a history of acute respiratory illness [2].

In addition to detail examination of the participant's own exposures and symptoms, the Baseline Respiratory Disease and Smoke Exposure Questionnaire inquires about parental history of smoking and lung diseases (chronic bronchitis, emphysema, COPD, asthma, and lung cancer).

Via the Baseline E-Cigarette Use Assessment questionnaire, also being used by COPDGene and SPIROMICS, SOURCE will capture detained information into vaping behavior that could co-exist in our cohort with use of conventional cigarettes. Results will yield data on specific delivery devices (including via a photograph of the actual product), self-reported use of cannabis, delta-9-tetrahydrocannabinol (THC), and cannabidiol (CBD), and healthcare utilization events compatible with vaping-induced lung injury.

The baseline employment history form capture extensive information about the participants current job and longest held job since age 16 (if different), including military service, paid, self-paid, or unpaid (i.e., volunteer) positions involving at least 20 hours of work per week. It inquires specifically about 24 occupation classes, with follow-up questions about exposures to vapors, gas, dust, or fumes.

SOURCE is also collecting detailed data on SARS-CoV-2 immunization and COVID-19 infection history, using a form that will permit sharing of data with the Collaborative Cohort of Cohorts for COVID-19 Research (C4R) initiative (https://www.c4r-nih.org/).

Impulse Oscillometry (IO)

IO will be performed during the baseline visit and the follow-up visit at year 3. IO is an effort-independent assessment of airways obstruction, characterizing peripheral, central, or heterogeneous patterns. IO requires minimal participant cooperation, as is performed during resting tidal breathing. Thus, IO is an alternative to spirometry when the latter is difficult for the participant to complete properly.

IO measures respiratory impedance (Zrs) during resting tidal breathing. Zrs is commonly represented by the resistance (Rrs), which accounts for obstruction in the central airways, and reactance (Xrs), which accounts for capacitance and inertance in the lung periphery. The reported sensitivity of IO to detect airway obstruction is similar or superior to that of spirometry [3-5]. Several studies have also shown its potential utility to separate the contributions of inspiration and expiration to Rrs and Xrs. This purpose uses an approach known as within-breath analysis of Zrs, allowing the calculation of inspiratory resistance and reactance (Rinsp and Xinsp, respectively) and expiratory resistance and reactance (Rexp and Xexp, respectively).

We will use the 2003 ERS task force document as guidance in conducting IO measurements. IO will be performed with disposable low-resistance filters. Participants will be seated after loosening tight or restrictive clothing and removing loose dentures, wearing a nose clip, with the chin slight elevated. If forced respiratory maneuvers precede an IO test, at least three minutes of quiet breathing should be allowed for recovery. During measurements, the participant's cheeks and floor of the mouth are supported by the technician using both hands. Participants are encouraged to perform relaxed tidal breathing at baseline and throughout the 30-60 seconds of data collection.

We will collect a total of three to five acceptable measurements. Each measurement will continue until 10 breaths meeting quality criteria have been captured. Between successive measurements, the participant should come off the mouthpiece to rest for at least 30 seconds. To assess peripheral closing volume, we will perform an additional IO recording consisting of five breaths, followed by a slow expiratory vital capacity.

Data management plan

Data will be entered directly into the Carolina Data Acquisition and Reporting Tool (CDART), a custom web-based data management system (DMS). CDART allows editing during data entry plus generation by site teams and the CSCC of status reports and queries, all in real-time. When internet service is temporarily unavailable, data will be collected on paper forms and entered once access is restored. Via secure FTP, we regularly retrieve all data (including from reading centers), which is merged using SAS into clinical databases that will are rigorously reviewed for quality assurance.

All databases are housed in a secure, climate-controlled server facility at the CSCC, under an Information Technology (IT) Security Plan approved by NHLBI Contract Officers. This IT plan documents standard operating procedures required to secure the network and databases, including management, operational and technical controls. Also included are risk assessment, system continuity, and a disaster recovery plan. We employ the principle of "least access privilege" for study files.

Data confidentiality and security measures will be applied at all levels of data acquisition, transfer, and storage, and to all study agencies, including clinical and reading centers, central laboratories, and the CSCC. CDART meets exacting standards of confidentiality, including HIPAA requirements. Beyond password-controlled access to CDART, data collected at the clinical centers will be encrypted by the system and decrypted for display on-screen only by authorized study personnel. Personal identifiers will be collected in separate forms.

The CSCC will be responsive to data confidentiality requirements from providers of medical care and IRBs. SOURCE plans to collect all data electronically, but any paper forms used will be retained securely at the clinical centers, following follow local institutional procedures, until the Steering Committee acts on recommendations from the CSCC for disposal (e.g., after incremental data closure). As standard practice, data shared with reading centers, cores, or those analyzing SOURCE databases identifies participants only by ID number. Personally-identifying information collected in SOURCE includes name, address and address history, phone number, date of birth, dates of medical procedures, and social security number. These data will be stored on a secure server, in a highly restricted folder, following the plans described above.

Observational Studies Monitoring Board (OSMB)

The SOURCE OSMB is an independent group of scientists and clinicians required to provide recommendations about starting, continuing, and stopping the study to the Office of the Director, NHLBI. This OSMB operates under a written charter from NHBLI, which makes final decisions based on the written minutes of OSMB deliberation. The OSMB also monitors SPIROMICS, and accordingly is quite familiar with the similar designs of the two studies.

The OSMB is asked to make recommendations, as appropriate, to the NHLBI about: (a) benefit/risk ratio of procedures and participant burden; (b) selection, recruitment, and retention of participants; (c) adherence to protocol requirements; (d) completeness, quality, and analysis of measurements; (e) amendments to the study protocol and consent forms; (f) performance of individual centers and core labs; (g) participant safety; (h) notification of and referral for abnormal findings.

The Board has the responsibility of reviewing all Ancillary Study proposals to determine whether the Ancillary Study could provide harm to the conduct of the main study. The Ancillary Study review by the Board can be done during the annual Board meeting or by email review. An Executive Secretary for the Board is an NHLBI staff member not associated with the study, who provides for all interaction between the study and the Board.

Communication with OSMB members will be primarily through the NHLBI Program Office and the Genomics and Information Center (GIC). It is expected that study investigators will not communicate with OSMB members about the study directly, except when making presentations or responding to questions at OSMB meetings or during conference calls.

For each meeting, the Collaborative Studies Coordinating Center (CSCC) managing SOURCE, with input from NHLBI staff, will prepare summary reports and tables to facilitate the oversight role of the OSMB. The OSMB should discuss at the first or subsequent meetings what data they wish to review and how the data should be presented.

SUPPLEMENTAL REFERENCES

1. Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. Combined impact of smoking and early life exposures on adult lung function trajectories. *Am J Respir Crit Care Med*. 2017:1021-1030. doi:10.1164/rccm.201703-0506OC
2. Martinez FJ, Han MK, Lopez C, et al. Discriminative accuracy of the CAPTURE tool for identifying chronic obstructive pulmonary disease in US primary care settings. *JAMA*. 2023;329(6):490-501. doi:10.1001/jama.2023.0128
3. van Noord JA, Wellens W, Clarysse I, Cauberghs M, Van de Woestijne KP, Demedts M. Total respiratory resistance and reactance in patients with upper airway obstruction. *Chest*. 1987;92(3):475-80. doi:10.1378/chest.92.3.475
4. Zerah F, Lorino AM, Lorino H, Harf A, Macquin-Mavier I. Forced oscillation technique vs spirometry to assess bronchodilatation in patients with asthma and COPD. *Chest*. 1995;108(1):41-7. doi:10.1378/chest.108.1.41
5. Faria AC, Costa AA, Lopes AJ, Jansen JM, Melo PL. Forced oscillation technique in the detection of smoking-induced respiratory alterations: diagnostic accuracy and comparison with spirometry. *Clinics (Sao Paulo)*. 2010;65(12):1295-304. doi:10.1590/s1807-59322010001200012
6. Tomaszewski EL, Atkinson MJ, Janson C, et al. Chronic Airways Assessment Test: psychometric properties in patients with asthma and/or COPD. *Respir Res*. 2023;24(1):106. doi:10.1186/s12931-023-02394-6
7. Lange P, Celli B, Agusti A, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. *N Engl J Med*. 2015;373(2):111-22. doi:10.1056/NEJMoa1411532

tables

Table 1. Synopsis of the scientific questions in Early COPD that will be addressed by this protocol.

Aim 1	Aim 2	Aim 3
(Entire cohort)	(Entire cohort)	(Bronchoscopy sub-study)
Can baseline PRM^{fSAD} predict future lung damage including emphysema?	Do sputum total mucins and concentrations of MUC5AC and MUC5B correlate with: 2a. baseline PRM^{fSAD}? 2b. FEV_1 change & future lung damage?	Does the IL-17 epithelial gene signature correlate with PRM^{fSAD} (1° endpoint), and with longitudinal FEV_1 change, future lung damage, and airway mucin concentration (2° endpoints)?

Table 2. SOURCE inclusion and exclusion criteria

Inclusion
age 30-55 years;
healthy controls defined as:
no smoking history (< 100 cigarettes in lifetime, including vaping and cannabis use);
pre-bronchodilator $FEV_1/FVC \geq 0.70$, pre-bronchodilator $FEV_1 \geq 80\%$ predicted, pre-bronchodilator FVC ≥ 80% predicted;
Chronic Airway Assessment Test (CAAT) score [6] < 10, and additional willingness to participate in the bronchoscopy sub-study.
individuals at risk or with structural lung disease, defined as:
≥10 pack-years smoking history;
GOLD 0 (post-bronchodilator FEV1/FVC ≥0.70 and FEV1 >80% predicted); or
GOLD 1-2 (post-bronchodilator FEV1/FVC < 0.70 and FEV1 > 50% predicted); or
Preserved Ratio Impaired Spirometry (PRISm) participants: post-bronchodilator FEV1/FVC > 0.70 and FEV1 < 80% predicted.
Exclusion
severe adult asthma (GINA Step 4 or higher, > 3 unscheduled healthcare visits or one asthma hospitalization in past 12 months);
chronic respiratory condition other than COPD or asthma;
concurrent participation in blinded therapeutic trial;
illness expected to cause mortality in three years;
active pregnancy or planning to become pregnant during the course of the study;
cognitive dysfunction impeding completing study procedures;
BMI >35 kg/m^2 (due to impact of high BMI on imaging quality);
any implanted metallic device or prosthesis that could degrade HRCT imaging quality;
history of thoracic radiation or thoracic surgical resection of lung tissue;
known HIV/AIDS infection;
use of recreational or illicit substances, excluding marijuana, within the past 10 years.

Table 3. SOURCE baseline and follow-up assessments

Visit	Baseline (n=600)	Follow-up Phone Call/Email every 6 months (n=600)	Follow-up Phone Visit at 18 months (n=600)	Follow-up Visit at 3 years (n=600)
Assessment				
Demographics	X			
Modified 1946 birth cohort early life exposure questionnaire [a]	X			
Block Brief 2000 Food Frequency Questionnaire	X			
Stool Collection	X			
Exhaled Breath Condensate	X			
Adverse Events	X	X	X	X
Contact Information	X	X	X	X
Medical History	X		X	X
Exacerbation Questionnaire	X		X	X
Medication Use Questionnaire	X		X	X
Respiratory Disease and Smoke Exposure Questionnaire	X		X	X
CAPTURE Questionnaire	X			X
E-Cigarette Use Assessment	X		X	X
COVID-19 History Questionnaire	X		X	X
Chronic Airway Assessment Test	X		X	X

Employment History	X		X	X
Home Information Questionnaire	X		X	X
Modified Medical Research Council Dyspnea Scale	X		X	X
St. George's Respiratory Questionnaire	X		X	X
Anthropometry	X			X
Blood Pressure	X			X
Spirometry	X			X
Oscillometry	X			X
HRCT Imaging	X			X
Blood Collection	X			X
Sputum Collection	X			X
Hospital Anxiety and Depression Scale	X			X
Follow-up Phone Questionnaire		X		

a, [1]

LIST OF APPENDICES

SOURCE – SPIROMICS STUDY OF EARLY COPD PROGRESSION also known as Origins of Early COPD Progression

MASTER PROTOCOL

VERSION 3.5

February 6, 2024

Table of Contents

1. Table of Abbreviations

ASL: Airway surface liquid

ATS: American Thoracic Society

BMI: Body mass index

BP: Blood pressure

BSP: Biospecimen Processing Facility

CAPTURE: COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk

CAT: COPD Assessment Test

CAAT: Chronic Airway Assessment Test

CanCOLD: Canadian Cohort Obstructive Lung Disease

CBC: Complete blood count

COPD: Chronic obstructive pulmonary disease

CT: Computerized tomography

CTDI: Computerized tomography dose index

DMS: Data Management System

DNA: Deoxyribonucleic acid

EBC: Exhaled breath condensate

EDTA: Ethylenediaminetetraacetic acid

Emph: Emphysema

ER: Emergency room

ERS: European Respiratory Society

FEV_1: Forced expiratory volume in first second

fSAD: Functional small airway disease

FVC: Forced vital capacity

GIC: Genomics and Informatics Center

GINA: Global Initiative for Asthma

GOLD: Global Initiative for Chronic Obstructive Lung Disease

HIPAA: Health Insurance Portability and Accountability Act

HRCT: High-resolution computed tomography

ICS: Inhaled corticosteroid

IL-17: Interleukin 17

IO: Impulse oscillometry

IR: Inversion recovery

IRB: Institutional Review Board

LABA: Long-acting β_2 adrenoceptor agonist

LAMA: Long-acting muscarinic antagonist

LASSO: Least absolute shrinkage and selection operator

LDMS: Laboratory Data Management System

mAs: Milliampere second

MRC: Medical Research Council

MOP: Manual of Procedures

NIH: National Institutes of Health

NHLBI: National Heart, Lung, and Blood Institute

OSMB: Observational Studies Monitoring Board

PaO_2: Partial pressure of oxygen

PCA: Principal component analysis

PD: Project director

PLSDA: Partial least squares discriminant analysis

PLSR: Partial least squares regression

PFT: Pulmonary function test

PHI: Personal health information

PI: Principal investigator

PPRN: Patient Powered Research Network

PRISm: Preserved Ratio Impaired Spirometry

PRM: Parametric Response Mapping

QA: Quality assurance

QC: Quality control

R_{insp}: Inspiratory resistance

R_{exp}: Expiratory resistance

R_{rs}: Respiratory resistance

RETHINC: REdefining THerapy in Early COPD for the Pulmonary Trials Cooperative

RNA: Ribonucleic acid

RT-PCR: Reverse transcription polymerase chain reaction

RV: Residual volume

SAA: Small airway abnormality

SAD: Small airway disease

SaO_2: Oxygen saturation

SGRQ: St. George's Respiratory Questionnaire

SOP: Standard Operating Procedure

SPIROMICS: Subpopulations and Intermediate Outcome Measures in COPD Study

TLC: Total lung capacity

X_{insp}: Inspiratory reactance

X_{exp}: Expiratory reactance

X_{rs}: Respiratory reactance

Z_{rs}: Respiratory impedance

2. Introduction

SOURCE is studying the clinical characteristics and biological underpinnings of early chronic obstructive pulmonary disease (COPD). This study is recruiting a new cohort to complement the Subpopulations and Intermediate Outcome Measures in COPD Study II (SPIROMICS II) and employs the SPIROMICS II infrastructure. This protocol will expand our ability to define the nature of early COPD in younger, at-risk individuals. To study early COPD, we will recruit and study participants no older than 55 years of age, with ≥ 10 pack-years smoking history, and with either: (a) mild to moderate airflow obstruction; or (b) respiratory symptoms in the absence of airflow obstruction. Previous data suggest that these subjects are more likely to experience more rapid disease progression as evidenced by FEV_1 decline and CT changes.

3. Background and Specific Aims

COPD is a highly prevalent disorder with rising morbidity and mortality[1, 2]. Pharmacotherapy may improve symptoms and decrease exacerbation frequency but does not alter lung function decline or mortality [3, 4]. Other than smoking cessation, no current therapy can slow COPD progression.

Studies of disease modification in COPD have failed due in part to their focus on late-stage disease. Moreover, patients with established COPD demonstrate significant variability in symptom presentation, rates of disease progression, exacerbation frequency, and histologic abnormalities. Inability to access well-characterized research participants for targeted therapies and the lack of good intermediate endpoints have impeded early-stage development research. Because COPD is a slowly progressive disease, determining an “early” signal in Phase 2 studies has proven to be incredibly challenging.

In those who eventually develop COPD, the onset of respiratory symptoms and lung function decline begins in early adulthood. However, airflow obstruction in COPD may represent either accelerated lung function decline or failure to attain peak function in adulthood with subsequent age-appropriate decline [5]. Only those with accelerated lung function decline would be anticipated to respond to interventions aiming to slow progression.

The design of the current study was informed by recently published epidemiological studies. The MRC National Survey of Health & Development analyzed 5,362 subjects born during one week in 1946 and followed for six decades. In those with ≥ 10 pack-years smoking history, increased respiratory symptoms were evident by 36-43 years of age [6]; accelerated lung function decline was evident by 43 years of age, particularly in those with respiratory symptoms[6, 7]. That research group noted that escalating symptom prevalence during middle age was unlikely to be explained by rising cigarette consumption alone [6]. Similarly, in the Framingham Offspring Study, faster lung function decline was associated with the presence of respiratory symptoms in the mid-30s [8]. A separate group also found an association of progression with increased symptoms in smokers in their late 50s [9]. Thus, smoking, particularly when associated with respiratory symptoms, is strongly associated with later impaired lung function; the impact in susceptible smokers is detectable by 30-40 years of age with exposures as little as 10 pack-years smoking history.

Supporting the feasibility of identifying those with accelerated lung function decline, early histological studies confirm anatomical abnormalities in smokers by early adulthood. Older necropsy studies showed that histological emphysema [10] or airway remodeling [11] became prominent by ages 40s to early 50s. Detailed histological analyses confirmed airway-centered changes to be the first lung abnormality associated with smoking [12], suggesting that they could be the source of subsequent emphysema [13].

Small airway abnormality (SAA) is a pathological feature of mild COPD. A cross-sectional study confirmed that the number of terminal and transitional bronchioles was decreased in mild-moderate COPD compared with control smokers; 40-60% decreases were noted in GOLD I or II individuals [(14)]. Remaining small airways showed thickened walls and narrowed lumens. With greater degrees of airflow obstruction, emphysematous changes were increasingly seen. Importantly, loss and changes in bronchioles were demonstrated in areas with no emphysematous destruction. The PRM technique, a CT voxel-based imaging biomarker validated by our group, employs dynamic image registration and separate density thresholds for inspiratory and expiratory voxel measurements. PRM distinguishes regions of "normal" lung from "functional small airways disease" (PRM^{fSAD}) and "emphysema" (PRM^{Emph}) [(15)]. From baseline and five-year follow-up data from COPDGene, we established an association between PRM^{fSAD} and subsequent FEV_1 decline in those with established COPD [(16)]. Importantly, among GOLD 0 subjects, baseline PRM^{fSAD} correlated significantly with later FEV_1 decline. Using an innovative cryo-microCT methodology, we demonstrated the association of PRM^{fSAD} with histological findings of narrowing and loss of terminal and transitional bronchioles [(17)]. PRM^{fSAD} correlated very strongly with decreased circularity, decreased luminal area, and complete obstruction. In contrast, PRM^{Emph} uniquely associated with decreased alveolar surface area and fewer alveolar attachments[(17)].

In recently published analyses of SPIROMICS subjects, we showed a small but significant increase in PRM^{fSAD} with age among healthy, asymptomatic non-smokers. This change likely reflects normal aging [(18)]. In addition, due to a significant age interaction, the ability of PRM^{fSAD} to predict lung function loss is greater in younger individuals. Similarly, PRM^{fSAD} precedes PRM-defined emphysema development. In a separate analysis, PRM^{fSAD} was higher in milder grade COPD disease, while increased emphysema was seen more prominently in later grade COPD disease. These longitudinal analyses show that COPD progression is typified by increases in PRM^{fSAD} in milder stage disease, followed by increases in PRM^{Emph}[(16)]. Lastly, in preliminary data among SPIROMICS subjects who have smoke exposure, normal spirometry, and age < 55 years, increased symptoms, as indicated by higher COPD Assessment Test (CAT) score, is associated with greater changes in PRM^{fSAD} over three years (unpublished data).

Increasing mechanistic insights have been gleaned that may be applicable to SOURCE. Bronchoscopic sampling suggests that the earliest detectable step induced by smoking is reprogrammed gene expression by the basal epithelial cells that maintain the airway epithelial barrier [(19-21)]. However, these studies were performed in older subjects and lacked imaging parameters or longitudinal evaluation. Epithelial reprogramming causes an ecological transition of the small airway epithelium from a distal community of diverse cell-types well-suited to self-defense and efficient clearance [(22, 23)] to one dominated by mucus hypersecretion, squamous metaplasia, and damaged cilia. These changes impair mucus clearance, facilitating retention of microbes on airway epithelial cells, rather than speeding their elimination. However, epithelial reprogramming alone cannot explain why only some smokers progress rapidly as reprogramming occurs in almost all smokers initially without epithelial invasion by inflammatory cells. Thus, those changes are <u>necessary</u> but <u>insufficient</u> for SAA development. Instead, auto-aggressive immune responses appear to determine which small airways are damaged [(24-26)].

In older individuals with established COPD, an IL-17 signature has been associated with reduced lung function [(27, 28)]. Findings from the SPIROMICS bronchoscopy sub-study imply that the epithelial responses to IL-17-driven inflammation may causally contribute to SAA. Data from that sub-study demonstrated significant association between increased PRM^{fSAD} at baseline and detection in airway epithelial brushing of the IL-17 gene signature.

Epithelial reprogramming induces multiple alterations to airway surface liquid (ASL). In SPIROMICS subjects, smoking changed the homeostatic balance between ASL volume [(29, 30)] and the relative concentration of mucus and periciliary layers [(31)] balance inducing mucus hyperconcentration [(32)]. Furthermore, total sputum mucin concentrations in older SPIROMICS subjects correlated significantly with spirometric and imaging markers of SAA. In a subset of

SPIROMICS subjects (mean age 66 years) with GOLD 0-II COPD who had baseline sputum and follow-up imaging at three years, greater total mucin concentration at baseline correlated with increased PRM^{fSAD} (and hence SAA) at three years. Similarly, in preliminary data, greater total mucin at baseline was associated with increased PRM^{Emph} at three years; this association was more significant when analyses were limited to GOLD I/II. Collectively, these data on airway epithelial cell gene reprogramming, associated deficits in lung host defense that lead to immune-mediated inflammation, and changes in mucus characteristics that favor bacterial colonization of the small airways provide a plausible hypothesis of the mechanisms underlying SAD progression in early COPD *(25)*. This study will test the validity of that hypothesis.

Developing and testing novel, disease-modifying treatments will require reliable biomarkers to identify those with progressive disease at a younger age than studied to date. Most COPD studies to date focus on subjects ≥ 60 years old *(33)*. This limitation is true of cohorts with detailed imaging and biologic data collection, including COPDGene *(34)*, SPIROMICS *(35)*, and CanCOLD *(36)*. The high prevalence of their subjects with "mild" disease (GOLD 0-I) at that age range is consistent with the pathological process in many of their participants being defective lung growth rather than accelerated loss of lung function. Hence, new cohorts are needed to define the mechanisms of early COPD.

To standardize future studies, an international group proposed a working definition for research on early changes leading to COPD (early COPD). This group defined the optimal study population as those younger than 50 years with ≥ 10 pack-years smoking history and any of these abnormalities: (1) early airflow limitation; (2) compatible CT abnormalities; or (3) rapid decline in FEV_1 (≥ 60 mL/y) that is accelerated relative to FVC *(25)*. This study follows that recruitment strategy and provides a unique opportunity to redefine a rapidly evolving field.

Specific Aims: COPD is one of a few chronic disorders with rising morbidity and mortality, yet physicians currently have limited ability to identify those at risk for rapid progression and lack reliable, disease-modifying therapies. Epidemiological studies convincingly pinpoint the onset of accelerated FEV_1 decline in susceptible smokers to the ages we propose to study. In older smokers with established COPD, publications from our group and others, as well as our preliminary data, offer compelling candidates for responsible pathological processes, which are unstudied in younger smokers. This project will yield two highly significant deliverables (see Synopsis table): we will identify the biological mechanisms essential to develop rapidly progressive COPD at an early age; and will hasten the transition to clinical practice of non-invasive biomarkers with superior discriminatory power to identify progression.

Synopsis of questions in Early COPD that will be addressed by this protocol

Aim 1 (Entire cohort)	**Aim 2 (Bronchoscopy sub-study)**	**Aim 3 (Entire cohort)**
Can PRM^{fSAD} predict future lung damage including emphysema?	Does IL-17 epithelial gene signature correlate with PRM^{fSAD} (1° endpoint), DC absolute numbers, & airway mucin concentration (2° endpoint)?	Do sputum total mucins correlate with: 3a. baseline PRM^{fSAD}? 3b. FEV_1 change & future lung damage?

This protocol is relevant to the NHLBI strategic vision to: *(a)* investigate pathobiologic mechanisms important to lung disease onset; *(b)* identify factors that account for individual differences in pathobiology; and *(c)* develop novel diagnostic strategies. We precisely address goals from the NHLBI COPD National Action Plan to: *(a)* define the pathogenesis of COPD heterogeneity by developing a biomarker to further characterize COPD subtypes; and *(b)* develop strategies to prevent the onset and progression of COPD by studying the progression of subclinical disease to overt disease. Finally, as we are using a standardized imaging protocol shared by other cohort studies including SPIROMICS and MESA, data form this study has the potential to be integrated with other imaging-based research efforts.

Aim 1a. To determine the relationship between CT-defined SAA and disease progression in early COPD, our primary analysis will use linear mixed effects models to estimate and perform inference on trajectories of PRM^{Emph}, based on PRM^{fSAD} and PRM^{Emph} at baseline CT imaging.

Aim 1b. In secondary analysis of Aim 1, we will similarly explore the relationship between baseline PRM^{fSAD} and: (1) further increases PRM^{fSAD}; (2) FEV_1 % decline over three years; (3) changes in oscillometry-based indices; (4) changes in patient-reported outcomes such as CAAT score; and (5) exacerbations. We will also examine the relationship between baseline emphysema and further development of emphysema at three years. Additional analyses will be performed using multivariable linear mixed models to adjust for age, sex, racial and ethnic groups, early life exposures, smoking history and ongoing smoking status (as a time-dependent covariate where appropriate), baseline pulmonary function, and concomitant medications. We will also investigate potential interactions between any of these variables and PRM metrics with the primary outcome in case relationships between PRM metrics and development of emphysema are modified by any of these parameters.

Aim 2. To define the biological basis of SAA in early COPD via lung-derived biomarkers.

We will correlate baseline PRM^{fSAD} with IL-17 gene signature in airway epithelium. Epithelial brushes and AMø will undergo RNA isolation and sequencing on an Illumina HiSeq 4000 platform at the Weill Cornell Sequencing Core. Sequence data will be analyzed to calculate values for each subject's distal and proximal airway samples for the IL-17 gene signature[27, 28]. We anticipate using distal sample values for our primary analysis, but proximal samples will also be of interest to compare to distal samples and to data from SPIROMICS.

BAL leukocytes will be enumerated and studied without and with ex vivo stimulation with agonists recognized by specific host receptors (TLR4, TLR2, TLR3) to activate innate responses, or by direct T cell activation (anti-CD3 plus anti-CD28). They will be stained for cell-type informative surface phenotypic markers and for intracellular cytokine production (three per cell type); supernatants will be reserved for multiplexed measurement of inflammatory mediators tailored to the cell panel and the stimulus. Separate panels of phenotypic markers will permit identification of AMø, monocytes, and DC subsets and multiple subsets of T cells. Flow cytometry will be performed and analyzed as described[56, 57].

Total mucin concentrations will be measured by size exclusion chromatography with multi-angle light scattering analysis and differential refractive index detection (SEC/MALS/dRI)[32].

Aim 3a. To determine whether sputum can serve as a non-invasive biomarker of early COPD.

The primary analysis for Aim 3 will be to correlate baseline total mucin concentrations from sputum samples and PRM^{fSAD}. Further analyses will be performed using multivariable models to adjust for age, sex, racial/ethnic groups, early life exposures, smoking history, and smoking status (as a time dependent covariate where appropriate), concomitant medications, and baseline disease FEV_1 % predicted; potential interactions/effect modification will be explored.

Aim 3b. Investigate the association between baseline mucin concentration and three-year change in mucin concentrations with change in FEV_1 and progression of PRM-defined CT abnormalities.

4. Study Design and Methods

4.1. Overview

We will use 14 clinical centers to enroll up to 1000 participants, 30-55 years old, both sexes, all races, and ethnicities. The participants will include never-smokers (n=40) and GOLD 0-2 participants (n=960).

- University of Alabama at Birmingham
- University of California at Los Angeles

  - Also the PFT Reading Center
- Johns Hopkins University
- University of Michigan
- National Jewish Health
- Columbia University
- University of California at San Francisco
- University of Utah
- Temple University
- University of Illinois at Chicago
- Wake Forest University Health Sciences
- University of Iowa
  - Also the Radiology Reading Center
- Weill Cornell Medicine

The University of North Carolina at Chapel Hill is not a clinical center enrolling participants into this study. UNC serves as the Regulatory (via sIRB) and Data Coordinating Center, with the latter known as the Genomics and Informatics Center (GIC).

- Also the Biospecimen Repository
- Also the Sputum Reading Center

4.2. Participant Inclusion and Exclusion Criteria

4.2.1. Inclusion Criteria

4.2.1.1. SOURCE cohort (n=1000)

- 40 of the 1000 will be healthy controls: ages 30-55 years; with no smoking history (< 100 cigarettes in lifetime), including vaping and cannabis use; pre-bronchodilator $FEV_1/FVC \geq 0.70$; pre-bronchodilator $FEV_1 \geq 80\%$ predicted; pre-bronchodilator $FVC \geq 80\%$ predicted; Chronic Airway Assessment Test (CAAT) score < 10. Willingness to also participate in the bronchoscopy sub-study is only required of the 20 healthy controls recruited from the clinical centers participating in the sub-study.
- Approximately one-third of the 960 will be GOLD 0 participants: ages 30-55 years; with ≥ 10 pack-year smoking history; post-bronchodilator $FEV_1/FVC \geq 0.70$ and $FEV_1 \geq 80\%$ predicted. This early COPD group has been chosen because it is not currently well-represented in COPDGene and SPIROMICS cohorts.
- Approximately one-third of the 960 will be Preserved Ratio Impaired Spirometry (PRISm) participants: ages 30-55 years; with ≥ 10 pack-year smoking history; post-bronchodilator $FEV_1/FVC \geq 0.70$ and $FEV_1 < 80\%$ predicted. This early COPD group was enrolled in small numbers in COPDGene but was excluded from SPIROMICS.
- Approximately one-third of the 960 will be GOLD 1-2 participants: ages 30-55 years; with ≥ 10 pack-year smoking history; post-bronchodilator $FEV_1/FVC < 0.70$ and $FEV_1 \geq 50\%$ predicted. This early COPD group has been chosen because it is also not currently well-represented in the first funded phase of the COPDGene and SPIROMICS cohorts.

4.2.2. Exclusion Criteria (presence of any of the following excludes the participant)

4.2.2.1. SOURCE (n=1000)

- Severe asthma, which is defined as any of the following:
  - Current (i.e., at the time of the visit) Global Initiative for Asthma (GINA) Step 4 or higher therapy (medium dose ICS/LABA or high dose ICS or add-on LAMA; Medium dose ≥ 250 fluticasone propionate, = 100 fluticasone furoate, > 200 beclomethasone, > 400 budesonide, > 220 mometasone). We will accept low-dose ICS/LABA or medium dose ICS;
    or
  - Three or more unscheduled healthcare visits (provider/urgent care/ER) for asthma in the past 12 months;
    or
  - One asthma hospitalization in the past 12 months.
- Concurrent participation in a therapeutic trial where treatment is blinded.
- Active pregnancy at the time of the baseline visit or planning to become pregnant during the course of the study. This special population is being excluded to minimize potential for fetal radiation exposure.
- Cognitive dysfunction that prevents the participant from completing study procedures.
- BMI > 35.0 kg/m^2 at baseline, due to the effects of body weight on CT scan imaging quality.
- The presence of a respiratory condition other than COPD (including chronic bronchitis and emphysema) or asthma, such as interstitial lung disease or pulmonary fibrosis, or of a comorbid condition that in the judgment of the investigator may be the principal cause of respiratory symptoms (e.g., dyspnea or decreased exercise tolerance).
- Any illness expected to cause mortality in the next three years.
- Any implanted metallic devices or prosthesis above the waist that could degrade thoracic CT scan image quality.
- History of thoracic radiation or thoracic surgery with resection of lung tissue.
- Known HIV/AIDS infection.
- Current illicit substance abuse, excluding marijuana.
- History of or current use of IV Ritalin.
- History of or current use of heroin.
- History of illegal IV drug use within the last 10 years or more than 5 instances of illegal IV drug use ever.

4.2.2.2. Additional *Temporal* Exclusion Criteria

- Participants who present with an acute exacerbation of COPD, either solely participant-identified or that has been clinically treated, in the last 30 days can be rescreened for the study once the 30-day window from end of drug therapy has passed.
- Participants who present with current use of acute antibiotics or steroids can be rescreened for the study ≥ 30 days after discontinuing acute antibiotics/steroids. This temporal exclusion does not apply to participants who are on chronic prednisone or participants who are currently on chronic, prophylactic, or suppressive antibiotic therapy.

4.2.2.3. Additional exclusions from SOURCE Bronchoscopy sub-study cohort (n=100 of the above n=1000)

- History of cardiac disease or other comorbid condition severe enough to significantly increase risks, based on bronchoscopist discretion.
- PaO_2 or SaO_2 that qualifies the participant for supplementary oxygen at rest (PaO_2 < 60 mmHg or SaO_2 < 88% while breathing ambient air).
- Use of anticoagulation. Participants on warfarin or clopidogrel will be excluded, but participants on aspirin alone can be included even with concurrent use.

5. SOURCE Study Procedures

5.1. Recruitment

This study will employ a mixture of strategies designed to maximize the efficiency of recruitment and retention of younger individuals. We appreciate the challenge of this process in a group, which is anticipated to be predominately actively employed and potentially also busy with child-care responsibilities. The foundation of our approach is the set of recruitment strategies successfully employed for the SPIROMICS cohort. These traditional methods include use of existing patient databases at each of the clinical centers, recruiting patients from clinic, and the use of newspaper and television advertisements, fliers, mailings, and posters.

Importantly, however, we will also use a series of innovative methods. These approaches derive from three sources: first, the highly successful ongoing efforts by our collaborator, Dr. Wedzicha, to recruit a young smoking population in the UK; second, an adapted format developed by Drs. Han and Woodruff for the ongoing NHLBI-sponsored RETHINC study, which is recruiting smokers with respiratory symptoms but without airflow obstruction; and third, a similar approach used by Drs. Kaner and Crystal in New York City to recruit younger smokers to undergo research bronchoscopy. These methods include robust online and social media marketing, by which we will be able to target specific geographic locations of recruiting clinical centers and the age range of our desired participants. Such recruitment methods are particularly suited to younger participants. The COPD Foundation has agreed to partner in these endeavors including the use of its robust COPD Patient Powered Research Network (COPD PPRN), COPD360social, the COPD Foundation website, the COPD Foundation social media outlets, the Faces of COPD Newsletter, and Facebook advertisements and events. The success of these programs is highlighted by the recruitment of over 450 individuals for a COPD study of respiratory infections in which the mean age of participants was under 50 and in a separate study with a narrowly defined geographic population.

Retention strategies for this cohort will include continued engagement via telephone call, text message, and/or email every six months from the baseline visit until the grant period ends and at 18 months. Participants will also be compensated for the baseline visit and follow-up visit at three years, which include questionnaires, CT, spirometry, and induced sputum collection. Those undergoing bronchoscopy will receive additional compensation for those sub-study visits. The amount of compensation will be appropriate for the geographic location of the recruiting clinical center, therefore, may vary slightly.

Study coordinators will contact participants by phone to conduct a screening questionnaire to assess potential eligibility. Participation is voluntary, and the participant's responses will not be captured for data use purposes and will remain strictly confidential.

5.2. Consent Process

All study participants will sign a SOURCE informed consent form before any research-related questionnaire administration or study involvement (other than screening questions). Clinic staff members will provide a copy of the informed consent form to individuals who meet inclusion criteria and allow them an opportunity to read the document. They will then review the document with the potential participants, verbally explaining each section of the informed consent and answering any questions. If at the end of this review an individual feels comfortable proceeding, the clinic staff member will obtain the participant's signature. Clinic staff will also sign the consent form. A copy of the signed form will be given to the participant and the original will be stored by the clinical center.

The above consent process may be performed in-person at the baseline visit or over the phone. To minimize in-person interactions as much as possible, it is preferable for all consents, if feasible, to be administered remotely and electronically, if local institutional guidelines permit it.

5.3. Schedule of Activities

All participants will undergo study related questionnaires, nasal swab, blood, stool, and urine collection, spirometry, sputum induction, and CT imaging as outlined in Table A.

Table A. Schedule of Activities	**Baseline Visit (n=1000)**	**Follow-up Telephone Call/Email every 6 months (n=1000)**	**Follow-up Phone Call at 18 months (n=1000)**	**Follow-up Visit at 3 years (n=1000)**
Procedures				
Informed Consent	X			
Demographic Information Form	X			
Personal Identifiers Form	X			X
Contact Information Form	X	X	X	X
Inclusion/Exclusion Criteria Form	X			
Participant Eligibility Status and Stratum Form	X			
Medical History Form	X		X	X
Follow-up Phone Questionnaire		X		
Follow-up Status Form			X	X
Exacerbation Questionnaire	X		X	X
Medication Use Questionnaire	X		X	X
Respiratory Disease and Smoke Exposure Questionnaire	X		X	X
CAPTURE Questionnaire	X			X
E-Cigarette Use Assessment	X		X	X
COVID-19 History Questionnaire	X		X	X
Chronic Airway Assessment Test (CAAT)	X		X	X
Modified 1946 birth cohort early life exposure questionnaire [(7)]	X			
Employment History Form	X		X	X
Home Information Questionnaire	X		X	X

Pulmonary Function Eligibility Form	X			X
Spirometry Data Form	X			X
Anthropometry Form	X			X
Blood Pressure Form	X			X
CT Image Acquisition Form	X			X
Induced Sputum Worksheet / Sputum Processing Worksheet / Sputum Slide Form / Sputum Slide Label ID Form	X			X
Oscillometry Data Form	X			X
Biospecimen Collection Form	X			X
Exhaled Breath Condensate Collection Form	X			
CBC Results Form	X			X
Clinically Significant Findings Form	X			X
Adverse Events Form	X	X	X	X
Modified Medical Research Council (MRC) Dyspnea Scale	X		X	X
St. George's Respiratory Questionnaire	X		X	X
Hospital Anxiety and Depression Scale (HADS)	X			X
Block Brief 2000 Food Frequency Questionnaire / NutritionQuest Questionnaire Label ID Form / NutritionQuest Tracking Form	X			
Stool Sample Collection Form / Stool Kit Label ID Form	X			
Reason for Study Withdrawal Form	X	X	X	X

5.4. Description of Study Exams, Specimen Collection, and Questionnaires

The study will heavily leverage the protocols and procedures currently in use in SPIROMICS. Hence, the time from regulatory approval to study initiation is anticipated to be minimal.

5.4.1. Clinical Data Collection

5.4.1.1. Data collection forms (most are the same as SPIROMICS forms)

Demographic Information Form
Personal Identifiers Form
Contact Information Form
Medical History Form
Follow-up Phone Questionnaire
Exacerbation Questionnaire
Medication Use Questionnaire
Respiratory Disease and Smoke Exposure Questionnaire
COVID-19 History Questionnaire
Chronic Airway Assessment Test (CAAT)

Employment History Form
Home Information Questionnaire
Pulmonary Function Eligibility Form
Spirometry Data Form
Anthropometry Form
Blood Pressure Form
CT Image Acquisition Form
Induced Sputum Worksheet
Sputum Processing Worksheet
Sputum Slide Form
Sputum Slide Label ID Form
Oscillometry Data Form
Biospecimen Collection Form
Exhaled Breath Condensate Collection Form
CBC Results Form
Clinically Significant Findings Form
Informed Consent Tracking Form
Adverse Events Form
Reason for Study Withdrawal Form
E-Cigarette Use Assessment
Modified Medical Research Council (MRC) Dyspnea Scale
St. George's Respiratory Questionnaire
Hospital Anxiety and Depression Scale (HADS)
Block Brief 2000 Food Frequency Questionnaire
NutritionQuest Questionnaire Label ID Form
NutritionQuest Tracking Form
Stool Sample Collection Form
Stool Kit Label ID Form

5.4.1.2. Data collection forms (new for SOURCE)

CAPTURE Questionnaire
Modified 1946 birth cohort early life exposure questionnaire
Inclusion/Exclusion Criteria Form
Participant Eligibility Status and Stratum Form
Follow-up Status Form

5.4.2. Physiological Data Collection

5.4.2.1. Laboratory-Based Spirometry

Laboratory-based pre- and post-bronchodilator spirometry will be performed on all subjects at the baseline visit and again at the three-year follow-up visit. The 2019 ATS/ERS guidelines for pulmonary function testing and interpretation will serve as the primary guidance for the conduct and interpretation of the spirometry measurements [(37-40)].

The between-maneuver repeatability between the two highest readings, which 90% of consecutive patients can meet, is 120 mL (6.1%) for FEV_1 and 150 mL (5.3%) for FVC [(41)]. The established target is 150 mL for both measures (or 100 mL if the FVC is < 1 L). The short-term (24.9±17.1 days) reproducibility of these measurements in participants with mild COPD is 113 mL (CV 4.1%) for FEV_1 and 150 mL (CV 3.5%) for FVC [(42)]. The minimal clinically-significant difference for FEV_1 is about 100 mL [(43)].

5.4.2.2. Oscillometry

Impulse Oscillometry (IO) will be performed during the baseline visit and the follow-up visit at year 3. IO is an effort-independent assessment of airways obstruction, characterizing peripheral, central, or heterogeneous patterns. IO requires minimal cooperation by the subject, as is performed during resting tidal breathing. Thus, IO is an alternative to spirometry when the latter is difficult for the subject to complete properly.

IO measures respiratory impedance (Z_{rs}) during resting tidal breathing. Z_{rs} is commonly represented by the resistance (R_{rs}), which mainly accounts for the obstruction in the central airways, and reactance (X_{rs}), which accounts for capacitance and inertance in the peripheral parts of the lungs. IO has been reported to have a sensitivity to detect airway obstruction similar or superior to that of spirometry [(44-46)]. Several studies have also shown the potential utility of IO to separate the contributions of inspiration and expiration to R_{rs} and X_{rs}. This purpose uses an approach known as *within-breath analysis* of Z_{rs}, allowing the calculation of inspiratory resistance and reactance (R_{insp} and X_{insp}, respectively) and expiratory resistance and reactance (R_{exp} and X_{exp}, respectively).

The 2003 ERS task force document will be used as guidance for conducting IO measurements. Measurements are performed in the sitting position with the head in a neutral or slightly extended position. At least three minutes of quiet breathing should be allowed for recovery if forced respiratory maneuvers have been made before an IO test is performed. A nose clip is worn by the participant, whose cheeks and the floor of the mouth are firmly supported during the measurement by the technician using both hands. The subject is instructed to breathe quietly from end-expiratory lung volume or functional residual capacity. A total of three to five acceptable measurements will be collected for each participant. The subject should come off the mouthpiece between successive measurements and should rest for at least 30 seconds. Each measurement will continue until 10 breaths have been captured, all of which meet quality criteria. To assess peripheral closing volume, an additional IO recording consisting of five breaths, followed by a slow expiratory vital capacity, will be performed.

IO will be performed with disposable low-resistance filters, nose clips, seated with the chin slightly up, after loosening tight or restrictive clothing and removing loose dentures. PFT technicians will encourage the subjects to perform relaxed tidal breathing at baseline and throughout the 30-60 seconds of data collection. A minimum of three acceptable and two repeatable maneuvers will be obtained lasting no more than 30 seconds each. Up to five maneuvers are permitted to meet criteria.

5.4.3. Imaging Data Collection

With the evolution of CT scanners, it is now possible to obtain images with improved spatial resolution at significantly lower radiation dose, while still attaining equivalent or improved quantitative measures [(48-52)]. Participants will undergo two low-dose lung CT scans (at baseline and at three-year follow-up) using dose modulation to adjust for body size on a slice-by-slice basis. Because dose modulation is being used, the doses will, by design, automatically fluctuate slightly between participants, dependent upon the individual's body build. To further support lower dose imaging, iterative reconstruction will be employed. Protocols vary according to scanner make and model with the goal of keeping all scanners within a similar level of noise and image spatial resolution. These considerations mean that target mAs and IR parameters will vary between individual participants. The first scan for each participant at each visit will be performed at a breath hold at total lung capacity (TLC); the estimated CTDI volume and effective dose is estimated for an average-size person with a 30 cm scan length. The last scan for each participant at each visit will be performed at a breath hold at residual volume (RV).

5.4.4. Biological Sample Collection

5.4.4.1. Blood Collection

Blood will be banked for future analysis. In addition, a complete blood count with differential will be performed as evidence suggests white blood cell count and platelet count may be important markers of inflammation in COPD; eosinophil count has also been associated with exacerbation frequency, lung function response, and response to inhaled corticosteroids [(53, 54)].

Instructions for Specimen Preparation, Handling, and Storage

The following blood samples should be collected, limited to no more than eight tubes of blood drawn per day. The recommended order of the blood draw is as follows:

- 2 tubes of blood (8.5 mL red top tubes) allowed to clot at room temperature to obtain serum. These tubes will be processed within 2 hours of collection. The serum portion of the tubes will be split into 14 aliquots at the clinical site for batch transfer to the biospecimen repository on dry ice.
- 2 tubes of blood collected (10 mL lavender top tubes) in EDTA for plasma. These tubes will be processed within 2 hours of collection. The plasma portion of the tubes will be split into 14 aliquots at the clinical site for batch transfer to the biospecimen repository on dry ice.
- 1 tube of blood collected (3 mL lavender top tube) in EDTA for submission directly to the local clinical laboratory for complete blood count with white blood cell differential and platelet count.
- 1 tube of blood collected (8.5 mL P100 red top tube) for preservation of plasma proteins in anticipation of proteomics analyses. This tube will be processed within 2 hours of collection. The plasma portion of the tube will be split into 14 aliquots at the clinical site for batch transfer to the biospecimen repository on dry ice.
- 1 tube of blood collected (8.5 mL blue top PAXgene DNA tube). This tube will be shipped to the biospecimen repository and sent in batches for DNA extraction.
- 1 tube of blood collected (2.5 mL red top PAXgene RNA tube). This tube will be shipped to the biospecimen repository in batches for RNA extraction (including preservation of microRNA species). RNA will be suitable for all down-stream applications (transcriptomics, RT-PCR).

5.4.4.2. Exhaled Breath Condensate

EBC will be collected at individual clinical centers during study visits using the commercially available RTube (Respiratory Research, Inc., Charlottesville, VA), a handheld, self-contained, single-use device. The device consists of a mouthpiece connected to a one-way valve via a T-connector that directs exhaled breath through a condenser tube cooled by a chiller sleeve. Gaseous phase liquid and aerosols in exhaled breath condense into liquid form on the inside surface of the chilled condenser tube and can be extracted using a plunger device. For EBCs, participants must have at least one hour between eating or drinking and the EBC collection. In order to be consistent with ATS/ERS recommendations[(55)], participants will wear a nose clip and will be coached to breath naturally without hyperventilation for approximately 10 minutes, but no more than 20 minutes, through a one-way valve into a cooled chamber that condenses and collects up to 2 mL of expired vapors, aerosols, and moisture. The participant should also be cautioned not to touch the chilled sleeve.

5.4.4.3. Nasal Swab Collection

A nasal swab for potential future gene expression studies and microbiome analysis will be

collected by sampling the nasal mucosal transcriptome as this may reflect the pathobiology occurring within the lower respiratory tract. The nasal transcriptome may be a minimally invasive "window" to view the respiratory tract of COPD patients. Relationships with gene expression in proximal and distal airways, alveolar macrophages, and systemic inflammatory patterns will be determined.

To correctly perform the nasopharyngeal swab, the participant must be seated comfortably with the back of their head against the headrest. If properly performed, this test should be minimally unpleasant and should not be painful. With the swab as caudal as feasible (i.e., on the floor of the nasal cavity adjacent to the roof of the mouth), the swab is inserted in the nose horizontally along an imaginary line between the nostril and the ear.

5.4.4.4. Sputum Sample Collection

The participant should be seated in a non-rolling chair and have last eaten at least 1 hour prior to sputum induction. Once at least 1 hour has elapsed, all participants will lightly brush/scrape their tongue 3-5 times. Following this, subjects will thoroughly gargle and rinse their mouths with water, then discard all contents from their mouth into the sink. Once this process is complete, sputum induction may begin. During the induction, the participant should be relaxed and asked to inhale through the mouth and exhale through the nose while breathing (semi-deep tidal breaths) in saline through a nebulizer. The participant should expectorate any saliva-salt water into a separate waste cup (i.e., not swallow any buildup of this fluid).

The induction procedure is dependent on the pre-sputum induction baseline FEV_1 obtained from the subject.

If the pre-sputum induction baseline FEV_1 is greater than or equal to 50% predicted, then put 15 mL of 3% saline into the disposable nebulizer. Turn the unit on and start the timer for 7 minutes. Ensure that the subject is comfortable and has the mouthpiece properly in their mouth. Adjust the output. If the subject has an urge to cough, they may do so, without scraping, and expectorate the sample into the sterile cup labeled with the subject number.

At the end of the 2 minutes, stop the timer and turn off the nebulizer, and have the subject come off the mouthpiece. Be careful not to spill or drain the saliva into the nebulizer – the saliva can build up in the tubing.

Perform spirometry. It is acceptable to obtain only one effort at this point, if it is technically acceptable and if it falls into the required range. This is to avoid subject fatigue. If there is any question about the quality of the effort, then allow the subject another minute or so of rest and repeat.

If the FEV_1 has not fallen 10% or more, restart the timer and the nebulizer, and continue until the 7 minutes have elapsed. Once the inhalation period is complete, turn off the nebulizer and perform the three-step cleansing routine as follows: (1) Bring the subject to the sink and have him/her to rinse mouth and gargle thoroughly and then spit into the sink; (2) Have the subject clear their throat 2-3 times (i.e., scrape the back of the throat and roof of mouth (demonstrate)), and again expectorate this into the sink; and (3) Have the subject blow their nose and discard tissue.

Finally, have the subject give a good cough effort from ONLY the chest (huffy type chest cough) with no coughing from the throat and without scraping the back of the throat, expectorate the sputum into the cup.

DO NOT HAWK OR SCRAPE when producing the sample.

Passively and quietly (no noise) move the sample from the back of the throat to the mouth for final expectoration into the cup. Once the subject can no longer bring up sputum, repeat spirometry with only one good effort.

If the FEV_1 falls less than 10% from the post-bronchodilator FEV_1 baseline, dispose of the remaining saline, and put 15 mL (or total amount of saline when mixed) of 4% saline into the cup. Repeat the procedure for 2 minutes, then stop the clock and repeat the spirometry. If the FEV_1 fall is still less than 10%, continue with the 4% saline until the 7-minute mark. If the FEV_1

fall is greater than 10% but less than 20%, do not continue with 4% but rather continue with 3% or less (0.9%, if the FEV_1 fall is 16-19%) until the 7-minute mark. Repeat the cleansing and cough steps as above followed by spirometry.

Again, if the FEV_1 falls less than 10%, continue to the final inhalation period with 15 mL of 5% saline for 2 minutes, then stop the clock. Repeat the spirometry. If the FEV_1 drops less than 10%, continue to completion and repeat the three-step cleansing routine followed by chest cough, then final spirometry. If the FEV_1 drop is 10-19%, do not continue with 5% but rather give 4% (or 3%, if the FEV_1 is 16-19%), then repeat cleansing and cough and final spirometry.

If the pre-sputum induction baseline FEV_1 is less than 50% but greater than or equal to 35% predicted, then put 15 mL of 0.9% saline into the disposable nebulizer. Turn the unit on and start the timer for 7 minutes. Ensure that the subject is comfortable and has the mouthpiece properly in their mouth. Adjust the output. If the subject has an urge to cough, they may do so, without scraping, and expectorate the sample into the sterile cup labeled with the subject number.

At the end of 1 minute, stop the timer and turn off the nebulizer, and have the subject come off the mouthpiece. Be careful not to spill or drain into the nebulizer the saliva which typically builds up in the tubing.

Perform spirometry. If the effort is technically acceptable and it falls into the required range, obtain only one effort at this point. This is to not fatigue the subject. If there is any question about the quality of the effort, then allow the subject another minute or so of rest and repeat. If the fall in FEV_1 is less than 20%, restart the timer and the nebulizer, and continue until 2 minutes have elapsed.

Repeat spirometry. Again, if the fall in FEV_1 is less than 20%, continue the induction until 5 minutes have elapsed. Repeat spirometry. If the fall in FEV_1 is still less than 20%, continue until the full 7 minutes have passed. Bring the subject to the sink and have them perform the three-step cleansing routine as described above: 1) Rinse mouth and gargle thoroughly and then spit into the sink; 2) Clear their throat (i.e., scrape the back of the throat and roof of mouth (demonstrate)), and again expectorate this into the sink; 3) Blow their nose and discard tissue. Finally, have the subject give a good huffy-type chest cough without scraping the throat, expectorate the sputum into the cup. **DO NOT HAWK OR SCRAPE** when producing the sample. Passively and silently move the sample from the back of the throat to the mouth for expectoration into the sample cup.

Repeat spirometry. If the fall in FEV_1 is less than 10%, dispose of the remaining saline, and put 15 mL (or total amount of saline when mixed) of 3% saline into the cup. If the fall in FEV_1 is between 10%-19%, add a new 15 mL of 0.9% saline. With either 3% or 0.9% in the cup, repeat the same procedure as above for another 7 minutes (i.e., performing FEV_1 checks at 1, 2, and 5 minutes). If the fall in FEV_1 is less than 20% at 1, 2, and 5 minutes, continue the procedure until the 7-minute mark, then perform the cleansing steps and cough attempts. Following the last cough attempt, perform spirometry.

If the fall in FEV_1 is less than 10% and you had 3% saline in the cup for the previous inhalation, put 15 mL of 4% saline in the cup and repeat all spirometry steps (i.e., FEV_1 checks at 1, 2, and 5 minutes), cleansing and cough attempts as per the previous inhalation period. If you had 0.9% saline in the cup for the previous inhalations, put 15 mL of 3% saline in the cup and repeat all spirometry steps (i.e., FEV_1 checks at 1, 2, and 5 minutes), cleansing and cough attempts as per the previous inhalation period.

If the fall in FEV_1 is greater than 10% but less than 20%, repeat the final inhalation period with the same (or lesser) saline concentration that was previously used performing FEV_1 checks at 1, 2 and 5 minutes, cleansing steps, and cough attempts.

After the final cough attempt, perform the final spirometry check prior to discharge. FEV_1 should return to within 10% of pre-bronchodilator baseline prior to discharge.

We will analyze and process induced sputum using the “whole sputum” method as opposed to the “plug select sputum” method, which involves manually removing sputum plugs from the sample. "Whole sputum" is defined as the raw, unaltered, total expectorated secretions

collected from the participant at the conclusion of the induction. Participant must produce enough sputum to complete a cytospin slide in order for the collection to be considered complete. This amount typically is no less than 75 mg of total sputum material.

5.4.4.5. Urine Sample Collection

Urine will be banked for future analyses, and in the case of participants who are women of childbearing potential, for a pregnancy test performed and with results received before CT scanning.

5.4.4.6. Stool Sample Collection

Participants will be given a pre-packaged stool collection kit with barcode label, instruction forms, and mailers that allow for sample return directly to Michigan via USPS. Samples will be collected at home using the Zymo DNA/RNA Shield Fecal Collection Tube, which stabilizes degradation of nucleic acids at room temperature. Samples will be returned using provided packaging materials that include a small biospecimen bag, bubble material, a small cardboard envelope marked “exempt human specimen” and a larger shipping bag envelope (MO-A mail kits, DNAGenotek which meet all International Air Transport Association regulations).

6. Human Subjects Protection/Risks

6.1. Adequacy of Protection Against Risks

The risks associated with SOURCE are described here.

6.1.1. Risks Associated with Anthropometric Measurements

There is a low probability of participants experiencing an adverse or unanticipated event during anthropometric measurements. Clinical risks include syncope and vasovagal episodes. Participants may also experience feelings of embarrassment or discomfort with being measured. Efforts to minimize these risks include conducting the measurements in a quiet, private location. Study coordinators should approach participants in an open, tolerant manner, reassuring them during the procedures that they can stop at any time. Further, coordinators should carefully describe the measuring process before initiating it, so that the participant is not surprised at any step.

Risks Associated with Blood Pressure Measurements

There are no significant risks associated with measuring blood pressure. Participants may experience discomfort in the form of mild pressure, tingling, or numbness while the BP cuff is active.

6.1.2. Risks Associated with Blood, Urine, and Stool Collection

6.1.2.1. Blood Draws

Veins vary in size from one participant to another and from one side of the body to the other. Obtaining a blood sample from some people may be more difficult than from others.

Other risks associated with having blood drawn are slight but may include:

- Excessive bleeding
- Fainting or feeling light-headed
- Hematoma (blood accumulating under the skin)
- Infection (a slight risk any time the skin is broken)
- Discomfort related to needle stick
- Rarely, injury to adjacent structures

6.1.2.2. Urine

Urine collection is a benign procedure not associated with risk to the participant. Urine will be collected in a private location.

6.1.2.3. Stool

Any stool sample may contain germs that spread disease. It is important to carefully wash your hands and use careful handling techniques to avoid spreading infection. Some people may feel uncomfortable or embarrassed using the stool collection kit. There should be no pain while collecting the stool sample. However, if you are constipated, straining to pass stool may be painful.

6.1.3. Risks Associated with Pulmonary Function Tests (PFTs)

The following risk assessment applies to laboratory-based spirometry.

Spirometry is a common medical procedure of generally low risk. Some participants may experience breathlessness, cough, fatigue, dizziness/lightheadedness (hyperventilation), all of which are brief, and very rarely headache, syncope, musculoskeletal chest pain, rib fractures, or ear injury. An episode of stress incontinence (urine leakage) may be caused by the PFT maneuvers in susceptible individuals. A seated position has been specified to reduce risk related to dizziness or syncope. Transmission of airborne disease is rare and minimized or eliminated with single-use filters.

Instructions for withholding bronchodilator medications prior to testing will stress the continued use of rescue medication if needed. The use of albuterol or ipratropium will generally relieve any symptoms related to the trough effect of long-acting bronchodilators.

Albuterol has been reported to cause urticaria, angioedema, paradoxical bronchospasm, angina, arrhythmias, QT prolongation, hypertension, hypokalemia, seizures, tremor, nervousness, headache, tachycardia, muscle cramps, palpitations, insomnia, and dizziness. Ipratropium has been reported to cause cough, nausea, dry mouth, dizziness, headache, dyspnea, atrial fibrillation, tachycardia, paradoxical bronchospasm, laryngospasm, angioedema,

anaphylaxis, hypersensitivity, and exacerbate narrow-angle closure glaucoma.

The dose used in testing is twice the usual dose of albuterol (one dose every four hours) or ipratropium (one dose every six hours) used chronically. However, home management of exacerbations includes increasing the dose and/or frequency of bronchodilator therapy [(2)]. Doses in patients hospitalized or visiting the Emergency Department for exacerbations may be ten times the usual dose.

Data transmission for laboratory-based spirometry will be encrypted with a 128-bit VPN approach. The PFT software is designed to be HIPPA compliant for clinical use.

All study personnel are certified in the ethical conduct of human biomedical and genetics research and HIPPA information security.

6.1.4. Risks Associated with Sputum Induction

Inhalation of hypertonic saline for sputum induction may result in wheezing, coughing, or chest tightness, particularly in susceptible individuals such as those with asthma or hyperactive airways. COPD participants will be pre-medicated with albuterol in order to minimize this risk. Spirometry will be evaluated for all participants before induction as well as at prescribed intervals during each of the three levels of hypertonic saline. Participants may also experience transient throat irritation during the hypertonic saline inhalation, but this generally resolves post-induction when the participant is provided with a snack and juice or water.

6.1.5. Risks Associated with Questionnaires

The expected risks associated with completing study questionnaires are low. Potential risks include potential discomfort and embarrassment in answering questions. The study coordinator will make efforts to provide a safe, reassuring atmosphere and will emphasize that the participant does not have to answer any question he or she feels uncomfortable with.

6.1.6. Risks Associated with High Resolution CT (HRCT) Imaging

This research study involves exposure to radiation from CT scans. The X-ray exposure during these studies will be limited to roughly the area covered by the rib cage. This radiation exposure is not necessary for the participant's medical care and is for research purposes only. For a large subject receiving a maximum of this amount of radiation, this may involve a low, lifetime risk of cancer. If the participant is pregnant, she will not undergo a CT scan. The dosage considerations will be discussed with each participant explicitly in the consent form and consenting process.

These scanning images are not intended as a replacement for a physical examination or a substitute for a visit to the participant's doctor; nevertheless, they will be reviewed by a local radiologist. If there are any abnormalities observed by the clinical center radiologists, these will be reported to the clinical site Principal Investigator. Furthermore, the Radiology Reading Center personnel will report abnormal results found during the central read to the GIC. The GIC will in turn direct those results to the appropriate clinical center.

The risks of the abnormal findings are:

- False positive scan, which is an abnormality that initially is of concern for cancer that is later found not to be cancer. Anxiety may result from false positive results.
- Detection of abnormalities unrelated to lung cancer that could lead to unnecessary testing or treatment.

Another risk is failure to detect a lung cancer that is present and possibly miss an opportunity for care.

6.1.7. Risks Associated with Exhaled Breath Condensate Collection

Collecting EBC requires the subject to exhale into a chilled tube for approximately 10-15 minutes. There is a risk of the subjects becoming light-headed if they breath too forcefully, and there is also a risk of them sustaining freeze injury if they were to touch the chilled tube. The expected risks associated with completing study questionnaires are low.

6.1.8. Risks Associated with Nasal Swab

A soft swab will be inserted in your nose and gently brushed back and forth to collect cells and secretions. This may cause nasal irritation associated with itching or sneezing briefly. In rare cases, it may cause a mild nosebleed.

6.2. Potential Benefits of the Proposed Research to the Subjects and Others

The proposed study attempts to define the biological nature of early COPD. It is possible that study subjects will not receive any direct benefit by participating in this study. Study subjects could benefit through information gained by the early identification of clinical implications and biological underpinnings of early COPD. This information will be available to treating physicians and could aid in treatment decisions.

6.3. Importance of the Knowledge to be Gained

There is limited understanding regarding the prevalence, clinical impact, and biological underpinnings behind early COPD. The study of new treatments is hindered by the inability to correctly identify and quantify these features. Being able to ascertain and enroll subjects with or at risk for early COPD could speed the development of novel methods of treatment.

7. Statistical Approach

This is an observational study rather than a clinical trial, and a number of hypotheses will be investigated. Presentation of results will focus on estimation of parameters, such as regression coefficients and correlation coefficients, along with corresponding 95% confidence intervals. Where p-values are reported, actual values will be given rather than dichotomizing into significant versus inconclusive. For analyses in which the proportion of subjects with missing data is small (generally less than 10%), whether through participant drop-out or measurements not being available, there will be no adjustment for missing data. When the proportion is larger or other reasons exist to suspect the potential of bias, multiple imputation by chained equations will be used to address the missingness. Before analyses are undertaken for a manuscript, additional details of statistical analyses, particularly sensitivity analyses and handling of missing data, will be provided in a manuscript-specific Statistical Analysis Plan.

Among smokers, SOURCE will attempt to enroll approximately equal numbers in each of three groups: GOLD 0, GOLD 1-2, and PRISm (see section 3.2.1.1.). The primary analyses for the various specific aims will not stratify by or adjust for these categories. The groups are defined in terms of baseline pulmonary function and various sensitivity analyses will adjust for baseline pulmonary function. Further but stratification by and adjustment for group will be investigated in additional sensitivity analyses.

Aim 1 involves investigating the relationship between CT-defined small airway abnormality (SAA, the predictor) and three outcomes, progression of PRM^{Emph}, worsening PRM^{fSAD}, and lung function decline. The primary analysis will use linear mixed effects models to estimate the trajectories of the outcomes based on SAA (either PRM^{Emph} or PRM^{fSAD}) at baseline. Sensitivity

analyses will investigate the effect of adjusting for age, sex, racial and ethnic groups, early life exposures, smoking history, and ongoing smoking status (as a time-dependent covariate where appropriate), baseline pulmonary function, and concomitant medications. We will also investigate potential interactions between any of these variables and PRM metrics in case the relationships between the PRM metrics and development of emphysema are modified by any of these factors. Power analyses assumed an initial sample size of 600 with 10% loss to follow-up and a Type I error of 5%. Simulations were conducted in SAS using Proc Power and were based on estimates from the subset of SPIROMICS subjects who would have met the eligibility requirements for SOURCE. The effect size seen in SPIROMICS suggests that for every 10% higher baseline PRM^{fSAD}, there will be approximately a 1% added increase in PRM^{Emph} at three years. The simulation used to detect the increase in PRM^{Emph} attributed to baseline PRM^{fSAD} additionally adjusted for baseline PRM^{Emph}. The simulations indicated > 99% power to detect a 1% further increase in emphysema for every 10% higher PRM^{fSAD} at baseline; a result which is well within the range seen in our preliminary data.

Aim 2 will investigate the association between SAA and lung-derived biomarkers with the primary biomarker of interest being IL-17 gene signatures in airway epithelium from bronchoalveolar lavage samples. Analyses for this aim will combine data from SOURCE and SPIROMICS participants undergoing bronchoscopy. Among ever-smokers, SOURCE plans to contribute 80 participants and SPIROMICS 31 participants, while among never-smokers, SOURCE plans to contribute 20 participants and SPIROMICS 8. The primary analysis will use Spearman's correlation coefficient to estimate the correlation between PRM^{fSAD} and IL-17 gene signature. Secondary analyses include multivariable linear regression analysis of the relationship between IL-17 signature and PRM^{fSAD}, additionally adjusting for smoking status, age, batch (SPIROMICS I versus SOURCE), sex, race/ethnicity, smoking burden, and concomitant medication use (particularly inhaled corticosteroids). We will also test for potential interactions/effect modification between gene signatures and any of these factors. In the proposed sample size of n=139, assuming a Type I error of 5% with a Bonferroni correction for two correlation analyses, we will have > 90% power to detect a correlation of ≥ 0.27, which exceeds the correlation seen for the IL-17 signature in our preliminary data in phase I SPIROMICS subjects with established COPD.

Aim 3 will investigate whether sputum has value as a non-invasive biomarker of early COPD. Sub-aim 3a will estimate the association between baseline sputum mucin concentration and baseline SAA, and sub-aim 3b will estimate the association between change in sputum mucin concentration over three years and progression of PRM-defined CT abnormalities. For aim 3a, the primary analysis will be to estimate the correlation between baseline total mucin concentrations and PRM^{fSAD}. A conservative power analysis yields 90% power to detect a correlation of 0.13 between PRM^{fSAD} and mucin concentration, assuming a Type I error of 0.05. Further analyses will be performed using multivariable models to adjust for age, sex, racial/ethnic groups, early life exposures, smoking history, and smoking status (as a time dependent covariate where appropriate), concomitant medications, and baseline disease as measured by FEV_1 % predicted; we will also investigate potential interactions/effect modification. For aim 3b, the primary analysis will be to estimate the correlation between baseline total mucin concentrations and change in total mucin concentrations with: (a) change in PRM^{fSAD}; and (b) change in PRM^{Emph}. A conservative power analysis yields 90% power to detect correlations between baseline and three-year change in mucin concentrations with change in either PRM^{fSAD} or PRM^{Emph} of approximately 0.16, assuming 10% loss to follow-up and a Type I error of 0.0125 using a Bonferroni correction for the four comparisons of interest. In secondary analyses, we will use multivariable linear mixed effects models adjusted for covariates of interest, as outlined above, as well as examine the relationship between baseline and change in mucin concentrations with change in FEV_1 over three years.

Statistical analyses for specific aims 1-3 will be undertaken at the University of Michigan by Dr. Susan Murray's group. Additional analyses may be conducted at the University of North Carolina by Dr. David Couper's group and by investigators at other institutions.

8. Data Handling and Record Keeping

8.1. Data Collection

Data are collected at the in-person clinic visits and through follow-up telephone and/or email interviews. Spirometry data and CT imaging will be transmitted directly to a secure server, for the purposes of quality assurance, to investigators at the UCLA PFT Reading Center and the Iowa CT Reading Center, respectively.

8.2. Data Entry

Data collection for SOURCE clinic visits will be collected primarily through direct data entry by clinic staff using the SPIROMICS web-based data management system (DMS). The clinical database resides on servers located in a secure, climate-controlled server facility at UNC; confidentiality and data security of that system is described below. The use of web-based data collection, entry, and processing allows for real-time data edits at the time of data entry and the generation of real-time status reports and data queries for monitoring study data by clinic personnel.

Reading Center data are transferred via secure FTP to the GIC on a regular basis throughout the study and are merged with the clinical database.

8.3. Confidentiality and Security

8.3.1. GIC

The clinical database is housed in a secure, climate-controlled server facility at UNC. The CSCC has in place an IT Security Plan as required by NIH contracts and grants. The plan documents standard operating procedures required to secure the CSCC network and databases, including management, operational, and technical controls. As part of the plan, the principle of least access privilege for study files is implemented. Included in the plan are a risk assessment plan, a system continuity plan, and a disaster recovery plan.

Data confidentiality and security measures are applied at all levels of data acquisition, transfer, and storage, and applied to all study agencies, including the clinical centers, the Reading Centers, and the GIC. The DMS meets data management standards of confidentiality, as well as HIPAA requirements. Beyond the password-controlled access to the study equipment and the DMS, data collected at the clinical centers are encrypted (FIPS 140-2 compliant) by the system and can only be decrypted for display on-screen by authorized study personnel. Personal identifiers are collected on separate forms. The GIC is responsive to data confidentiality requirements originating from providers of medical care or IRBs, as needed, to enable the work of the clinical centers. It is the goal of the study to collect all data electronically, however, should paper data collection forms be used, they will be retained at secure locations at the clinical centers until the Steering Committee acts on recommendations from the GIC to dispose of such records (e.g., after incremental data closure). The secure storage and disposition of hard copy records at clinical centers will follow institutional procedures at each site.

As standard practice, output mailed to a clinical center identifies participants only by ID number. Printed material containing confidential information is discarded through supervised loading, transportation, and storage using a chain of custody control process, until the material can be recycled into paper pulp.

Personal identifying information will be collected as part of the study, including name, address and address history, phone number, date of birth, dates of medical procedures, and

social security number. These data and PHI will be stored on a secure server at the GIC, as well as at the Iowa Imaging Reading Center, following the procedures described above.

All the data management and statistical computations for the data management will be done at the University of North Carolina by Dr. David Couper's statistical analytic and data management programming groups.

8.4. Records Retention

8.4.1. Genomics and Informatics Center (GIC)

The GIC will comply with all local and federal regulations in maintaining study related documentation. This documentation includes financial records, supporting documents, and all records related to the award. NHLBI policy states that these must be kept for at least three years after study closure (NHLBI, 2013).

8.4.2. Biospecimen Repository

The Biospecimen Repository is not a general repository in that all samples that are stored are the property of each particular study. These samples are not available without permission from the study PI to non-study investigators. The repository collects no PHI on any samples that come into the lab. The GIC retains indefinitely data relating to specimen processing, such as time from collection to processing, DNA yield, volume of plasma, quality checks, etc.

Regarding the destruction of samples, it is the repository's policy that participants retain their rights to have their samples removed from the repository inventory at any time and have no further analytical disbursements performed. The withdrawal request must be made to the originating PI. This individual PI will transmit a signed, dated written request to the GIC (acting as honest broker), which will identify the link between the participant ID and the repository ID and generate a removal/destruction request for all samples and records associated with a specific repository ID. Samples already released to approved requesting investigators according to the repository's approved guidelines cannot be returned or destroyed. In addition, data generated prior to a participant's request for sample removal will not be destroyed.

The only other time the repository destroys samples is at the request of the study PI when a study participant turns out to be ineligible, in accordance with the IRB documentation. At this point, both the sample and any data associated with it are destroyed in the same manner as described above.

8.4.3. Clinical Sites

The secure storage and disposition of hard copy records at clinical centers will comply with local institutional procedures as well as federal regulations.

9. Study Monitoring, Auditing, and Inspecting

9.1. Study Monitoring Plan

As part of SOURCE, the GIC may conduct monitoring visits to clinical centers to assess compliance with the study protocol and MOPs, to help identify and resolve problems, and to verify correspondence between the study data and clinic records. Visits will be conducted once during the conduct of SOURCE or more often depending on the site's performance and the degree of risk, size, and complexity of the protocol.

Once date(s) for the monitoring visit have been finalized with site staff, site monitor(s) must email a site visit confirmation memo confirming the date(s) of the visit, a site monitoring visit meeting agenda identifying site staff that will need to be available during the visit, and what study locations, resources, and documentation the monitor will need access to. Monitor(s) should also confirm what the procedures are to gain access to source documentation while on-site, and complete any additional documentation needed for them to gain access (e.g., confidentiality forms, training certificates, etc.). All participant records need to be available for review by site monitor(s), as well as on-site and/or electronic source documents, regulatory files, and research facilities including imaging centers, laboratories, and reading centers. At the conclusion of the site monitoring visit, the site monitor should meet with the PI, study coordinator(s), lab technician(s), and any other necessary staff, to discuss any findings or recommendations resulting from the site visit and to answer questions and receive feedback from site staff.

After a site monitoring visit has been completed, monitor(s) must complete and route the site monitoring report to the GIC Project Director and NHLBI Program Office for review and finalizing within 7 business days. A finalized version of the report should be sent to the SOURCE PIs, Executive Committee, NHLBI Program Office, clinical site PI, and lead study coordinator(s) within 14 business days. Reports should be routed by the site to their local IRB as required.

9.2. Auditing and Inspecting

9.2.1. Clinical Center QA/QC

Clinic staff will be trained to safely perform physiological studies and sputum induction. Data quality will further be assured by adhering to the study protocol, including the quality control/quality assurance procedures outlined for each assessment. These instructions, along with step-by-step descriptions of study procedures, can be found in the Manual of Procedures.

9.2.2. GIC QA/QC

9.2.2.1. Training

The Investigative Team and the GIC will conduct web-based trainings, the first of which will be held prior to the start of study enrollment. The GIC will maintain a database of staff members with their corresponding data collector ID, certification (recertification) date, and procedures for

which they are certified. New staff members will receive certification via web-based video conference or monitoring visit.

9.2.2.2. Data Checks

Edit checks performed during web-based data entry at the clinical centers include range checks, correct execution of skip patterns, and across-variable consistency checks. In addition, the GIC will conduct complex across-form and across-visit data checks on a monthly basis to identify problem areas and assess data quality. These analyses may include but are not limited to:

1. Comparisons among clinical centers and among equipment at clinical centers to identify protocol violations, differences in interpretation, failure of standardization of methodology or equipment, and malfunction of measurement devices.
2. Descriptive statistics on selected variables by technician to identify differences in the application or interpretation of the study protocol.
3. Tabulations and listings of incomplete or inconsistent responses on data collection forms; tabulations and listings of expected forms not received in a timely manner; and tabulations of clinical center error rates in data entry.
4. Analyses of digit preference for clinical measurements, investigation of correlations between variables, and other evidence suggesting inadequate or fraudulent data collection.

9.2.2.3. Equipment Checks and Calibration

To ensure accurate and consistent data, it is essential that site equipment be in working order and correctly calibrated. Therefore, it is important that sites monitor how often equipment is checked and calibrated on at least an annual basis unless checks and calibrations are recommended more frequently. The PFT Reading Center will provide certified calibration syringes to each clinical site and replace these in event of any problem.

9.2.2.4. Biospecimen Repository QC

The UNC Biospecimen Processing Facility (BSP) has both an established QA/QC Policy and Manual of Procedures for ensuring the quality of all samples processed and stored in the lab. The BSP requires staff members to undergo annual reviews of BSP internal policy and to maintain certification on all regulatory training courses.

Standard BSP procedure states that all reagents are appropriately labeled and stored. Similarly, buffers are labeled with dates and are remade, as necessary. Recipes for buffers are maintained in a notebook in the lab.

Maintenance procedures for laboratory equipment are fully specified in the laboratory protocols or in manufacturers' manuals referenced in the protocols. Equipment is maintained on a regular basis and records of these checks and equipment performance are maintained in the lab. The laboratory protocol also fully specifies the sources of calibration standards and quality control materials, the procedures used to prepare and store calibration standards and quality control materials, to guarantee the stability of the material and the accuracy of the assay.

To assure sample and data quality, the BSP has established re-analysis cut points for samples with results outside of expected parameters. In addition, every six months, the staff checks that the location of at least 10% of samples in the freezers and cold boxes match the sample tracking system.

The software used by the facility for logging and tracking all samples stored and accessed is Laboratory Data Management System (LDMS). All repository freezers will remain locked, and

the keys will be in a secured location. Authorized personnel will record date, time, and reason for access to repository freezers. Additional specifics can be found in the Repository SOP for UNC-CEMALB.

10. Study Administration

10.1. Observational Study Monitoring Board (OSMB)

An OSMB is constituted to provide evaluation of the study with recommendations to the NHLBI regarding:

A. Participant safety, burden, confidentiality, and any other matter pertaining to protection of the study participants.

B. Study performance in terms of recruitment and retention, implementation of procedures and questionnaires, follow-up for events, and all aspects of quality control.

C. Study productivity in terms of significant research results in addressing the primary study aims.

The Board will meet after the first 25 participants have completed the baseline visit and then meet via web teleconference on an annual basis with members of the study Executive Committee (with other investigators if needed). The Board may be called to meet quarterly via teleconference for updates if requested by either the GIC, Board, or NIH. The GIC provides materials to inform the OSMB of study progress, and the investigators provide presentations and respond to any concerns addressed by the Board.

The Board has the responsibility of reviewing all Ancillary Study proposals to determine whether the Ancillary Study could provide harm to the conduct of the main study. The Ancillary Study review by the Board can be done during the annual Board meeting or by email review. An Executive Secretary for the Board is an NHLBI staff member not associated with the study, who provides for all interaction between the study and the Board.

The following NHLBI website describes the responsibilities of OSMBs:
http://www.nhlbi.nih.gov/funding/policies/osmb_inst.htm

10.2. Funding Sources

National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH),
COPD Foundation

11. References

1 Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007; 176:532-55.

2 Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, Lopez Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD Executive Summary. *Am J Respir Crit Care Med* 2017; 195:557-82.

3 Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, Yates JC, Vestbo J. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med* 2007; 356:775-89.

4 Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, Decramer M, UPLIFT Study Investigators. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med* 2008; 359:1543-54.

5 Lange P, Celli B, Agusti A, Boje Jensen G, Divo M, Faner R, Guerra S, Marott JL, Martinez FD, Martinez-Camblor P, Meek P, Owen CA, Petersen H, Pinto-Plata V, Schnohr P, Sood A, Soriano JB, Tesfaigzi Y, Vestbo J. Lung-function trajectories leading to chronic obstructive pulmonary disease. *N Engl J Med* 2015; 373:111-22.

6 Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. The presence of chronic mucus hypersecretion across adult life in relation to chronic obstructive pulmonary disease development. *Am J Respir Crit Care Med* 2016; 193:662-72.

7 Allinson JP, Hardy R, Donaldson GC, Shaheen SO, Kuh D, Wedzicha JA. Combined impact of smoking and early life exposures on adult lung function trajectories. *Am J Respir Crit Care Med* 2017; 196:1021-30.

8 Kohansal R, Martinez-Camblor P, Agusti A, Buist AS, Mannino DM, Soriano JB. The natural history of chronic airflow obstruction revisited: an analysis of the Framingham offspring cohort. *Am J Respir Crit Care Med* 2009; 180:3-10.

9 Chen S, Wang C, Li B, Shi G, Li H, Zhang J, Gu Y, Zhou J, Song Y, Bai C. Risk factors for FEV_1 decline in mild COPD and high-risk populations. *Int J Chron Obstruct Pulmon Dis* 2017; 12:435-42.

10 Anderson JA, Dunnill MS, Ryder RC. Dependence of the incidence of emphysema on smoking history, age, and sex. *Thorax* 1972; 27:547-51.

11 Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *N Engl J Med* 1961; 265:253-67.

12 Auerbach O, Forman JB, Gere JB, Kassouny DY, Muehsam GE, Petrick TG, Smolin HJ, Stout AP. Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. *N Engl J Med* 1957; 256:97-104.

13 Leopold JG, Gough J. The centrilobular form of hypertrophic emphysema and its relation to chronic bronchitis. *Thorax* 1957; 12:219-35.

14 Koo HK, Vasilescu DM, Booth S, Hsieh A, Katsamenis OL, Fishbane N, Elliott WM, Kirby M, Lackie P, Sinclair I, Warner JA, Cooper JD, Coxson HO, Pare PD, Hogg JC, Hackett TL. Small airways disease in mild and moderate chronic obstructive pulmonary disease: a cross-sectional study. *Lancet Respir Med* 2018; 6:591-602.

15 Galban CJ, Han MK, Boes JL, Chughtai KA, Meyer CR, Johnson TD, Galban S, Rehemtulla A, Kazerooni EA, Martinez FJ, Ross BD. Computed tomography-based biomarker provides unique signature for diagnosis of COPD phenotypes and disease progression. *Nat Med* 2012; 18:1711-5.

16 Bhatt SP, Soler X, Wang X, Murray S, Anzueto AR, Beaty TH, Boriek AM, Casaburi R, Criner GJ, Diaz AA, Dransfield MT, Curran-Everett D, Galban CJ, Hoffman EA, Hogg JC, Kazerooni EA, Kim V, Kinney GL, Lagstein A, Lynch DA, Make BJ, Martinez FJ, Ramsdell JW, Reddy R, Ross BD, Rossiter HB, Steiner RM, Strand MJ, van Beek EJ, Wan ES, Washko GR, Wells JM, Wendt CH, Wise RA, Silverman EK, Crapo JD, Bowler RP, Han MK, COPDGene Investigators. Association between functional small airway disease and FEV1 decline in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2016; 194:178-84.

17 Vasilescu DM, Martinez FJ, Marchetti D, Galbán CJ, Hatt C, Meldrum CA, Dass C, Tanabe N, Reddy RM, Lagstein A, Ross BD, Labaki WW, Murray S, Meng X, Curtis JL, Hackett TL, Kazerooni EA, Criner GC, Hogg JC, Han MK. Non-invasive imaging biomarker identifies small airway damage in severe COPD. *Am J Respir Crit Care Med* 2019; 200:575–81.
18 Martinez CH, Diaz AA, Meldrum C, Curtis JL, Cooper CB, Pirozzi C, Kanner RE, Paine R, 3rd, Woodruff PG, Bleecker ER, Hansel NN, Barr RG, Marchetti N, Criner GJ, Kazerooni EA, Hoffman EA, Ross BD, Galban CJ, Cigolle CT, Martinez FJ, Han MK. Age and small airway imaging abnormalities in subjects with and without airflow obstruction in SPIROMICS. *Am J Respir Crit Care Med* 2017; 195:464-72.
19 Staudt MR, Buro-Auriemma LJ, Walters MS, Salit J, Vincent T, Shaykhiev R, Mezey JG, Tilley AE, Kaner RJ, Ho MW, Crystal RG. Airway basal stem/progenitor cells have diminished capacity to regenerate airway epithelium in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014; 190:955-8.
20 Yang J, Zuo WL, Fukui T, Chao I, Gomi K, Lee B, Staudt MR, Kaner RJ, Strulovici-Barel Y, Salit J, Crystal RG, Shaykhiev R. Smoking-dependent distal-to-proximal repatterning of the adult human small airway epithelium. *Am J Respir Crit Care Med* 2017; 196:340-52.
21 Ghosh M, Miller YE, Nakachi I, Kwon JB, Baron AE, Brantley AE, Merrick DT, Franklin WA, Keith RL, Vandivier RW. Exhaustion of airway basal progenitor cells in early and established chronic obstructive pulmonary disease *Am J Respir Crit Care Med* 2018; 197:885-96.
22 Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK, Boucher RC, Rubinstein M. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. *Science* 2012; 337:937-41.
23 Polosukhin VV, Richmond BW, Du RH, Cates JM, Wu P, Nian H, Massion PP, Ware LB, Lee JW, Kononov AV, Lawson WE, Blackwell TS. Secretory IgA deficiency in individual small airways is associated with persistent inflammation and remodeling. *Am J Respir Crit Care Med* 2017; 195:1010-21.
24 Curtis JL. A hairline crack in the levee: focal secretory IgA deficiency as a first step toward emphysema. *Am J Respir Crit Care Med* 2017; 195:970-73.
25 Martinez FJ, Han MK, Allinson JP, Barr RG, Boucher RC, Calverley PMA, Celli BR, Christenson SC, Crystal RG, Fagerås M, Freeman CM, Gröenk L, Hoffman EA, Kesimer M, Kostikas K, Paine III R, Rafii S, Rennard SI, Segal LN, Shaykhiev R, Stevenson C, Tal-Singer R, Vestbo J, Woodruff PG, Curtis JL, Wedzicha JA. At the root: defining and halting progression of early chronic obstructive pulmonary disease. *Am J Resprir Crit Care Med* 2018; 197:1540-51.
26 Freeman CM, Curtis JL. It's complicated: lung dendritic cells in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2020; 202:479-81.
27 Christenson SA, Steiling K, van den Berge M, Hijazi K, Hiemstra PS, Postma DS, Lenburg ME, Spira A, Woodruff PG. Asthma-COPD overlap. Clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015; 191:758-66.
28 Christenson SA, van den Berge M, Faiz A, Imkamp K, Bhakta N, Bonser LR, Zlock LT, Barjaktarevic IZ, Barr RG, Bleecker ER, Boucher RC, Bowler RP, Comellas AP, Curtis JL, Han MK, Hansel NN, Hiemstra PS, Kaner RJ, Krishnan JA, Martinez FJ, O'Neal WK, Paine Iii R, Timens W, Wells JM, Spira A, Erle DJ, Woodruff PG. An airway epithelial IL-17A response signature identifies a steroid-unresponsive COPD patient subgroup. *J Clin Invest* 2019; 129:169-81.
29 Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. *Adv Drug Deliv Rev* 2002; 54:1359-71.
30 Tarran R, Grubb BR, Gatzy JT, Davis CW, Boucher RC. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J Gen Physiol* 2001; 118:223-36.
31 Boucher RC. Regulation of airway surface liquid volume by human airway epithelia. *Pflugers Arch* 2003; 445:495-8.
32 Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, Doerschuk CM, Alexis NE, Anderson WH, Henderson AG, Barr RG, Bleecker ER, Christenson SA, Cooper CB, Han MK, Hansel NN, Hastie AT, Hoffman EA, Kanner RE, Martinez F, Paine R, 3rd, Woodruff PG, O'Neal WK, Boucher RC. Airway mucin concentration as a marker of chronic bronchitis. *N Engl J Med* 2017; 377:911-22.
33 Rennard SI, Drummond MB. Early chronic obstructive pulmonary disease: definition, assessment, and prevention. *Lancet* 2015; 385:1778-88.

34 Regan EA, Hokanson JE, Murphy JR, Make B, Lynch DA, Beaty TH, Curran-Everett D, Silverman EK, Crapo JD. Genetic epidemiology of COPD (COPDGene) study design. *COPD* 2010; 7:32-43.
35 Couper D, LaVange LM, Han M, Barr RG, Bleecker E, Hoffman EA, Kanner R, Kleerup E, Martinez FJ, Woodruff PG, Rennard S. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). *Thorax* 2014; 69:491-4.
36 Bourbeau J, Tan WC, Benedetti A, Aaron SD, Chapman KR, Coxson HO, Cowie R, Fitzgerald M, Goldstein R, Hernandez P, Leipsic J, Maltais F, Marciniuk D, O'Donnell D, Sin DD, CanCOLD Study Group. Canadian Cohort Obstructive Lung Disease (CanCOLD): Fulfilling the need for longitudinal observational studies in COPD. *COPD* 2014; 11:125-32.
37 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J* 2005; 26:319-38.
38 Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. General considerations for lung function testing. *Eur Respir J* 2005; 26:153-61.
39 Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26:948-68.
40 Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G. Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26:511-22.
41 Enright PL, Beck KC, Sherrill DL. Repeatability of spirometry in 18,000 adult patients. *Am J Respir Crit Care Med* 2004; 169:235-8.
42 Wise RA, Connett J, Kurnow K, Grill J, Johnson L, Kanner R, Enright P. Selection of spirometric measurements in a clinical trial, the Lung Health Study. *Am J Respir Crit Care Med* 1995; 151:675-81.
43 Donohue JF. Minimal clinically important differences in COPD lung function. *COPD* 2005; 2:111-24.
44 van Noord JA, Wellens W, Clarysse I, Cauberghs M, Van de Woestijne KP, Demedts M. Total respiratory resistance and reactance in patients with upper airway obstruction. *Chest* 1987; 92:475-80.
45 Zerah F, Lorino AM, Lorino H, Harf A, Macquin-Mavier I. Forced oscillation technique vs spirometry to assess bronchodilatation in patients with asthma and COPD. *Chest* 1995; 108:41-7.
46 Faria AC, Costa AA, Lopes AJ, Jansen JM, Melo PL. Forced oscillation technique in the detection of smoking-induced respiratory alterations: diagnostic accuracy and comparison with spirometry. *Clinics (Sao Paulo)* 2010; 65:1295-304.
47 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; 166:111-7.
48 Newell JD, Jr., Fuld MK, Allmendinger T, Sieren JP, Chan KS, Guo J, Hoffman EA. Very low-dose (0.15 mGy) chest CT protocols using the COPDGene 2 test object and a third-generation dual-source CT scanner with corresponding third-generation iterative reconstruction software. *Invest Radiol* 2015; 50:40-5.
49 Hammond E, Chan KS, Ames JC, Stoyles N, Sloan CM, Guo J, Newell JD, Jr., Hoffman EA, Sieren JC. Impact of advanced detector technology and iterative reconstruction on low-dose quantitative assessment of lung computed tomography density in a biological lung model. *Med Phys* 2018; 45:3657-70.
50 Hammond E, Sloan C, Newell JD, Jr., Sieren JP, Saylor M, Vidal C, Hogue S, De Stefano F, Sieren A, Hoffman EA, Sieren JC. Comparison of low- and ultralow-dose computed tomography protocols for quantitative lung and airway assessment. *Med Phys* 2017; 44:4747-57.
51 Sieren JP, Newell JD, Jr., Barr RG, Bleecker ER, Burnette N, Carretta EE, Couper D, Goldin J, Guo J, Han MK, Hansel NN, Kanner RE, Kazerooni EA, Martinez FJ, Rennard S, Woodruff PG, Hoffman EA, SPIROMICS Research Group. SPIROMICS protocol for multicenter quantitative computed tomography to phenotype the lungs. *Am J Respir Crit Care Med* 2016; 194:794-806.
52 Sieren JP, Newell JD, Judy PF, Lynch DA, Chan KS, Guo J, Hoffman EA. Reference standard and statistical model for intersite and temporal comparisons of CT attenuation in a multicenter quantitative

lung study. *Med Phys* 2012; 39:5757-67.

53 Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Criner GJ, Frith P, Halpin DMG, Han M, Lopez Varela MV, Martinez F, Montes de Oca M, Papi A, Pavord ID, Roche N, Sin DD, Stockley R, Vestbo J, Wedzicha JA, Vogelmeier C. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. *Eur Respir J* 2019; 53:pii: 1900164.

54 Singh D, Bafadhel M, Brightling CE, Sciurba FC, Curtis JL, Martinez FJ, Pasquale CB, Merrill DD, Metzdorf N, Petruzzelli S, Tal-Singer R, Compton C, Rennard S, Martin UJ. Blood eosinophil counts in clinical trials for chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2020; 202:660-71.

55 Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jöbsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, Wirtz H. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005; 26:523-48.

56 Freeman CM, Crudgington S, Stolberg VR, Brown JP, Sonstein J, Alexis NE, Doerschuk CM, Basta PV, Carretta EE, Couper DJ, Hastie AT, Kaner RJ, O'Neal WK, Paine R, 3rd, Rennard SI, Shimbo D, Woodruff PG, Zeidler M, Curtis JL. Design of a multi-center immunophenotyping analysis of peripheral blood, sputum and bronchoalveolar lavage fluid in the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS). *Journal of translational medicine.* 2015;13:19. Epub 2015/01/28. doi: 10.1186/s12967-014-0374-z. PubMed PMID: 25622723; PMCID: PMC4314767.

57 Martinez CH, Li SX, Hirzel AJ, Stolberg VR, Alexis NE, Barr RG, Bleecker ER, Carretta EE, Christenson SA, Cooper CB, Couper DJ, Doerschuk CM, Han MK, Hansel NN, Hastie AT, Hoffman EA, Kaner RJ, Martinez FJ, Meyers DA, O'Neal WK, Paine R, 3rd, Putcha N, Rennard SI, Woodruff PG, Zeidler M, Curtis JL, Freeman CM. Alveolar eosinophilia in current smokers with chronic obstructive pulmonary disease in the SPIROMICS cohort. *The Journal of allergy and clinical immunology.* 2018;141:429-32. Epub 2017/09/17. doi: 10.1016/j.jaci.2017.07.039. PubMed PMID: 28916185; PMCID: PMC5758424.

SOURCE
SPIROMICS Study of Early COPD Progression

MOP 2

PULMONARY FUNCTION TESTING

Version 4.4

October 13, 2023

Abbreviations

AE: Adverse event

ATS: American Thoracic Society

BTPS: Body temperature and pressure, saturated

CDC: Center for Disease Control

CO: Carbon monoxide

HbCO: Carboxyhemoglobin

CV: Coefficient of variation

ERS: European Respiratory Society

eSP: Electronic short path spirometry software

FDA: Federal Drug Administration

$FEF_{25\%-75\%}$: Forced expiratory flow between 25% and 75% of forced vital capacity

FET: Forced expiratory time

FEV_1: Forced expiratory volume in first second

FIO_2: Fraction inspired oxygen

FRC: Functional residual volume

FVC: Forced vital capacity

HFA: Hydrofluoroalakane propellant, HFA-134a (1,1,1,2-tetrafluoroethane)

MDCT: Multi-detector computed tomography

IC: Inspiratory capacity

ISP: Communications software for the eSP KoKo spirometer

LLN: Lower limit of normal

MCID: Minimal clinically important difference

MDI: Metered dose inhaler

NHANES: National Health and Nutrition Examination Survey

PEF: Peak expiratory flow

PEFT: Peak expiratory flow time

PFT: Pulmonary function testing (spirometry)

PO_2: Partial pressure of oxygen

ppm: Parts per million

RB: Rebreathing

RV: Residual volume

SBP: Systolic blood pressure

SpO_2: Oxygen saturation measured by pulse oximetry

TLC: Total lung capacity

VC: Vital capacity, may be modified as slow or forced

VEXT: Volume, extrapolated (spirometry)

VO_2 max: Maximum oxygen uptake

1 PROTOCOL SUMMARY

1.1 Background and Rationale

The 2019 ATS/ERS guidelines for pulmonary function testing and interpretation will serve as the primary guidance for the conduct and interpretation of the spirometry and if performed, lung volumes and diffusing capacity [1,2,3,4,5]. The FDA preferred primary endpoint for assessment of alteration in disease progression is serial measurements of FEV_1 over three years [6]. Other objective physiologic assessments considered by the FDA, in the draft guidelines, are RV/TLC and exercise test results such as six-minute walk. FEV_1 has been the primary outcome for most trials attempting to demonstrate disease modification for COPD. FEV_1 is also the usual outcome for studies of bronchodilation. The between maneuver repeatability, which 90% of consecutive patients can meet, is 120 mL (6.1%) for FEV_1 and 150 mL (5.3%) for FVC [7]. The established target is 150 mL for both measures (or 100 mL if the FVC is < 1.0 L) [3]. The short-term (24.9±17.1 days) repeatability in mild COPD participants is 113 mL (CV 4.1%) for FEV_1 and 150 mL (CV 3.5%) for FVC [8]. The minimal clinically important difference for FEV_1 is about 100 mL [9].

1.2 Inclusion Criteria Specific to Pulmonary Function Testing

- None (all included in study enrollment criteria).

1.3 Exclusion Criteria Specific to Pulmonary Function Testing Resulting in Postponement or Modification of Testing

- Myocardial infarction, eye, chest, or abdominal surgery within six weeks.
- Upper or lower respiratory tract infections including untreated tuberculosis; chest, abdominal, oral, or facial pain within three weeks.
- Those with prior significant difficulties with pulmonary function testing.
- Recent use of bronchodilators is recorded, and specific withholding is suggested but not mandatory.

1.4 Methods

Brief testing sequence and methods for SOURCE:

- Impulse Oscillometry using the Medgraphics® Resmon Pro™.
- Spirometry (FVC) will be performed using standardized equipment [Easy on-PC spirometer (ndd Medical Technologies, https://nddmed.com/)].
- In addition to ndd software inclusion of automated quality assurance (QA) for each effort and selection of best effort according to ATS guidelines, a dedicated centralized quality assurance center (UCLA PFT Reading Center) will perform over-read and/or quality assurance, append notes, forms, and documents to the test subjects' folder.
- Forced vital capacity pre-bronchodilator.
- Bronchodilation with four puffs of ipratropium bromide HFA and four puffs of albuterol sulfate HFA (30-minute waiting period before post-bronchodilator testing).
- Impulse oscillometry.
- Forced vital capacity post-bronchodilator.

1.5 Primary Outcome:

Change in post-bronchodilator FEV_1 as percent reference (Hankinson 1999[10]) with baseline value (% reference) as a covariate.

1.6 Secondary Outcomes:

- Oscillometry, Rrs, Xrs
- FVC
- Bronchodilator responsiveness of FEV_1 and FVC
- FEV_1 /FVC
- S_pO_2 at rest

1.7 Human Participants Protection

PFTs are a common medical procedure of generally low risk. Some participants may experience breathlessness, cough, fatigue, dizziness/lightheadedness (hyperventilation), all of which are brief. Very rarely participants might experience headache, syncope, musculoskeletal chest pain, or ear injury. A seated position has been specified to reduce risk related to dizziness or syncope.

Transmission of airborne disease is rare and minimized or eliminated with single-use filters. Instructions for withholding bronchodilator medications prior to testing will stress the continued use of rescue medication if needed. The use of albuterol or ipratropium will generally relieve any symptoms related to the trough effect of long-acting bronchodilators. Albuterol has been reported to cause urticaria, angioedema, paradoxical bronchospasm, angina, arrhythmias, QT prolongation, hypertension, hypokalemia, seizures, tremor, nervousness, headache, tachycardia, muscle cramps, palpitations, insomnia, and dizziness. Ipratropium has been reported to cause cough, nausea, dry mouth, dizziness, headache, dyspnea, atrial fibrillation, tachycardia, paradoxical bronchospasm, laryngospasm, angioedema, anaphylaxis, hypersensitivity, and exacerbate angle closure glaucoma.

The dose used in testing is twice the usual 4-hourly maintenance dose of albuterol or the 6-hourly dose of ipratropium. However, home management of exacerbations includes increasing the dose and/or frequency of bronchodilator therapy [11]. Doses in patients hospitalized or visiting the Emergency Department for exacerbations may be 10 times the usual dose. Redosing, after at least 3 hours, is unlikely to result in any additional side effects.

Data transmission is FDA 21 CFR Part 11 compliant. The date of the procedure and of birth are the only HIPPA protected data to be transmitted, but the public data sharing data set will use the visit identifier and the ordinal age. The PFT software is designed to be HIPPA compliant for clinical use.

The participant may benefit from treatment or secondary prevention after study identification of unrecognized pulmonary disease. All study personnel are certified in the ethical conduct of human biomedical and genetics research and HIPPA information security.

2 EQUIPMENT

A standardized spirometer (ndd Easy on-PC) and oscillometer (Resmon Pro) will be installed at each clinical site. Every effort will be made to assure the same equipment is available throughout the study. A chair without wheels for the participant will be provided adjacent to a table for the spirometer and laptop. A medical waste receptacle will be available to dispose of any items contacting the participant's oral secretions etc. (e.g., mouthpieces).

2.1 Supplies

Disposable, one-time use low-resistance (0.5 cm H_2O at 60 L/min) spirettes, filters, adapters, and nose clips with extra supplies for wastage and training. Site supplied alcohol hand gel and germicidal equipment wipes will be used to meet recommended infection control measures.

2.1 Spirometry

The ndd Easy on-PC spirometer connects to a dedicated PC with at least Windows 10.0 installed. The accuracy error is $\leq 2\%$ meeting the ATS/ERS standards. Data suggest the repeatability of the ndd spirometers is comparable or superior to rolling seal spirometers.

Repeatability: the difference between 95% of pairs of measurements is expected to be less than this value.
Bias: mean and 95% CI for the difference between measured and expected.
Imprecision: the difference between 95% of measurements and the true value are expected to be less than this value after correction for bias.

2.3 Spirometry Data Storage and Back-up:

Immediate data transfers after each test from the individual sites to the secure over read server at UCLA (Box, secure, HIPAA-compliant UCLA cloud server) will provide for data storage (FDA 21 CFR Part 11 compliant). Coordinators and PFT technicians are to transfer data from their spirometer to the UCLA server weekly (every Tuesday). Online/offline data acquisition, audit trail, and remote upgrade capabilities are available. The UCLA Reading PFT Center transfers monthly to the GIC via secure FTP.

3 PERSONNEL

3.1 Qualifications

All technicians who perform pulmonary function tests will be required to meet the recommendations for personnel qualifications for pulmonary function testing issued by the American Thoracic Society [12] and updated by the ATS/ERS Task Force [2]. Minimum requirements include sufficient education and training to assure that the technician understands the fundamentals of the tests, the common signs of pulmonary diseases, and the management of the acquired pulmonary function data. Each PI will certify the qualifications of the technicians at their site. Each technician will certify they have reviewed the pulmonary function MOP.

3.2 Training

Two to three technicians will be trained for each site. Initial central training at a coordinators' meeting prior to beginning the study (protocol specific theory and practice) will be conducted assuming all technicians have previously performed PFTs. Additional training sessions (live and recorded) will be available online.

3.3 Certification

Enablement on the spirometer system, technicians will complete:

- Security statement
- Sample calibration
- Sample linearity check
- Sample FVC
- Technician certification checklist

These items will be uploaded to the UCLA PFT Reading Center for review. After review, the technician will be enabled for testing on the local system. *Certification* of each technician will be based on the independent performance of PFTs on two separate individuals prior to any study PFTs. Each technician will have the initial PFTs evaluated for quality and protocol compliance by the UCLA PFT core. Training of new interim personnel will follow a similar plan. Site visits will include specific review of PFT quality. Development of spirometry skills will emphasize: 1) demonstration of the FVC maneuver before participant's first attempt; 2) vigorously coaching to obtain a full inspiration followed by a "blast" at outset of maneuver and constant encouragement of complete exhalation ("squeeze everything out"); 3) observation of participant throughout the maneuver; and 4) enthusiastic feedback to encourage maximal efforts. Physiology, spirometry repeatability and acceptability, calibration verification, quality assurance, infection control, and troubleshooting will also be addressed.

4 INFECTION CONTROL

4.1 Filters

Disposable filters/mouth pieces and nose clips will be used for each participant [13].
Materials in contact with participant mucosal surfaces will be disposed of as medical waste.

4.2 Cleaning

Measures will include hand hygiene (alcohol gel) and daily external cleaning of spirometer with germicidal disposable wipes after each patient testing. CDC guidelines do not recommend routinely sterilizing or disinfecting the internal machinery of the PFT machine [13].

4.3 Hand Washing

Hand washing (soap and water or alcohol based "waterless" hand cleaner) before and after each patient contact.

5 CALIBRATION AND LINEARITY VERIFICATION

Each day of subject testing the spirometer will be calibrated at three target flows with a biologic filter in place. The tracings will be stored in a binder at the site (may be archived to a file when full). The calibration factors will be reviewed centrally.

- Low flow rate cycle (0-4 L/sec)
- Medium flow rate (4-8 L/sec)
- High flow rate cycle (8-12 L/sec)

Each week a verification of linearity will be performed with three strokes at each of the above flows. The tracings will be stored in a binder at the site (may be archived to a file when full).

6 SCHEDULE OF EXAMS

Subjects in SOURCE will be assigned a unique subject number and will have tests performed at the baseline clinic visit and at the 3-year follow-up clinic visit. Ideally the spirometry will be performed at the same time of day to obviate the effect of diurnal variation, but a 4-hour window is acceptable.

7 QUALITY ASSURANCE

7.1 Local

7.1.1 Technician feedback

Biannual feedback on the performance of each technician will include:

1) information concerning the nature and extent of unacceptable maneuvers and non-repeatable tests (goal < 5%)
2) corrective action that the technician can take to improve the quality and number of acceptable maneuvers
3) positive feedback to technicians for good performance
4) comments on the calibration. Immediate feedback on acceptability and repeatability will be provided by the spirometer software.

7.1.2 Spirometry

No attempt will be made to hand calculate values from tracings. Tracings generated from digital data are unlikely to yield useful corrections or validation. Avoiding this will also shorten the training time.

7.1.2.1 Mechanical Standards

Spirometer/pneumotach: Daily **calibration verification** will be performed with a 3 L syringe (with a biologic filter in line) at 3 flow rates. Immediate feedback will be the ±1%, 2%, & 3% volume at each flow rate. Calibration syringes will be recertified bi-annually (3 L ± 15 mL).

7.1.2.2 Repeatability Goals

A continuous feedback program to the technicians will target > 95% of spirometry tests meeting the repeatability criteria (NHANES III achieved 90% for FVC and 92% for FEV_1, reported by ATS 2019).

7.1.3 Entered demographic/atmospheric data

Entered participant data (date of birth, height, sex, and race) will be crosschecked against the main database. The barometric pressure temperature and humidity is entered by the technician. nViro values will be verified at the site visits by comparison to a reference barometer at site visits.

7.2 Centralized Quality Assurance Core (UCLA PFT Reading Center)

A database query of the centralized spirometry (Figure 1) will evaluate the acceptability and repeatability of all spirometry. Data transfer procedures are described in detail in the appendices (**Appendix A**).

An over read QA program will review all spirometry. A secondary review will be made of:

- Initial PFTs for each technician (certification)
- Statistical outliers at baseline of FEV_1, FVC
- Statistical outliers in change of the same parameters (review of baseline and changed follow-up)
- Discordant changes (e.g., fall in FEV_1 and improvement in symptoms if identified by GIC analysis)
- Those flagged as poor quality by database query
- Random sample stratified by site enrollment.

Calibration records, syringe certification, and mechanical standards for each site will be reviewed on a regular basis. This core will not provide clinical interpretations.

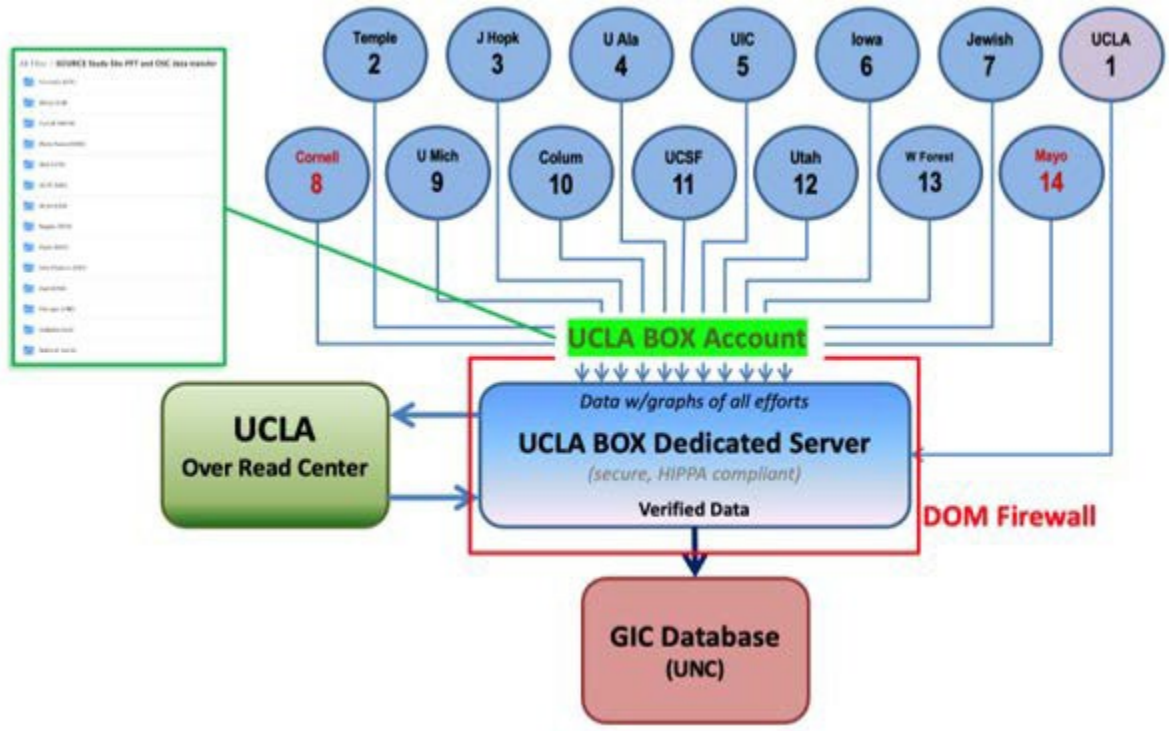


Figure 1: UCLA Centralized PFT Reading Center Network

8 PARTICIPANT PREPARATION

8.1 Safety

PFTs will be deferred or not performed for those with: recent (6 weeks) upper or lower respiratory tract infections including TB; chest, abdominal, oral, or facial pain; stress incontinence; dementia; recent myocardial infarction (6 weeks); chest or abdominal surgery (6 weeks); those with prior significant difficulties with spirometry; or participant refusal.

8.2 Instruction

Prior to PFTs, participants will be asked to withhold/refrain from vigorous exercise (30 minutes), smoking (1 hour), eating a large meal (2 hours), alcohol (4 hours), caffeine (6 hours), inhaled albuterol (6 hours), inhaled ipratropium (8 hours), and other bronchodilators (twice the usual dosing frequency, see Table 1). Practicality may require some long-acting bronchodilators to simply be noted rather than withheld. Instructions for withholding bronchodilator medications prior to testing will stress the continued use of rescue medication if needed. The use of albuterol or ipratropium will generally relieve any symptoms related to the trough effect of long-acting bronchodilators. Failing to withhold/refrain from the above activities will not exclude a participant from continuing with PFTs.

The PFT values after bronchodilators are the most important outcomes. Sites will vary in the ability to consent/instruct potential subjects over the telephone for withholding prior to written consent due to local IRB policies. Withholding may be different at sequential visits. Some subjects, in spite of trying, will be unable to withhold drugs prior to visits.

Table 1: Target duration of abstinence from bronchodilators

Drug	Brand names containing the drug	Duration of abstinence
Albuterol	ProAir HFA, Proventil HFA, Ventolin HFA, AccuNeb, Generic nebulizer solutions, Non-sustained release oral tablets	6 hours
Epinephrine	Epipen, Twinject, S2, generic	6 hours
Levalbuterol	Xopenex HFA, Xopenex solution, Generic solution	6 hours
Metaproterenol	Generic nebulizer solutions, Non-sustained release oral tablets or syrup	6 hours
Pirbuterol	Maxair Autohaler	6 hours
Terbutaline	Generic tablets	6 hours
Ipratropium	Atrovent HFA, Combivent, DuoNeb, Generic nebulizer solutions	8 hours
Theophylline immediate release	Elixophyllin, Theolair, Generic	12 hours

Albuterol (sustained release)	VoSpire ER, Sustained release oral tablets	24 hours
Arformoterol	Brovana	24 hours
Formoterol	Dulera, Foradil Aerolizer, Foradil Certihaler, Perforomist, Symbicort	24 hours
Salmeterol	Advair Diskus, Advair HFA, Serevent Diskus	24 hours
Theophylline extended 12-hour release	Theochron, Generic q12 hour ER	24 hours
Theophylline 24-hour release	Theo-24, Uniphyl	48 hours
Tiotropium	Spiriva	48 hours

Combination drugs are listed as the drug with the longer withholding time. Drugs available in the US (Orange Book) are listed http://www.accessdata.fda.gov/scripts/cder/ob/docs/queryai.cfm. Inhaled steroids, cromolyn, intranasal steroids, antihistamines, leukotriene receptor blockers (montelukast, zafirlukast, Accolate, Singulair), and 5-LO inhibiters (zileuton, Zyflo, Zyflo CR) have no withholding parameters. Beta-blockers will be noted but not restricted.

Caffeine up to 200 mg (see Table 2) is permitted prior to the procedures (note the NPO requirements for the blood).

Table 2: Caffeine equivalents

Caffeine equivalent to 200 mg

- Coffee up to 16 oz.
- Espresso up to 3 shots
- Energy drinks: Rock star, Amp Red Bull, Full Throttle up to 20 oz.
- High caffeine cola drinks (Jolt) 24 oz.
- Instant coffee or tea up to 25 oz.
- Brewed tea up to 33 oz.
- Vault up to 34 oz.
- Non-cola soft drinks up to 43 oz.
- Diet cola or Diet Dr. Pepper up to 51 oz.
- Cola or Dr. Pepper up to 65 oz.
- Chocolate milk up to 2.5 gallons
- Cocoa (Swiss Miss) up to 83 gallons
- Chocolate covered coffee beans up to 16 beans
- Chocolate chips, semisweet up to 1.75 cups
- Chocolate chips, milk up to 5.95 cups
- M&Ms, plain up to 6.87 cups
- M&Ms, peanut up to 11.76 cups
- Butterfinger bars, bite size up to 2857 bars

Small container energy drinks:

- Charge! Super Shot (200 mg/ 59 mL)
- Upshot (200 mg/ 74 mL)
- Fuel Cell (180 mg/ 59 mL)
- Ammo (171 mg/ 30 mL)
- Mana Energy Potion (160 mg/ 40 mL)
- Jolt Endurance Shot (150 mg/ 59 mL)
- NOS Powershot (125 mg/ 59 mL)
- Slam Energy Drink (107 mg/ 59 mL)
- Kore Energy Shot (100 mg/ 51 mL)
- Powershot (100 mg/ 30 mL)
- Sky Rocket Syrup (100 mg/ 28 mL)

Energy drinks over the 200 mg limit in a single container:

- Redline Power Rush (350 mg/ 74 mL)
- Wired X344 (344 mg/ 455 mL)
- Spike Shooter (300 mg/ 248 mL)
- Cocaine Energy Drink (280 mg/ 248 mL)
- Extreme Energy 6-hour shot (220 mg/ 59 mL)

9 PREPARATION AND CALIBRATION

Room temperature will be ideally maintained at 23±1.5°C. The temperature, humidity, and barometric pressure should be recorded from current site weather stations (not provided in this study) or an online weather station. Volume/flow verification will be performed daily with a three-liter syringe at three flow rates. The height measured from the anthropomorphic portion of the protocol will be entered into the spirometer.

10 OSCILLOMETRY

10.1 Definition/Description

Oscillometry is a non-invasive method to assess airway function by imposing oscillatory pressure signals to the respiratory tract. The technique enables the determination of airway resistance at different oscillation frequencies (e.g., R5, R11, and R19) as well as reactance at the same frequencies (e.g., X5, X11, and X19).

10.2 Equipment and Supplies

Each SOURCE clinical site is provided with an oscillometer (ResMon Pro™) on loan for this study from Monitored Therapeutics Inc, Minneapolis, MN.

10.3 Method of Assessment

Oscillometry will be performed with disposable low-resistance filters. Each site will receive a special adaptor that will enable the same usage of PFT filters supplied for the PFTs nose clips, seated with the chin slightly up, after loosening tight or restrictive clothing and removing loose dentures.

The oscillometry method used for SOURCE. PFT technicians will emphasize:

- demonstration of baseline breathing.
- coaching to perform quiet resting breathing during testing.
- observation of participant throughout the maneuver.

A minimum of three acceptable maneuvers will be obtained. Up to five maneuvers are permitted to meet criteria. See **SOURCE PFT RC Memo 10** (Appendix C) for study specific users guide for screen shots and step by step instructions for software.

10.4 Acceptability and Repeatability Criteria

The Resmon Pro™ tracks resting tidal breathing until a predetermined number of quality breaths have been accumulated (usually 5-7).

10.5 Quality Assurance

- Training and certification initially and annually or as needed for difficulties.
- Protocol compliance prompts in software.
- Quality assurance prompts in software.
- Central over read/QA review of each maneuver (acceptability and repeatability).

10.6 Derivation or calculation of variable

- R5, R11, R19, X5, X11, X19
- Resonant frequency. Expiratory flow

10.7 Normal range, protocol limits, significant change, and adverse event grading

- Precision and units: X.XX Liters BTPS
- Protocol inclusion criteria: NA
- Clinically significant change: unknown
- AE grade: NA

10.8 Data validation

- Electronic data transfer
- Expert over read and electronic validation methods

11 SPIROMETRY: FORCED VITAL CAPACITY

11.1 Definition/Description

Improvement in FEV_1 is the standard for regulatory approval of bronchodilator drugs and the most widely accepted criterion for judging disease progression or disease modification. Because the test is readily available and inexpensive, the results may be easily generalized.

11.2 Equipment and Supplies Setup and Configuration

Refer to **SOURCE PFT RC Memo 9.5** (Appendix B) and **SOURCE PFT RC Memo 12** (Appendix D) for ndd Easy on-PC setup, configuration, training, and certification.

- ndd Easy on-PC spirometer
- Computer with ndd EasyOne Connect software installed and at least Windows 10.0 or greater
- 3-liter calibration syringe
- Participant spirettes, filters and adapters or EasyOne Filter, and nose clips
- ndd Easy on-PC Operator's Manual V08 (4 December 2020)
- ndd Easy on-PC Operator's Manual V09 (29 November 2021); integration of EasyOne Filter

11.3 Method of Assessment

Spirometry will be performed with disposable, one-time use low-resistance spirettes, filters, adapters, and nose clips, seated with the chin slightly up, after loosening tight or restrictive clothing and removing loose dentures.

Forced expiratory vital capacity (spirometry) will be performed after completing oscillometry. PFT technicians will emphasize:

1) demonstration of the FVC maneuver before participant's first attempt.
2) vigorously coaching to obtain a full inspiration followed by a "blast" at outset of maneuver and constant encouragement of complete exhalation ("squeeze everything out").
3) observation of participant throughout the maneuver.

4) enthusiastic feedback to encourage maximal efforts. A minimum of three acceptable and two repeatable maneuvers will be obtained.

A minimum of three acceptable maneuvers will be performed. Additional maneuvers (up to 8 total) will be performed until the repeatability criteria are met or it is not safe for the participant to continue.

11.4 Acceptability and Repeatability Criteria

Acceptability Criteria: (applied to each maneuver)

1) Back-extrapolated zero-time is less than 5% of the FVC or 100 mL (whichever is greater)
    i) Slow start of test. The participant did not begin their initial peak flow early enough; repeat, coaching for a more forceful and abrupt start ("BLAST it out").
2) Rapid rise to PEF. Time to peak < 120 ms will generate a prompt to "blow harder" but will not result in the rejection of the maneuver. Rise time and dwell time will be evaluated for PEF but are not mandated.
    i) Low peak flow. The patient did not achieve an adequately forceful blast. Repeat the effort, coaching to blow harder and faster.
3) Absence of leaks or obstruction of mouthpiece.
4) Absence of glottic closure.
5) Absence of cough. Coughing causes abrupt irregularities in flow and is a reason to reject the test when it occurs during the first second of the effort. Coach the patient to make the effort without coughing. Sometimes it is helpful to have the patient blow just slightly less forcefully than the maximum to prevent a cough. Maneuvers with the cough occurring after the first second may still be usable for the FEV_1.
6) Smooth end of exhalation (plateau on volume time curve). The change in volume is less than 0.025 L over the last second of exhalation.
    i) Abrupt end of test. At the end of exhalation, the patient stopped blowing out too abruptly, ending their effort too soon. Coach the patient to maintain their expiratory effort to the very end.
7) Minimum six-second exhalation.
    i) Short expiratory time. Patient did not continue their expiration for at least 6 seconds or did not reach a volume plateau.

Repeatability Criteria: (between maneuvers for each participant)

FVC: the difference between largest and next largest FVC < 150 mL (100 mL if FVC < 1 L) [3].

a. FEV_1: the difference between largest and next largest value of FEV_1 <150 mL (100 mL if FVC < 1 L) [3].
b. Last FEV_1, not largest (trending up) will be evaluated, but not mandated.
c. PEF: the difference between largest and next largest value of PEF < 10% (or 6.6 L/min whichever is greater) (NHANES III criteria) and not trending up over subsequent maneuvers will not be mandated.

A minimum of three acceptable maneuvers will be expected [3]. In general, if you cannot obtain three acceptable and two repeatable tests within eight attempts, further testing will not be productive and may be terminated on the judgment of the technician. Many people who cannot perform spirometry, however, have either neurological or cognitive deficits that may not otherwise be obvious. Therefore, inability to perform spirometry should be reported to the investigators because it may affect participation in the study.

The largest acceptable FVC will be reported. The largest usable FEV_1 (not necessarily meeting acceptability criteria d to g above) will be reported. The PEF and $FEF_{25-75\%}$ will be reported from the maneuver with the largest sum of FEV_1 and FVC. The FET will be reported from the largest FVC maneuver.

11.5 Quality Assurance Messages and Flags

Individual Effort Quality (FVC)
Acceptable efforts must meet quality standards based on: EV, EOT, and TET (see below). The quality report status for these quality parameters is reported near the top of the current effort's volume time graph followed by:
- *Acceptable: (+) plus sign displays next to the Effort Quality Indicator.*
- *Unacceptable: (-) minus sign displays next to the Effort Quality Indicator.*

Defining the quality parameters:
- *EV (extrapolated volume): < 5% or 150 mL (whichever is greater) of FVC.*
- *EOT (end of test): volume change > 0.025 L during the last second of an effort.*
- *TET (total expiratory time): ≥ 6 seconds for patient's ≥ 10 yrs. As default.*
    - *> 3 seconds for patients age < 10 yrs. As default.*

The grading system (configurable) is based on ATS 2005 recommendations:
- *Acceptable repeatability is within 150 mL or within 100 mL if the FVC value is < 1 L.*
- *Reported Graphs and Loops will be checked for effort repeatability. The effort must meet all three acceptability criteria to be considered for repeatability.*

11.6 Grading System for FEV_1 (1)

Grade	Number of Measurements	Repeatability: Age >6 yr	Repeatability: Age ≤6 yr*
A	≥3 acceptable	Within 0.150 L	Within 0.100 L*
B	2 acceptable	Within 0.150 L	Within 0.100 L*
C	≥2 acceptable	Within 0.200 L	Within 0.150 L*
D	≥2 acceptable	Within 0.250 L	Within 0.200 L*
E	≥2 acceptable	>0.250 L	>0.200 L*
	OR 1 acceptable	N/A	N/A
U	0 acceptable AND ≥1 usable	N/A	N/A
F	0 acceptable and 0 usable	N/A	N/A

Definition of abbreviation: N/A = not applicable.
The repeatability grade is determined for the set of prebronchodilator maneuvers and the set of post-bronchodilator maneuvers separately. The repeatability criteria are applied to the differences between the two largest FVC values and the two largest FEV_1 values. Grade U indicates that only usable but not acceptable measurements were obtained. *Although some maneuvers may be acceptable or usable at grading levels lower than A, the overriding goal of the operator must be to always achieve the best possible testing quality for each patient.* Adapted from Reference 114.
*Or 10% of the highest value, whichever is greater; applies for age 6 years or younger only.

11.7 Maintenance

See ndd Easy on-PC Operator's Manual V08 (4 December 2020).

11.8 Quality Assurance

- Training and certification initially and annually or as needed for difficulties.
- Protocol compliance prompts in software. Quality assurance prompts in software.
- Central over read/QA review of each maneuver (acceptability and repeatability) and selection of best test.

11.9 Derivation or Calculation of Variable

FEV_1, FVC, PEF, FET, and $FEF_{25-75\%}$ calculated from the maneuver as for ATS/ERS standards.

FEV_1 /FVC Isovolume FEF: Average flow over reference volume a) pre-bronchodilator FVC and b) baseline visit FVC (post hoc).

For change over time:

- Absolute change: (follow-up value-baseline value)
- Percent initial value: (follow-up value-baseline value) / baseline value x 100
- Percent of predicted (reference) value: (follow-up value-baseline value) / (FEV_1 or FVC reference value)

Percent reference, Z score, and categorical LLN for Hankinson 1999 [10].

11.10 Normal Range, Protocol Limits, Significant Change, and Adverse Event Grading

- Lower/upper limit of normal: Hankinson/NHANES [10], non-smokers by race and sex [uses race, sex, age, and height, see Table 4 & 5 in reference]. Use Caucasian for all non-African American, non-Mexican American (Hispanic, non-African American).
- Precision and units: volumes X.XX Liters BTPS, flows X.XX L/s BTPS, ratios XX.X %. Protocol inclusion criteria: Ability to perform spirometry.

11.11 Data Validation

Electronic data transfer.
Electronic validation methods:

- Physiologically plausible human range of values in normal and disease: 15-130%
- Protocol inclusion criteria: strata above
- Change of > 10% absolute/year

11.12 Statistical Plan of Analysis

- Primary analysis: Change in post-bronchodilator FEV_1 as percent reference (Hankinson 1999 [10]) with baseline value (% reference) as a covariate.
- Secondary analysis: FEV_1/FVC
- Exploratory analysis: FEV_1 , FVC, PEF, FET, $FEF_{25-75\%}$, FEV_1 /FVC, isovolume $FEF_{25-75\%}$, baseline, $FEF_{25-75\%}$, pre-bronchodilator, as:
    1. change in post-bronchodilator percent reference with baseline percent reference as a covariate;
    2. change in post-bronchodilator Z score with baseline Z score as a covariate;
    3. change in pre-bronchodilator percent reference with baseline percent reference as a covariate;
    4. change in pre-bronchodilator Z score with baseline Z score as a covariate;
    5. change in bronchodilator response as percent reference FEV_1 with baseline as a covariate. Alternative reference equation Stanojevic [15], may have superior LLN and Z score characteristics.

11.13 Relation to Specific Aim/Study Objective

Serial measurements of FEV_1 over three years is the FDA preferred primary endpoint for assessment of alteration in disease progression [6]. Therefore, FEV_1 decline is the nominal comparator for novel outcomes.

12 BRONCHODILATION

12.1 Definition/Description

Assessment of acute bronchodilator response timed to target the peak drug effect. In COPD subjects, 4 puffs of ipratropium CFC reached 80% of the maximal response of 8 puffs before 0.6 hours and was sustained through 6.2 hours on average [16]. In asthma subject's albuterol HFA given as 1, 1, 2 puffs at 30-minute intervals achieved 83% of the 16-puff response at 30 minutes [17].

12.2 Equipment and Supplies

- Ipratropium bromide HFA (Atrovent HFA 12.9g (200 puff) canister NDC 0597-0087-17)
- Albuterol sulfate HFA (Ventolin HFA 18g (200 puff) canister NDC 0173-0682-20, or 8 g (60 puff) NDC 0173- 0682-21 or 0173-0682-24)
- Ventilator tubing (Figure 2), 15 cm (6 inches) segment used as spacer (100 feet/roll) cut with scissors

Figure 2: Ventilator tubing for use as a spacer

12.3 Method of Assessment

Repeated FVC following administration of:

Short-acting ß2-agonist and anticholinergic (SAMBA): ipratropium bromide HFA (Atrovent HFA), four puffs of 21 µg with spacer and albuterol sulfate HFA (Ventolin HFA), four puffs of 120 µg 30-180 minutes prior to "post" spirometry. A worksheet in the spirometry software will be used to document the time if the first puff of ipratropium. Please use the computer clock time.

Doses taken previously at home or other bronchodilators do not result in modification of this dose.

Good inhaler technique:

- Shake
- Slow deep breathe in over 4-5 seconds
- Technician will actuate MDI at beginning of breath
- Ten second breath hold
- Relax, catch breath, and repeat for next puff
- About 30 seconds total per puff

Remember, many of the control participants will never have used an inhaler.
Redosing: In the event that "post-bronchodilator" studies (including sputum induction and MDCT) are delayed, the participant may receive additional doses of bronchodilators as follows:

Table 3: Bronchodilator redosing

Time after initial re/dose (min)		Permitted testing window (minutes after redosing)
0 (initial dose)	4 puffs ipratropium and 4 puffs albuterol	30-180 mins
≥ 165 but less than 300	2 puff albuterol	15-180 mins
≥ 300	2 puffs ipratropium and 2 puffs albuterol	30-180 mins

12.4 Maintenance

Ipratropium MDI (Atrovent HFA) short acting anticholinergic (muscarinic), four puffs of 21 µg with spacer.

- Priming: initially and if not used for > 3 days.
- Storage: stored in an upright position in a secure area at room temperature.
- Washing: weekly (or if grossly contaminated) washing of mouthpiece (never canister) with warm water.
    - Allowed to dry completely prior to use. Cold sterilization (Cidex) should be used, if grossly contaminated.
- End of canister: Puff count for canister will use a manual tally on a file card.

Albuterol HFA (Ventolin HFA) short acting ß2 agonist, four puffs of 108 µg (90 µg albuterol base) with spacer.

- Priming: initially and if not used for > 2 weeks.
- Storage: stored in an upright position in a secure area at room temperature.
- Washing: weekly (or if grossly contaminated) washing of mouthpiece (never canister) with warm water.
    - Allowed to dry completely prior to use. Cold sterilization (Cidex) should be used, if grossly contaminated.
- End of canister: Puff count for canister will use the built-in mechanical counter (stop at 0).
- Expiration: expiration date on canister or 12-month limit after removal from the foil package.
- Dispose of after use in accordance with local policy.

Spacer is a 15 cm segment of ventilator tubing, single participant use. Dispose of as medical waste after use.

12.5 Quality Assurance

- Percent completed according to protocol
- Priming, expiration, and end of canister
- Adverse events

12.6 Derivation or Calculation of Variable

- Time before post-bronchodilator maneuvers
- Time to first post-bronchodilator FVC maneuver after administration of first puff of ipratropium (Criteria 30-120 minutes).

- For FEV_1 and FVC:
    - Absolute change: (post value-pre value)
    - Percent initial value: (post value-pre value) / pre value x 100
    - Percent of predicted (reference) value: (post value-pre value) / (FEV_1 or FVC reference value)

- For $FEF_{25-75\%}$:
    - Isovolume referenced to the pre-bronchodilator $FEF_{25-75\%}$
        - Absolute change: (post value-pre value)
        - Percent initial value: (post value-pre value) / pre value x 100
        - Percent of predicted (reference) value: (post value-pre value) / ($FEF_{25-75\%}$ reference value)

- For Oscillometry:
    - Rrs, Xrs, Resonate Frequency

12.7 Normal Range, Protocol Limits, Significant Change, and Adverse Event Grading

- Precision and units:
    - Absolute change: xxX mL
    - Percent initial value: xX.x %
    - Percent of predicted (reference) value: xX.X%

- Clinically significant change:
    - FEV_1 or FVC ≥ 12% (of baseline not percent reference) and 200 mL
    - < 8% (or < 150 mL) may be within the measurement error

12.8 Data Validation

Spirometry software prompt for time after bronchodilator Electronic validation methods for time between first dose of bronchodilator and FVC.

12.9 Statistical Plan of Analysis

Stratification by post-bronchodilator FEV_1 Bronchodilator response.

12.10 Relation to Specific Aim/Study Objective

Known predictor of decline in lung function.

13 CONTACT INFORMATION

Coordinators are to first contact the UCLA PFT Reading Center with any issues or questions related to spirometry, oscillometry, and PFT data transfer. In the case that there are technical difficulties with the spirometry equipment and first contact with Dr. Brett Dolezal is unsuccessful (by email and phone, remember he is on West coast time if you are calling early mornings), the site may follow up with ndd Medical Technologies support team for assistance. All oscillometry and PFT data transfer issues and questions should be directed to Dr. Brett Dolezal. The SOURCE GIC (source@unc.edu) and Dr. Brett Dolezal should be copied on all emails regarding PFT issues and questions.

UCLA PFT Reading Center – spirometry, oscillometry, and data transfer	Brett A. Dolezal, PhD bdolezal@mednet.ucla.edu (310) 741-8954
ndd Medical Technologies – only for Easy on-PC technical support	Michelle Dulany mdulany@nddmed.com +1 (978) 470-0923, ext. 117

14 BRONCHOSCOPY SUB-STUDY SPIROMETRY & OSCILLOMETRY PROCESSES

Spirometry and Oscillometry will be collected in the Bronchoscopy Sub-study; a separate Spirometry Data Form (SDF) and Oscillometry Data Form (OSC) should be entered using the Subject ID, respectively. The SDF will be present in the following form groups in addition to the main SOURCE visits: Pre-Bronchoscopy Visit 1 and Bronchoscopy Visit 2. The OSC will be present in the following form group in addition to the main SOURCE visits: Pre-Bronchoscopy Visit 1.

The Bronchoscopy Sub-study Label ID Form (BID) will be present in the Pre-Bronchoscopy Visit 1 and Bronchoscopy Visit 2 form groups.

When entering participant information in the EasyOne Connect software, please note the additional instructions in **SOURCE PFT RC Memo 9.5** (Appendix B) regarding the visit number and Bronchoscopy Sub-study ID (e.g., BRNxxxxxx). Visit # should be entered as B1 and B2 instead of V1 and V3 in the "Last Name" box, whereas the BRN ID should be entered in the "First Name" box. It is imperative that the letters are capitalized when entering.

Please note that when sending sub-study spirometry data via Box to the UCLA PFT Reading Center, the Bronchoscopy Sub-study ID (e.g., BRNxxxxxx) and Visit # should be used to save the .sqlite file to your desktop. The file name automatically saves to ID#_last name_first name_date of birth. Please rename the .sqlite file so that it looks like this: BRN123456_B#.sqlite, where 'BRN' is the bronchoscopy sub-study code used in this study for all applicable sites followed by the unique 6 digits (e.g., 123456) for that study participant. 'B#' applies to either the Pre-Bronchoscopy Visit 1 (i.e., 'B1') or the Bronchoscopy Visit 2 (i.e., 'B2'). See **SOURCE PFT RC Memo 9.5** (Appendix B). This Subject ID instruction also applies when sending the sub-study oscillometry data when exported as a PDF.

15 APPENDICES

A. Spirometry & Oscillometry data transfer to UCLA PFT Reading Center
B. ndd Easy on-PC Spirometer Setup, Configuration, Training & Certification, and Testing – refer to **SOURCE PFT RC Memo 9.5**
C. Updated Resmon Pro Oscillometry MOP - refer to **SOURCE PFT RC Memo 10**
D. NEWEST ndd Easy on-PC Spirometer Filter and Software Update – refer to **SOURCE PFT RC Memo 12**

16 REFERENCES

1. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, et al. (2005) Standardization of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 26: 720-735.
2. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, et al. (2005) General considerations for lung function testing. Eur Respir J 26: 153-161.
3. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. (2005) Standardization of spirometry. Eur Respir J 26: 319-338.
4. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, et al. (2005) Interpretative strategies for lung function tests. Eur Respir J 26: 948-968.
5. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, et al. (2005) Standardization of the measurement of lung volumes. Eur Respir J 26: 511-522.
6. (2007) Chronic Obstructive Pulmonary Disease: Developing Drugs for Treatment. In: (CDER) CfDEaR, editor. Draft Guidance ed. Rockville, MD: U.S. Department of Health and Human Services, Food and Drug Administration. pp. 14.
7. Enright PL, Beck KC, Sherrill DL (2004) Repeatability of spirometry in 18,000 adult patients. Am J Respir Crit Care Med 169: 235-238.
8. Wise RA, Connett J, Kurnow K, Grill J, Johnson L, et al. (1995) Selection of spirometric measurements in a clinical trial, the Lung Health Study. Am J Respir Crit Care Med 151: 675-681.
9. Donohue JF (2004) Minimal Clinically Important Differences in COPD Lung Function. COPD: Journal of Chronic Obstructive Pulmonary Disease 2: 111 - 124.
10. Hankinson JL, Odencrantz JR, Fedan KB (1999) Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med 159: 179-187.
11. (2007 Dec) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO workshop summary, updated 2007 London: Global Initiative for Chronic Obstructive Lung Disease (GOLD).
12. Gardner RM, Clausen JL, Epler G, Hankinson JL, Permutt S, et al. (1986) Pulmonary function laboratory personnel qualifications. Am Rev Respir Dis 134: 623-624.
13. Tablan OC, Anderson LJ, Besser R, Bridges C, Hajjeh R (2004) Guidelines for preventing health-care--associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. MMWR Recomm Rep 53: 1-36.
14. Nelson WB (2003) Recurrent Events Data Analysis for Product Repairs, Disease Recurrences and Other Applications. Schenectady, NY: American Statistical Association and the Society for Industrial and Applied Mathematics. 151 p.
15. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, et al. (2008) Reference ranges for spirometry across all ages: a new approach. Am J Respir Crit Care Med 177: 253-260.
16. Oren J, Tashkin DP, Gross NJ, Skorodin MS, Sambol N, et al. (1998) A pharmacodynamic dose-response study of ipratropium bromide in chronic obstructive pulmonary disease. Am J Respir and Crit Care Med 157: A802.
17. Kleerup EC, Tashkin DP, Cline AC, Ekholm BP (1996) Cumulative dose-response study of non-CFC propellant HFA 134a salbutamol sulfate metered-dose inhaler in patients with asthma. Chest 109: 702-707.

SOURCE
SPIROMICS Study of Early COPD Progression

MOP 3

CT IMAGING

Version 4.4

August 9, 2024

1 PURPOSE

Noninvasive measures (i.e., imaging) of airway structure and remodeling (e.g., thickened airway walls) as well as air trapping and other parameters of peripheral airway and parenchymal pathologies will allow us to longitudinally monitor the effects of various stimuli and treatments.

The University of Iowa (Eric A. Hoffman PhD, PI) will serve as the Radiology Center to provide standardization and harmonization of all CT imaging protocols performed in SOURCE. In addition, this center will provide initial quantitative CT analysis of all images following this protocol and provide this data to the SOURCE DCC. The Radiology Center will govern all imaging for SOURCE, review quality performance of imaging sites, and report to the SOURCE Steering Committee its recommendations. The Chair of the Radiology Committee (Eric Hoffman) will report to the SOURCE Steering Committee at regular scheduled meetings.

2 INTRODUCTION

Baseline and Follow-up scans: The CT scanning protocol for SOURCE consists of obtaining multi-detector CT (MDCT) images of the entire lung at full inspiration (TLC) and at Functional Residual Volume (RV). TLC & RV scans will be performed at baseline and three-year follow-up visit. At the visit subsequent to the baseline visit, participants will be scanned on the same scanner used at baseline (unless that scanner is no longer available at the site) with the same CT imaging and reconstruction parameters, such as the same kV and dose modulation settings, the same reconstruction kernel, and the same iterative reconstruction version and settings as well as the same diameter field of view (DFOV) used during the baseline CT visit. Follow-up visit should match the same DFOV used for the TLC scan at baseline (which should have matched the RV DFOV at baseline). In the case of inconsistent DFOV at baseline, the follow-up will match the baseline DFOV for each respective lung volume.

If the original scanner is no longer available at the site or becomes permanently unavailable during the study, the Radiology Center will work with the site to determine the best scanner to use along with an appropriate protocol.

Breathing instructions are required to obtain appropriate images at full inspiration (TLC) and full expiration (RV). To minimize dose, SOURCE has adopted a scanning protocol which takes advantage of dose modulation and iterative reconstruction at those sites with appropriate scanner models and follows a protocol initially established for the NIH supported PrecISE asthma study. The images from the MDCT chest will be stored locally and will be sent to the Imaging Core (University of Iowa) via DISPATCH software (University of Iowa) which securely transmits anonymized image data stored in a standardized format known as DICOM (Digital Imaging and Communications in Medicine) for protocol compliance verification.

3 INCLUSION/EXCLUSION

All participants meeting primary inclusion and exclusion criteria for entrance into SOURCE will have a CT scan.

A form will be completed for each participant to track any known changes/surgeries to the lungs

since the last scan. Those events include but are not limited to:

1. Pneumonectomy
2. Lobectomy
3. Wedge Resection
4. Lung Volume Reduction Surgery (LVRS)
5. Pleural Surgery
6. Indwelling Pleural Catheter (in place at time of visit)
7. Lung Transplant

Participants with known lung changes/surgeries will still undergo a CT scan to be used for ancillary study analysis. The data gathered will be flagged as to keep it out of the core CT dataset.

Participants at the baseline visit should NOT be scanned if they have metal implants anywhere within the chest scan range. This includes, but is not limited to, shoulder prostheses, spinal implants, pacemakers, surgical clips, etc. However, if metal is implanted in between the baseline visit and three-year follow-up visit, it is okay to scan with metal implants at follow-up.

Participants at the baseline visit should NOT be scanned if they cannot bring their arms above their head. For the three-year follow-up visit, if the technologist has tried everything possible to get the arms up enough to fit (**the chest must stay iso-centered!),** they can scan with the arms straight down at their sides. This must be noted in the comments section of the PVS form.

4 CT SCANNING PARAMETERS

Radiaton Exposure Specification: Scanners of different makes and models deliver differing number of photons to the participants for a given mAs setting because of differences in beam filtration, variances in tube potential, etc. Furthermore, each manufacturer implements dose modulation differently, and by definition, dose modulation serves to deliver radiation dose optimal to the individual participant. Thus, the SOURCE imaging protocol includes the requirement for documenting the scanner reported CTDIvol located on the scanner console to determine an individual participant's radiation exposure.

BMI-Based Sites (only one site without a scanner supporting the dose modulation and iterative reconstruction methods needed to transition to the lower dose protocol: GE LightSpeed VCT)

BMI and scan type will determine the CTDIvol and subsequently the effective mAs or mA setting. Five levels of CTDIvol are recommended as a function of 1) large, medium, or small BMI and 2) scan type (i.e., TLC or RV). This mechanism allows for optimal doses to the various size participants. The BMI values will be entered into PVS (University of Iowa, see section 6) during registration by the study coordinator. PVS will generate a protocol form based on stored baseline parameters and corresponding dose protocol for the given BMI size. This form will then be printed by the coordinator and provided to the CT Technologist prior to scanning.

Scout scans will be obtained to assure that the full lung will be imaged and at the same time to assure minimization of the participant's exposure to radiation. The scout scans must be performed

at the lung volume for which anatomical boundaries are being evaluated. Each study site should perform scouts as deemed locally appropriate to specify a TLC and RV scan such that when spirally scanned, the full extent of the lung is acquired at the respective lung volumes and over-scanning is kept to a minimum (no more than 3 cm cephalad to the apical or 6 cm caudal to the basal lung borders). Inadequate scout scans may be repeated as per site-specific determination (Spiral scans, however, may *NOT* be repeated). The exposure factors (kV and mA) for the topogram should be set to the lowest available on the CT scanner that provides an acceptable image. For GE scanners, 80 kv and 20 mA is sufficient to achieve satisfactory scout image quality.

The specific CT scanning parameters are provided upon the patient being registered into SOURCE via the Procedure and Verification (PVS) software discussed in section 7 below. The scanner settings utilized in SOURCE should be maintained consistent throughout the duration of the study. The image data will be reconstructed with the reconstruction algorithms as standardized for each scanner make and model. The data shall be displayed using a display field of view (DFOV) that includes the lungs and approximately 2 cm or less chest wall in the right/left dimension so that resolution of the lung is maximum. The Standard reconstruction algorithm (Q30/Qr40, Standard) data is all that will be used for further computer analysis. An additional reconstruction of the TLC scan can be added to meet the specific preferences of the site radiologist for a visual read if the study specified reconstructions do not satisfy the site radiologist's preference. This reconstruction will not be sent through DISPATCH but must be available to the radiologist and can be modified based on the site radiologist's preference.

Dose-Modulated Sites (Siemens: SOMATOM Definition AS+, Definition Flash and SOMATOM Force; GE: Revolution CT, Discovery 750 HD, Revolution GSI & Revolution HD)

Sites using a dose-modulated scanner will utilize an automated exposure control system (CARE Dose4D, automA/smartmA) which adapts the dose to compensate for differing densities within the scan range (shoulders, breasts, upper abdomen) and individual body builds while maintaining a specified image quality. Due to advancements in scanner technology and with the use of iterative reconstructions (ADMIRE, ASiR-V), we are able to use a very low-dose reference protocol: ~1.49-2.36 mSv scans for an average (75 kg/ 25 BMI) size adult using dose modulation to harmonize image noise across the study population.

A lateral topogram will be obtained for each participant and performed at the lung volume for which anatomical boundaries are being evaluated to minimize the participant's exposure to radiation. **One AP topogram will be performed at the beginning of the exam to provide additional information for the dose modulation algorithm**. Because dose modulation is being used, it is very important that the participant is at isocenter and does not move between the topogram and actual scan. If the participant's position needs to be adjusted at all, a new topogram must be taken. Each lateral topogram should be performed to specify a TLC and RV scan such that when spirally scanned, the full extent of the lung is acquired at the respective lung volumes and over-scanning is kept to a minimum (no more than 3 cm cephalad to the apical or 6 cm caudal to the basal lung borders). Inadequate topograms may be repeated as per site-specific determination (Spiral scans, however, may *NOT* be repeated). The exposure factors (kV and mAs) for the topogram should be set to the manufacturer's default topogram parameters.

The scanner settings utilized in SOURCE should be maintained consistent throughout the duration of the grant. The image data will be reconstructed with the reconstruction algorithms as outlined. The data shall be displayed using a consistent display field of view (DFOV) between the two scans for the baseline visit, and the baseline DFOV values should be used at the three-year follow-up visit as well.

5 BREATH HOLD TECHNIQUE FOR SCANNING PROCEDURE

CT scanning should take place less than 165 minutes after albuterol administration and less than 300 minutes after ipratropium administration or else bronchodilator redosing should occur as indicated in *MOP 2 – Pulmonary Function Testing*.

In order to obtain appropriate image data, unique breathing instructions are required. These instructions are found on the *CT Tech Form*. Before the scans are acquired, the coordinator (or trained CT technologist) will review the breathing instructions with the participant and emphasize the importance of following them as closely as possible during the actual imaging of the lungs. In summary, the participant will be instructed to inhale deeply and exhale two times and then hold their breath one of two different ways: with the lungs full of air (total lung capacity: TLC) and at the end of a full expiratory effort (residual volume: RV).

In order to extract the desired information from the CT images, it is *very important* that the breathing instructions are followed closely to ensure the participant is not arching back, to ensure the participant is breathing all the way in and out, and to make sure the participant reaches peak breath hold (TLC or RV) before imaging. An example of the breathing instructions can be found the Appendix.

6 PROPER IDENTIFICATION OF THE SCAN RECONSTRUCTIONS

Scan reconstruction identifiers (this is information the techs can add to each reconstruction) should specify the lung volume (Inspiration (TLC) / Expiration (RV)), slice thickness, and reconstruction kernel.

Here is an example of the labeling scheme for each of the SOURCE reconstructions on a SIEMENS scanner:

- Inspiration 0.75 Qr40_5
- Expiration 0.75 Qr40_5

7 PVS AND IMAGING FORMS

An automated web portal system (Procedural Verification Software or PVS) is used by the Radiology Center to track and verify the data. It provides mechanism for obtaining information for SOURCE scans including violations and scan parameters as well as the appropriate dose and baseline DFOV information (for three-year follow-up) for a given participant's visit. PVS users at each site will register the participants for their scans using PVS, which will in turn generate a CT Image Acquisition Form (hereinafter referred to as "PVS Form") to be used for that participant's scan. This form provides the site specific scanner settings based upon the scanner make and model

qualified for scanning in SOURCE. For dose-modulated sites, the PVS form also serves as an exposure guide to ensure the consented radiation dose is never exceeded. The coordinator will enter in the values used for the scan after the acquisition is complete and submit the form for review at the Radiology Center. Additionally, the coordinator will enter the CTA (CT Image Acquisition Form) with the date of the CT scan in the Electronic Data Management System maintained by the GIC for data matching purposes with the PVS entered data.

The *PVS Form* is completed by the technologist before and after each participant is scanned. When the CT technologist completes and signs the form legibly with their full name and no abbreviations, the technologist is taking responsibility for carrying out the scanning as specified by this form, as approved by the IRB, and as agreed upon in the consent form. The *PVS Form* will be generated via SOURCE PVS website and printed by the coordinator prior to the scan.

The *CT Tech Form* will help ensure the CT technologists have the proper information to complete the CT scans in accordance with the protocol. Each site will maintain a copy of this reference sheet so that it is readily available at the scanner. The reference sheet details proper breathing instructions and scan parameters for the sites specific scanners. Forms will be provided by the Radiology Center at the beginning of the study and can be requested at any time.

8 DATA ANALYSIS

Recorded information from the PVS Form should be entered into PVS and submitted within two business days of the scan date to catch any dosing errors in a timely manner. The CT image data must be transmitted to the Radiology Center within five working days of performance of the CT via the provided DISPATCH software. Raw data should be corrected to remove PHI, replacing any identifiers with the correct SOURCE ID *(NOTE: Do not use the "anonymize" feature on the scanner as it strips important DICOM tags)* and exported to an external hard drive. Raw data will be kept at the site and available so that image data can be corrected in the event a reconstruction-based error is detected *(NOTE: If the site is unable to keep the anonymized raw data on an external hard drive, then it is up to the radiology department to ensure the raw data is protected until the Radiology Center confirms data has been processed correctly).*

At the Radiology Center (University of Iowa), CT scans will be analyzed using automated, quantitative airway evaluation software designed to reconstruct 3-D lungs, lobes, and airway trees from MDCT images (VIDA|vision, VIDA Diagnostics, Coralville, Iowa). Analysis will provide airway and parenchymal-based metrics which will be transmitted from the Radiology Core to the data center.

Using existing techniques, the lungs will be segmented to identify left and right lungs along with their associated lobes. Air and tissue volumes will be reported for the whole lung, right and left lungs, and for each individual lobe. Local statistical measures of lung parenchymal attenuation values will be computed for each lobe and sublobar region. Parenchymal measures from the TLC scans will include voxels below -950, -910, and -856, density histogram-based means, modes, skewness, kurtosis as well as measures of air and non-air volumes for the whole lung, left and right lung, and lobes. The airway tree will be segmented to include five primary paths (RB1, RB4, RB10, LB1, LB10). The VIDA|vision software automatically labels airway segments according to

standard bronchoscopic terminology. Airway segment maximum and minimum diameters will be reported for the middle third of each segment (avoiding the branch point saddles); segment lengths will be included which permit the calculation of segment luminal volumes. Airway wall thickness will be reported for the middle third of each segment. Airway wall thickness will be normalized to the lumen plus wall area to provide a wall area percent measure for each found segment, and a Pi10 measure will be reported for each of the above named five paths. This measure is derived from a plot of the inner perimeter (x-axis) vs Square Root of the airway wall area (x-axis), identifying a regression line and identifying the modeled airway wall area associated with an inner perimeter of 10 mm using the relationship defined by the regression. Air trapping on the RV scans will be defined as the percent voxels (on a whole lung, left and right lung, and lobar basis) falling below -856 HU. Airways will not be assessed on the RV scans. Other metrics will include a measure of functional small airways disease (fSAD) along with measures of regional lung mechanics derived from VIDA's Disease Probability Measure (DPM) which derives it's information via the matching of TLC to RV lung anatomy. Additionaly, an assessment of the central airway branching pattern will be provided, including an index of airway dysanapsis. A full set of metrics are provided in the online supplement to *Sieren JP; Am J Respir Crit Care Med. 2016 Oct 1;194(7):794-806.*

9 CERTIFICATION OF IMAGING TECHNOLOGISTS

It is a strong reccomendation that the technologist be a CT certified radiologic technologist, and they must have specific training and certification detailed to the SOURCE MOP. The certification regarding this study can come from the Radiology Center or from a SOURCE site-certified technologist who will report new certifications to the Radiology Center. An online PowerPoint based training tutorial will be provided by the Radiology Center. A test will be administered at the end of the online tutorial. Technologist privileges with respect to SOURCE CT scanning can be revoked as deemed necessary by a consensus opinion of the Radiology Center, Site Radiologist, Site PI, and Study PI.

10 QUALITY CONTROL

The site study coordinator will take responsibility to provide the radiology technologist with participant specific SOURCE protocol upon delivery of the participant to the CT suite and will remain in the CT control room during scanning to assure that appropriate breath hold instructions are being given. Because of the reliance of the study coordinator to the on-site "eyes and ears" for SOURCE at the time of participant scanning, the study coordinator should be familiar with the radiology protocol requirements.

The CT scanner needs to be operated according to the recommendations of the manufacturer; including the use of weekly water phantoms and daily air calibrations. The SOURCE CT protocol needs to be pre-loaded into the protocol list of the designated CT scanner with each study acquired using this preset protocol.

<u>All CT scanners used in SOURCE must be certified with a phantom (COPDGene Phantom) scan.</u> This phantom scan will provide checks for protocol adherence and proper calibration of the CT scanner. Each site will acquire a COPDGene phantom for the duration of the study. The initial

phantom scan from any new CT scanner must be sent to the University of Iowa via DISPATCH for scanner approval prior to scanning human participants. In order to establish a baseline threshold for the given scanner, we will have the site scan the phantom 3 times in a row during the first month. After the first month, the site will only need to scan the phantom once a month. Phantom scans not meeting the scanning protocol defined by this MOP will be automatically rejected by DISPATCH with an explanation. The explantion will provide feedback regarding the nature of the failure and how to correct for it. If it is a post processing issue such as the wrong DFOV, slice thickness or slice spacing, DISPATCH will inform what parameter failed and the Radiology Reading Center will include how to fix it without a rescan. If the problem is related to scan acqusition, i.e. wrong mA/mAs, kV, pitch, or exposure time, the phantom must be re-scanned and DISPATCH will explain the parameter that failed as well as provide the correct parameter to use. Scanners may also be rejected if the scanner does not meet calibration criteria. Once the scanner is approved, monthly scans must be completed for quality assurance purposes and transmitted via DISPATCH. These ongoing phantom scans should be continued through the course of the studies. If phantom scans show a scanner to deviate from the approved baseline measures, decisions regarding continuation of scanning will be made through a consultation between the Radiology Center, site Radiologist and Site PI. Questions or concerns regarding scanning of the phantom may be referred to the University of Iowa.

11 DATA AND SAFETY MONITORING PLAN

All SOURCE sites agree to perform research CT imaging on participants using the approved SOURCE Imaging Manual of Procedures.

All SOURCE CT datasets and CT Image Acquistion Forms (CTA) from participants and phantoms will be transferred to the University of Iowa Radiology Center within five working days of its performance to ensure protocol compliance. A quarterly compliance report will be generated and sent by the Radiology Center to the DCC and each site's PI and lead coordinator. Participant specific deviations will also be sent as outlined below. Protocol deviations will be classified as Type A, Type B, or Type C.

Type A – Dose related deviations: Reported as detected.

- Excessive current or voltage setting resulting in radiation exposure greater than approved limits.
- Repeating either lung volume scan resulting in radiation exposure greater than approved limits.

Type B – Scan specific deviations: Documented as detected; reported to site monthly. Site will be contacted immediately if the same deviation occurs three or more times within a week's time.

- Incorrect current or voltage setting that does not result in non-approved radiation exposure.
- Whole lung not scanned (excessive clipping – up to the Radiology Center's discretion).
- Excessive scan length (scan length exceeds 3 cm above apices or 6 cm past base of lung).
- CT Technologist not certified.

Type C – Data related deviations: Documented as detected; reported if determined unfixable or unfixed after three weeks of detection.

- PVS form completed incorrectly or not completed.
- Incorrect reconstruction kernel used.
- Incorrect slice thickness or spacing.
- CT data lost.
- CT data not anonymized.

If an event occurs that is not listed above, the Radiology Center will use their discretion to determine the type of deviation. Any additions will be addressed during the following SOURCE Steering Committee meeting.

Any Type A deviation detected by the University of Iowa, in which radiation exposure exceeds 10% of the approved range, will result in suspension of CT scanning at that site until the cause of the deviation is reported and the SOURCE Steering Committee and Radiology Center have approved the remediation plan.

Type B and C deviations from the SOURCE Imaging Manual of Procedures detected by the University of Iowa will be reported to the SOURCE Steering Committee if there are more than 10% occurrences at a particular site within the previous 12 months.

All SOURCE sites agree to report any protocol deviation or violation, consent violation, or adverse event that results in suspension of CT imaging to the SOURCE Steering Committee and to the SOURCE DCC. The SOURCE DCC will relay the report to NHLBI. In addition, SOURCE sites will follow individual institutional IRB policies and procedures.

12 DATA TRANSFER & STORAGE

DISPATCH (DICOM Selection Parser and Transfer Check, University of Iowa) is an automated system that imports medical image data that has been stored in a standardized file format known as DICOM (Digital Imaging and Communications in Medicine) and provides a mechanism of transmitting that data elsewhere via the internet in a secure manner. At the same time, it allows for the automated and customized interrogation of the header record of the image data to ensure the data has been gathered with the appropriate parameter ranges specified by the protocol.

DISPATCH runs on the transferring site's local machine and uses a web-based application to transfer imaging data. The input of study-specific criteria parses the data and restricts the upload of files which do not fit the protocol, nor will it allow uploads of duplicate data; thus, making the process more efficient on the part of the sender and the receiver. It is important that the DISPATCH user (i.e., study coordinator) only send the scan data needed for the study; otherwise, the system will parse unnecessary data which will not pass the study-specific criteria previously determined. Study-specific scan reconstructions can be referenced above in section 5.

DISPATCH allows the user to ensure data was acquired according to a specific study protocol and

check for errors prior to data transmission.

There are two training methods on the use of DISPATCH. The traditional method is a user manual which outlines the installation and set up as well as the process of using the tool. The second training tool is an online PowerPoint based tutorial which describes DISPATCH, how to use it, and demonstrates its use. The Radiology Center will oversee the setup, training, and maintenance of DISPATCH for the SOURCE imaging sites.

Medical Image File Archive and Retrieval (MIFAR, University of Iowa) is a web-based system that allows for secure storage of SOURCE data. MIFAR currently holds over 100,000 volumetric lung image data sets providing storage and retrieval of research records together with image data. MIFAR consists of a distributed set of software components that communicate with each other over a TCP/IP network. This system allows inter-site data exchange and enables security and quality control.

All SOURCE reconstructions must be archived and stored at the site. Additionally, raw data must be kept at the site or at least protected on the scanner until the Radiology Center confirms data has been processed correctly.

Authorized Use Permission of DISPATCH

Use of the DISPATCH is allowed for SOURCE purposes only. Unauthorized usage of the system and making unauthorized copies of data, software, reports, and documents is strictly prohibited. If you wish to obtain waiver use or copy permissions, please contact the University of Iowa, Division of Physiologic Imaging at 319-356-1381.

13 PLAN FOR DE-IDENTIFICATION OF DICOM IMAGE DATA

This plan has 4 parts:

- **PART 1:** Study sites will de-identify DICOM CT images prior to sending the images to the University of Iowa Imaging Center as per above.
- **PART 2:** The Radiology Reading Center will keep the original DICOM files on a secure University of Iowa file server with a back-up copy on a second secure University of Iowa file server. Access to these original image datasets will be limited to a small number of staff members at the University of Iowa Radiology Reading Center tasked with image data management for this study. Starting now, all incoming images will be scrubbed of PHI in the same way and stored in this same secure server environment. A copy of the data in this original format is kept in case information stored in the DICOM private tags, that is machine related, is of importance to a particular research question.
- **PART 3** is to scrub or remove all DICOM file private tags and keep only a finite set of DICOM file public tags with known non-PHI needed for image analysis and appropriate

linking of images to other data sets. The participant name field will house the unique study identifier. We will keep the study date in order to be able to distinguish between scans from a participant on different days or time points. Other tags that will be retained include details of the image reconstruction parameters, tube current, kV, and other scan parameter details. This copy, dubbed "mini-DICOM-1," will be sent to investigators needing continued access to the image datasets. The existing Data and Materials Distribution Agreement (DMDA) already states that they cannot redistribute these image datasets outside of their immediate research personnel at their institution.

- **PART 4** of the plan is to create a "mini-DICOM-2" file when non-study centers have been approved by the GIC to receive image data. Here, the Radiology Center will replace the study visit dates with a code identifying study visit number and time point. As needed, the study number with a unique identifier assigned to each participant, matching the identifier used in the modified core data set provided to the outside recipient.

14 APPENDIX

Breathing Instructions

Use the breathing instructions to perform:

- A practice breathing session
- Scouts - as needed - to position the FOV to cover the entire lung and as little soft tissue as possible
- The Inspiration CT scan (TLC)

Inspiratory CT (TLC)

BREATHING INSTRUCTIONS:

"For this scan I am going to ask you to take a couple of deep breaths in and out before we have you breathe all the way in and hold your breath.

Ok, let's get started
Take a deep breath in *(watch chest to ensure a deep breath in)*
Let it out *(watch chest to ensure air is out)*
Take a deep breath in *(watch chest to ensure a deep breath in)*
Let it out *(watch chest to ensure air is out)*
Now breathe all the way IN... IN... IN and HOLD IT IN *(watch chest to ensure a deep breath in as far as possible)*
Keep holding your breath – DO NOT BREATHE
At end of scan or practice - **Breathe and relax**."

Use the breathing instructions to perform:

- A practice breathing session
- Scouts - as needed - to position the FOV to cover the entire lung and as little soft tissue as possible
- The Expiration CT scan (RV)

Expiratory CT (RV)

BREATHING INSTRUCTIONS:

Designate and practice hand signal before beginning.

"For this last scan, I am going to ask you to take a couple of deep breaths in and out before letting your breath all the way out. You will hold your breath out when you have no more air left and quickly signal to me.

Now we're ready again so please,

Take a deep breath in *(watch chest to ensure a deep breath in)*

Let it out *(watch chest to ensure air is out)*

Take a deep breath in *(watch chest to ensure a deep breath in)*

Let it out *(watch chest to ensure air is out)*

Take another deep breath in *(watch chest to ensure a deep breath in)*

Now let it all the way OUT... OUT... OUT... as much as possible, and signal to me when you have no more air left and HOLD IT OUT *(watch chest to ensure all air is out and for hand signal before starting the scan)*

Keep holding your breath – DO NOT BREATHE

At end of scan or practice - **Breathe and relax**."

SOURCE
SPIROMICS Study of Early COPD Progression

MOP 4

BIOSPECIMEN COLLECTION AND PROCESSING

Version 4.4
October 13, 2023

1 BIOSPECIMEN COLLECTION AND PROCESSING: BLOOD, URINE, NASAL SWAB, AND EXHALED BREATH CONDENSATE

The study entitled "SOURCE – SPIROMICS Study of Early COPD Progression" supports the prospective collection and analysis of phenotypic, biomarker, genetic, genomic, and clinical data from an observational cohort of participants with or at risk of COPD for the purpose of identifying subpopulations and intermediate outcome measures. It is funded by the National Heart, Lung, and Blood Institute (NHLBI) and the COPD Foundation and is coordinated by the University of North Carolina at Chapel Hill.

Research participants for SOURCE will be enrolled, phenotyped, and followed at 12 clinical centers. Molecular fingerprinting and extensive participant phenotyping will be performed to identify disease subpopulations and to identify and validate surrogate markers of disease severity that will be useful as intermediate outcome measures for future clinical trials. Secondary aims are to clarify the natural history of COPD, to develop bioinformatic resources that will enable the utilization and sharing of data in studies of COPD and related diseases, and to create a collection of clinical, biomarker, radiographic, and genetic data that can be used by external investigators for other studies of COPD.

Samples of blood, urine, nasal swab, induced sputum, and exhaled breath condensate (EBC) will be collected at enrollment from all SOURCE participants. Aliquots of serum, plasma, and cells from peripheral blood and urine, sputum, and EBC will be prepared at the field centers and shipped to the Alexis Biospecimen Repository, where it will be stored. The Alexis Biospecimen Repository is located at the University of North Carolina at Chapel Hill. The Alexis Biospecimen Repository at the University of North Carolina at Chapel Hill will set-up, operate, and maintain a repository/biobank of biospecimens collected from SOURCE participants. The purpose of this particular repository/biobank is to store samples for future research.

NOTE: This MOP specifically covers the acquisition of serum, plasma, and cells from peripheral blood, as well as urine, EBC, and nasal swab. A complete list of the aliquots to be prepared for sputum samples is located in ***Box 2 of MOP 5 – Sputum Induction and Processing****, which is provided separately.*

Investigators will, in the future, conduct a wide variety of analyses on the biospecimens banked in the Alexis Biospecimen Repository. Some of the analyses will include genetic and genomic analyses to look for relationships between genes, between genes and non-genetic factors (e.g., environment, behaviors), and between genes and other diseases. Other kinds of analyses will include studying the concentrations and mixture of proteins, cells, and RNA found in the various biospecimens.

The most important step in maintaining a successful repository and often the most difficult to standardize is the collection and clinical center processing of the biological samples. If the blood, urine, nasal swab, induced sputum, or EBC samples are not collected or processed correctly, laboratory results may not accurately reflect true differences between study participants, even if the laboratory tests are performed accurately. Standardized, quality sample collection and processing are critical to maintaining baseline reproducibility. For this or

any study to succeed, biospecimens must be collected, processed, and stored in a standardized, safe, and appropriate manner at all clinical sites, as well as in the repository. Thus, it is important that all staff be well-trained, certified, and fully compliant with the protocol for drawing and processing the biospecimens.

2 PREPARATION FOR BIOSPECIMEN COLLECTION

Because participation in this study is voluntary and as it asks a lot of each participant, all SOURCE staff should make a concerted effort to make the entire visit as easy and comfortable as possible. As always, the phlebotomists should be calm and confident, particularly when the participant is uneasy or nervous. One way to accomplish this goal is to be thoroughly knowledgeable about all aspects of the procedures to be conducted.

In total, the SOURCE study will collect approximately 58-76 mL of blood in eight tubes from each participant. The phlebotomists should reinforce the idea that the volume of blood being collected is only about 4-5 tablespoons, even if it looks like a lot more, and they should add that this is only about a 10^{th} of the amount of blood that is drawn when a pint of blood is donated.

2.1 Staff Certification Requirements

Blood drawing and processing will be performed by a trained phlebotomist/blood draw technician that has been certified on the SOURCE MOP. The technicians will complete a training course taught by either SOURCE staff or SOURCE trained clinical lab certified staff. Each technician must complete the training and must pass a written exam before becoming SOURCE certified. Recertification should take place annually and will be authorized by clinical center supervisory personnel. Based on individual job duties, biospecimen certification may be necessary in one or all the following areas:

- Blood drawing and urine collection
- Blood and urine processing and preparation of aliquots
- Packaging and shipping of samples

2.2 Blood and Urine Collection Trays and Tubes

One day before a scheduled participant visit, the clinical center processing technician will prepare one tray to hold the urine and blood collection supplies and the blood collection tubes. In addition, six racks will be prepared to hold intermediate products and final aliquots as outlined in section 2.2.4. Before the visit, all urine cups, blood vacutainer tubes, processing tubes, and final product aliquot tubes should be tagged with labels provided by the Genomics and Informatics Coordinating Center (GIC) containing the appropriate code numbers for the participant. Label rolls will include the following:

- 9 primary labels with Laboratory ID (Lab ID) and specimen type for the eight blood tubes and the single urine specimen cup.
    - Note that the 3 mL EDTA tube for on-site clinical lab processing may need additional labeling according to local lab requirements.

- 14 labels with Lab ID, visit, specimen type, and aliquot number for each blood tube processed. These secondary labels are for the aliquots of serum or plasma from blood tubes #1-4 and #6.

- 1 label with Lab ID, visit, and specimen type for the preserved urine conical tube.

- 20 labels with Lab ID, visit, specimen type, and aliquot number for urine specimen aliquots.

- 25 labels with Lab ID, visit, specimen type, and aliquot number for the sputum collection vessels and processed specimen aliquots. Note that if sputum is processed in a separate location from the blood processing lab, use two generic labels for the -80°C sputum aliquot box until these samples are transferred to the main lab for inclusion in box 2 of 2 for this participant. For aliquot numbering scheme, refer to *MOP 5 – Sputum Induction and Processing*.

- 5 labels with Lab ID, visit, specimen type, and aliquot number for EBC collection, including one for the collection tube and up to four aliquots. For aliquot numbering scheme, refer to Appendix 9.

- 2 labels with Lab ID for the top and bottom of the 4°C mucin aliquot sputum storage box.

- 4 labels with Lab ID, visit, and box numbers for the tops and bottoms of the two aliquot-containing -80°C storage boxes. These are labeled "Box 01 of 02" and "Box 02 of 02".

- 2 labels with Lab ID for the top and bottom of the 3x3 sputolysin-containing -80°C storage box.

- 4 labels with Lab ID for the tops and bottoms of the PAXgene DNA and RNA blood tube boxes. These are labeled "PXGR – StorDNA" and "PXGR – StorRNA." Sites will need to add the appropriate box numbers.

- 20 generic labels with Lab ID and visit number to be used at the discretion of the technician for items such as blood sample redraw, dropped aliquot tube, etc. These labels should be appropriately hand-labeled with specimen type, aliquot number, etc.

- Labels for blood collection and tube processing racks are not included. Sites are free to use generic labels for this purpose.

2.2.1 Blood and urine collection tray

Prior to the visit, the technician organizes and prepares the blood collection tray. The blood collection tray should be made of materials that can be easily cleaned. The tray should have an appropriate amount of space to hold the following supplies:

- urine specimen collection cup
- one-step pregnancy test
- rack that holds at least eight blood collection tubes (exact tubes to be used are described in the next section)
- 12-inch blood collection set (mandatory for the P100 plasma collection tubes), which includes 21-gauge butterfly needle with Luer adapter and plastic tube guide
- alcohol wipes
- gauze sponges
- tourniquet
- adhesive bandages

A sharps container must be accessible for immediate needle and glass disposal. Finally, smelling salts, ice packs, and washcloths should be readily available in the blood collection area for participants who become faint during the blood collection.

2.2.2 Blood collection tubes

Technicians must be familiar with the arrangement of blood collection tubes, the order in which the tubes are to be filled (draw order matches the numbering sequence), the type of anticoagulant/preservative in each tube, and the possible sources of error in handling each tube. The tubes are organized in the test tube rack in the following sequence:

Tubes #1 and #2 are 8.5 mL red stoppered serum tubes. Although these tubes do not contain anticoagulant, they do have a clot activator and therefore require mixing immediately following collection. The serum from each of these tubes will be aliquoted into 14 aliquots as described below. Serum will be used for analysis of known biomarkers or hypothesis-driven biomarkers where it has been previously determined or reasonably speculated that serum will serve as the best source for analyte measurement.

Tubes #3 and #4 are two, 10 mL lavender stoppered tubes containing a sprayed on K_2EDTA anticoagulant. The plasma from both tubes will be used for hypothesis-driven, specifically defined, proteomic analyses.

Tube #5 is a 3 mL lavender stoppered tube containing a sprayed on K_2EDTA anticoagulant. This tube will be sent directly to the clinical lab at each clinical site for a Complete Blood Count (CBC) with differential.

Tube #6 is an 8.5 mL P100 red stoppered plasma collection tube with a mechanical separator and sprayed on K_2EDTA anticoagulant and proprietary additives. Plasma from this tube will be used for large-scale discovery proteomic analyses.

Tube #7 is a blue stoppered PAXgene DNA tube containing K_2EDTA anticoagulant and DNA stabilizers. This tube must be filled completely to standardize the blood to liquid anticoagulant ratio. A full tube is equivalent to collection of 8.5 mL of blood. This tube will be used by the Alexis Biospecimen Repository for DNA extraction for potential future epigenetic analyses.

Tube #8 is a 2.5 mL red stoppered PAXgene RNA tube containing anticoagulant and RNA

stabilizers. The PAXgene tube is the size of a 10 mL collection tube, but because of the liquid stabilizers, only 2.5 mL of blood is collected. This tube must be filled completely to standardize the blood to liquid anticoagulant ratio. Partially filled tubes will result in erroneous test results. RNA will be isolated from the white blood cells and used for gene expression studies. **Because there is a large volume of liquid in this tube, be sure to hold the tube below the participant's arm during collection. There is a risk, although extremely small, that the liquid in the tube could flow into the participant's vein if the tube is not held below the arm during collection.**

2.2.3 Blood collection tubes: Labeling and set-up

Blood collection tubes should be set up in advance of the participant visit. Determine participant ID from the site coordinator and obtain biospecimen labels with the Lab ID from GIC provided label rolls (section 2.2).

1. Because there will not be paper manifests in this study, be sure that the Lab ID for this participant is scanned into the DMS to setup the participant ID/Lab ID link. See example of this sheet in Appendix 8.

2. Apply pre-numbered barcoded SOURCE participant Lab ID labels to each blood collection tube. **Handle only one participant's tubes at a time so the chance of mislabeling is minimized.**

3. Arrange the blood collection tubes in the test tube rack in the same order in which they are to be collected. The eight tubes are to be collected in the following order:

   Tube #1: 8.5 mL red stoppered serum tube
   Tube #2: 8.5 mL red stoppered serum tube
   Tube #3: 10 mL lavender stoppered EDTA tube
   Tube #4: 10 mL lavender stoppered EDTA tube
   Tube #5: 3 mL lavender stoppered EDTA tube
   Tube #6: 8.5 mL red stoppered P100 plasma tube
   Tube #7: 8.5 mL blue stoppered PAXgene DNA tube
   Tube #8: 2.5 mL red stoppered PAXgene RNA tube

2.2.4 Processing tubes, sample aliquot rack, and storage container setup

The blood draw/biospecimen technician needs to prepare the following test tube racks (described in detail below) prior to participant arrival. Each rack will be used for a different type of aliquot or intermediate container (urine) and should have the following capacities:

- one test tube rack capable of holding nine 15 mL conical tubes/vacutainer blood tubes
- four test tube racks (suggested Fisher cat. no. 50-212-351) capable of holding at least 28 x 1.5 mL screwcap tubes. It is strongly suggested that each rack holding the aliquot tubes be, at a minimum, labeled with the source tube and, if possible, be a different color or be labeled with a different colored tape that matches the top of the source vacutainer tube.

Label racks with the Lab ID as well as with the aliquot type as shown below in the rack

diagrams.

Rack 1 will hold 28 vials for the 14 aliquots of serum from each of blood collection tubes #1 and #2 (8.5 mL red stoppered serum tubes).

Rack 2 will hold 28 vials for the aliquots of plasma from the blood collection tubes #3 and #4 (10 mL lavender stoppered EDTA tubes).

Rack 3 will hold one intermediate 15 mL conical tube for transfer of urine from the participant collection vial into a tube containing preservative. This 15 mL tube should be prepared ahead to contain 20 mg of preservative (ascorbic acid in crystalline from). Rack 4 will also hold blood collection tube #5 for transfer to the on-site clinical lab for CBC analysis and blood collection tubes #7 and #8 (PAXgene DNA and RNA, respectively) to be frozen for batch shipment to the Alexis Biospecimen Repository.

Rack 4 will hold 20 vials for the aliquots from the urine specimen cup and the intermediate urine tube containing the ascorbic acid preservative.

Rack 5 will hold 14 vials for the aliquots of plasma from blood collection tube #6 (8.5 mL red stoppered P100 plasma tube).

Box 1 of 2 will contain aliquots from aliquoting racks 1 and 2 (see section 5.8 for map).

Box 2 of 2 will contain aliquots from aliquoting racks 4 and 5, sputum aliquots (see section 5.8 for map), and EBC aliquots.

2.2.5 Organization and labeling

Label the screwcap aliquot tubes with the Lab ID and arrange in the racks as follows:

Rack 1 – Serum aliquots from 8.5 mL red stoppered Tubes #1 and #2

Tube #1 Serum aliquot 1 150 µL	Tube #1 Serum aliquot 2 150 µL	Tube #1 Serum aliquot 3 150 µL	Tube #1 Serum aliquot 4 150 µL	Tube #1 Serum aliquot 5 150 µL	Tube #1 Serum aliquot 6 150 µL	Tube #1 Serum aliquot 7 150 µL	Tube #1 Serum aliquot 8 150 µL	Tube #1 Serum aliquot 9 150 µL	Tube #1 Serum aliquot 10 150 µL
Tube #1 Serum aliquot 11 150 µL	Tube #1 Serum aliquot 12 150 µL	Tube #1 Serum aliquot 13 150 µL	Tube #1 Serum aliquot 14 remainder up to 1000 µL						

Tube #2 Serum aliquot 1 150 µL	Tube #2 Serum aliquot 2 150 µL	Tube #2 Serum aliquot 3 150 µL	Tube #2 Serum aliquot 4 150 µL	Tube #2 Serum aliquot 5 150 µL	Tube #2 Serum aliquot 6 150 µL	Tube #2 Serum aliquot 7 150 µL	Tube #2 Serum aliquot 8 150 µL	Tube #2 Serum aliquot 9 150 µL	Tube #2 Serum aliquot 10 150 µL
Tube #2 Serum aliquot 11 150 µL	Tube #2 Serum aliquot 12 150 µL	Tube #2 Serum aliquot 13 150 µL	Tube #2 Serum aliquot 14 remainder up to 1000 µL						

Rack 2 – Plasma aliquots from 10 mL lavender stoppered EDTA Tubes #3 and #4

Tube #3 Plasma aliquot 1 150 µL	Tube #3 Plasma aliquot 2 150 µL	Tube #3 Plasma aliquot 3 150 µL	Tube #3 Plasma aliquot 4 150 µL	Tube #3 Plasma aliquot 5 150 µL	Tube #3 Plasma aliquot 6 150 µL	Tube #3 Plasma aliquot 7 150 µL	Tube #3 Plasma aliquot 8 150 µL	Tube #3 Plasma aliquot 9 150 µL	Tube #3 Plasma aliquot 10 150 µL
Tube #3 Plasma aliquot 11 150 µL	Tube #3 Plasma aliquot 12 150 µL	Tube #3 Plasma aliquot 13 150 µL	Tube #3 Plasma aliquot 14 remainder up to 1000 µL						
Tube #4 Plasma aliquot 1 150 µL	Tube #4 Plasma aliquot 2 150 µL	Tube #4 Plasma aliquot 3 150 µL	Tube #4 Plasma aliquot 4 150 µL	Tube #4 Plasma aliquot 5 150 µL	Tube #4 Plasma aliquot 6 150 µL	Tube #4 Plasma aliquot 7 150 µL	Tube #4 Plasma aliquot 8 150 µL	Tube #4 Plasma aliquot 9 150 µL	Tube #4 Plasma aliquot 10 150 µL
Tube #4 Plasma aliquot 11 150 µL	Tube #4 Plasma aliquot 12 150 µL	Tube #4 Plasma aliquot 13 150 µL	Tube #4 Plasma aliquot 14 remainder up to 1000 µL						

Rack 3 – Intermediate preserved urine tube and blood tubes with alternative shipments

15 mL conical tube for preserved urine		Tube #5 3 mL EDTA for on-site clinical lab		Tube #7 PAXgene DNA		Tube #8 PAXgene RNA

Rack 4 – Urine aliquots

Urine not preserved aliquot 1 1 mL	Urine not preserved aliquot 2 1 mL	Urine not preserved aliquot 3 1 mL	Urine not preserved aliquot 4 1 mL	Urine not preserved aliquot 5 1 mL	Urine not preserved aliquot 6 1 mL	Urine not preserved aliquot 7 1 mL	Urine not preserved aliquot 8 1 mL	Urine not preserved aliquot 9 1 mL	Urine not preserved aliquot 10 1 mL
Urine preserved aliquot 1 1 mL	Urine preserved aliquot 2 1 mL	Urine preserved aliquot 3 1 mL	Urine preserved aliquot 4 1 mL	Urine preserved aliquot 5 1 mL	Urine preserved aliquot 6 1 mL	Urine preserved aliquot 7 1 mL	Urine preserved aliquot 8 1 mL	Urine preserved aliquot 9 1 mL	Urine preserved aliquot 10 1 mL

Rack 5 – Plasma aliquots from 8.5 mL red stoppered P100 Tube #6

Tube #6 P100 Plasma aliquot 1 150 µL	Tube #6 P100 Plasma aliquot 2 150 µL	Tube #6 P100 Plasma aliquot 3 150 µL	Tube #6 P100 Plasma aliquot 4 150 µL	Tube #6 P100 Plasma aliquot 5 150 µL	Tube #6 P100 Plasma aliquot 6 150 µL	Tube #6 P100 Plasma aliquot 7 150 µL	Tube #6 P100 Plasma aliquot 8 150 µL	Tube #6 P100 Plasma aliquot 9 150 µL	Tube #6 P100 Plasma aliquot 10 150 µL
Tube #6 P100 Plasma aliquot 11 150 µL	Tube #6 P100 Plasma aliquot 12 150 µL	Tube #6 P100 Plasma aliquot 13 150 µL	Tube #6 P100 Plasma aliquot 14 remainder up to 1000 µL						

2.2.6 Preparation for biospecimen collection

In the morning, before drawing blood from the participant:

1. Check to make sure the blood collection tray is properly equipped. Every item on the checklist (section 2.2.1) must be ready before proceeding (see Appendix 7 for checklist).

2. Check that the urine collection vial and that each vacutainer tube is properly labeled with the correct SOURCE participant ID label.

3. Check that the sample aliquot racks are properly labeled and equipped. Every item on the aliquoting/processing checklist must be ready and in its proper position using the rack outlines above.

4. Check that each aliquot storage container (two 2", 9x9 boxes) is labeled with the correct SOURCE participant ID/box labels (on both top and bottom of the box).

5. Perform and record temperature check on centrifuge (21°C ± 2°C). If centrifuges do not meet these specifications, call for repair and find an alternative centrifuge to use.

6. Perform and record temperature check on refrigerator temperature (4°C ± 2°C). If refrigerator does not meet these specifications, call for repair and find an alternative refrigerator to use.

7. Perform and record temperature check on ultra-cold freezer temperature (-75°C ± 5°C). If freezer does not meet these specifications, call for repair and find an alternative freezer to use.

8. Record room temperature.

2.2.7 At participant arrival

1. Open the DMS (Data Management System). Confirm that this participant has been appropriately consented and that the consent form data have been entered. **Confirm that the SOURCE Laboratory ID on the Biospecimen Label ID Form (LAB) in the DMS matches the label on the collection tubes and aliquot containers** (see Appendix 2 for a sample LAB form).

2. Note on the Biospecimen Collection Form (BIO) whether the participant has fasted before the blood draw. Not fasting does not disqualify a participant from having their blood drawn or their urine collected but needs to be noted.

2.3 Urine Collection

Before drawing the participant's blood, have the participant collect a clean catch urine specimen of at least 20 mL into an appropriately labeled specimen cup provided by the biospecimen technician (see Appendix 6 for clean catch urine instructions). When the urine specimen is less than 20 mL and the participant is unable to provide an additional sample, the non-preserved should be prioritized. A portion of the labeling on this specimen cup should have an area for writing down the time of the void.

Some clinics may have clean catch collection instructions in the restroom. However, SOURCE instructions should be printed and given to each participant as clean catch procedures may vary across institutions. It is important to make sure the participant understands the procedure. The participant should be instructed to void in the cup, filling it at least half-full if possible, place the lid securely on the cup, and then follow the clinic specific instructions for recording the time of collection and transporting the specimen to the biospecimen processing technician. The biospecimen processing technician should check that the top is secured and the time of the collection is recorded on the cup, and then place the specimen in an appropriate refrigerator until processing.

If the participant cannot void at this time or if at least 20 mL was not collected, they may try again after the blood draw. Every effort should be made to void at least 20 mL at the initial void. If a second void is made, this should be added to the BIO form.

2.4 Nasal Swab Collection

Please ask the participant to blow their nose. Open the Zymo nasal sampling kit. Please note that you should wear gloves at all times when handling biospecimens. Remove the swab from the wrapper by pulling the two ends of the wrapper apart (like you would a Band-Aid) and pull out the swab. Be careful to only touch the handle, not the soft cotton tip. Do NOT let the cotton swab side of the applicator touch anything except the inside of the participant's nose or the inside of the test tube. Gently insert the ENTIRE soft tip of the swab into one of the participant's nostrils until you feel a bit of resistance but no more than an inch (you should not be able to see the cotton tip anymore) and rub it in a circle around the participant's nostril gently and slowly for 15 seconds (at least 4 times). Next, gently insert the same swab into the other nostril and repeat. Put the swab in the tube by lowering the tube tip first into the provided tube. Once the tip is at the bottom, break the swab handle at the top of the tube by bending it back and forth. Then, screw the cap on tightly. Throw away the remaining sample kit items.

Nasal swab tubes are to be affixed with one of the miscellaneous (MISC) lab sample barcode labels. Tubes from the individual participants should be kept upright, to prevent the sample from leaking if initially placed on their side, then placed horizontally into the current "Nasal Swab" (non-sectioned) 2" box once frozen. Keep adding tubes to this box as additional participants are seen until the box is filled (30 tubes should fit) or until your next shipment to the Alexis Biospecimen Repository. Label these boxes starting with a new box number (i.e., 2, 3, etc.) each time you either fill a box or ship a box to the Alexis Biospecimen Repository. These boxes should be stored frozen (-80°C) and will be shipped frozen on dry ice (see Section 7.1) to the Alexis Biospecimen Repository.

3 VENIPUNCTURE PROCEDURE

3.1 Venipuncture Safety Precautions

All biospecimens should be treated as potentially infectious for laboratory workers. Bloodborne pathogens, such as hepatitis B and human immunodeficiency virus (HIV), can be transmitted following contact of a tainted blood sample through "broken skin" or intact mucous membrane (mouth, eyes, or nose) or as a result of an inadvertent needle stick. Examples of "broken skin" include open cuts, nicks and abrasions, dermatitis, and acne. Occupational Safety and Health Administration (OSHA) rules mandate that technicians always wear disposable protective gloves when collecting, processing, and handling biospecimens.

Gloves worn during venipuncture must be new and intact (e.g., you cannot tear off the tip of the glove to locate the venipuncture site). If the phlebotomist accidentally sustains a stick with a contaminated needle, clean the wound thoroughly with disinfectant soap and water, notify a supervisor, consult the SOURCE physician, and follow any local regulations regarding reporting needle sticks or visiting employee health. Never take lab coats worn during the collection and processing of samples outside of the laboratory area, except for laundering. Before leaving the laboratory, the technician will remove the lab coat and disposable gloves and wash hands with a disinfectant soap.

Use OSHA approved cleaning solution to clean up any spills of blood, plasma, serum, or urine. Use this solution to clean all laboratory work surfaces at the completion of work activities. OSHA regulations require that all needles and sharp instruments be discarded into puncture resistant containers. Do not attempt to bend, break, or recap any needle before discarding it. Appropriately discard the butterfly set following each biospecimen collection.

3.2 Phlebotomy Room

The blood drawing takes place in an area that offers privacy to the study participant. This phlebotomy area should be equipped with all the necessary blood drawing supplies. A separate work area is equipped with all the supplies that are used in the blood processing. The biosafety cabinet (or protective shields), centrifuge, refrigerator, and freezers should be nearby.

3.3 Participant Preparation

The phlebotomist should either confirm in the DMS that the participant has completed the informed consent or should check with the study coordinator to ensure the participant has been appropriately informed of the reason for venipuncture and the associated risks. The consent form also explains the number of tubes and amount of blood that will be drawn.

If possible, the participant should be in the seated position for a minimum of five minutes prior to the venipuncture. This allows the participant to relax before the procedure. Perform venipuncture with a 21-gauge butterfly needle and 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small thin-walled needle that minimizes trauma to the skin and vein. The use of 12 inches of tubing allows tubes to be changed without any movement of the needle in the vein. Give the participant time to relax after the blood collection.

If the participant is nervous or excited, the technician can briefly describe the procedure and purpose (e.g., "I am going to draw 4-5 tablespoons of blood. This blood will be used in a range of blood tests including CBC, platelets, and chemistries. We hope to be able to use the results of these tests to better understand the health issues of participants with COPD which may lead to the development of better treatments in the future.").

Do NOT under any circumstances force the participant to have blood drawn. It may help to explain to the participant that the blood draw is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms without actually drawing blood. If the participant is very anxious, they may lie down during the blood collection. A reclining individual will undergo an extravascular water shift resulting in a dilution effect on lipid values. If this option is taken, note it on the BIO form in the DMS by placing an "X" in the appropriate boxes.

NOTE: If a participant is unable to have blood drawn, discuss the participant's viability with the site Principal Investigator. This should be done as soon as the coordinator has concerns to avoid unnecessary testing (e.g., it may be preferable to address this before continuing with the visit). If necessary, blood can be drawn at a subsequent visit if the visit is completed within the study visit window. If the coordinator and/or the Principal Investigator feel it is unlikely the participant will be able to provide blood specimens, the study visit should be stopped. Participants must contribute at least one tube of blood to be enrolled in the study.

If a participant is unwilling to have biospecimens drawn or is unwilling to allow those biospecimens to be used for research purposes, the study visit should be stopped.

3.4 Venipuncture

NOTE: Complete the BIO form in the DMS or on a hard copy printout as blood is drawn and urine is collected. If data is collected on a hard copy, transfer this data into the DMS on the same day it is collected.

The participant should be asked if they are on any anticoagulants (blood thinners) or if they have a bleeding disorder. If so, the phlebotomist should apply pressure at the site for five minutes after the needle is removed from the vein.

With jacket or sweater removed, have the participant sit upright with the sleeves rolled up to expose the antecubital fossa (elbow). Use a tourniquet to increase venous filling. This makes the veins more prominent and easier to enter. The preferred arm to draw from is the left arm. Use the right arm only if blood collection is not possible from the left arm. This does not mean you must stick the left arm. Only do so if an adequate vein is apparent. Additional reasons to not use the left arm:

- If the participant has had lymph node dissection or radiation therapy to the left axilla (armpit) for both males and females.
- If the participant has tubes, rashes, dressings, casts, open sores hematomas, wounds, an arteriovenous (AV) shunt, or any other IV access device.

PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. Never leave the tourniquet on for longer than two minutes. To do so may result in hemoconcentration or a variation in blood test values. If a tourniquet must be applied for preliminary vein selection and it remains on the arm for longer than two minutes, it should be released and reapplied after a wait of two minutes.

Instruct the participant that they should not clench their fist prior to the venipuncture. Doing so could cause fluctuations in the results in several of the analytes being measured. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

A. Apply tourniquet.

1. Wrap the tourniquet around the arm 3-4 inches (7.5-10 cm) above the venipuncture site.

2. Tuck the end of the tourniquet under the last round.

3. If a Velcro tourniquet is used, adhere the ends to each other.

B. Identify vein.

1. Palpate and trace the path of veins several times with your index finger. Unlike veins, arteries pulsate, are more elastic, and have a thick wall. Veins lack resilience, feel cord-like, and roll easily. If superficial veins are not readily apparent, lowering the extremity over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.

C. Assemble the butterfly-vacutainer set.

1. Attach the Luer adapter to the vacutainer holder.

2. Attach the Luer end of the butterfly needle set to the Luer adapter.

D. Cleanse the venipuncture site.

1. Remove alcohol prep from its sterile package.

2. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.

3. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the participant when the venipuncture is performed.

4. If venipuncture becomes difficult, the vein may need to be touched again with a gloved hand. If this happens, cleanse the site again with alcohol.

E. Perform venipuncture.

1. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This maneuver anchors the vein, making it less likely to roll. The thumb should be 1-2 inches (2.5-5 cm) below the venipuncture site.

2. With the needle bevel upward, enter the vein in a smooth continuous motion.

3. Once blood appears in the butterfly tubing, place Tube #1 into the vacutainer holder. Grasp the flange of the holder and push the blood tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.

4. Make sure the participant's arm is in a flat or downward position while maintaining the tube below the site when the needle is in the vein. It may be helpful to use a support cushion or have the participant make a fist with the opposite hand and place it under the elbow for support. <u>DO NOT HAVE THE PARTICIPANT MAKE A FIST IN THE HAND OF THE ARM FROM WHICH BLOOD IS TO BE DRAWN.</u>

5. After Tube #3 fills, <u>remove the tourniquet</u>, while leaving the 21-gauge butterfly needle inserted in the participant’s arm. Once the remaining draws have started, do not change the position of a tube until it is withdrawn from the needle. The tourniquet may be reapplied if blood flow is slow without it. If the color of the arm turns red or blue, the tourniquet is applied too tightly. Loosen it and continue. If the tourniquet is loosened or reapplied, note this on the BIO form.

6. Keep a constant, slight forward pressure (in the direction of the adapter) on the end of the tube. This prevents release of the shutoff valve and stopping of blood flow. Do not vary pressure nor reintroduce pressure after completion of the draw.

7. Fill each vacutainer tube as completely as possible (i.e., until the vacuum is exhausted and blood flow ceases). If a vacutainer tube fills only partially, remove the tube, and attach another without removing the needle from the vein.

8. When the blood flow into the collection tube ceases, remove the tube from the holder. The shutoff valve covers the needle point, stopping blood flow until the next tube is inserted (if necessary). Immediately following removal of the tube from the adapter and while the next tube is filling, gently invert Tubes #1-2 five times, Tubes #3-6 eight times, and Tubes #7-8 8-10 times (see section 3.6 for mixing instructions).

9. When collecting Tube #6 (P100) and Tubes #7-8 (PAXgene), be sure to keep the tube positioned vertically while also below the participant's arm. When collecting the P100 tube, observe the following instructions to minimize premature separation of the mechanical separator from the stopper (Appendix 10):

    - Push tube onto non-participant end of the needle in one swift action.
    - Hold tube on non-participant end during drawing. If the mechanical separator moves in the P100 tube during blood collection, discard the tube and redraw.
    - **Extreme care should be taken when drawing these tubes due to their high cost.**

10. To remove the needle, first lightly place clean gauze over the venipuncture site. Next, remove the needle quickly and immediately apply pressure to the site with the gauze pad. Discard the needle with its cap into the sharps container. DO NOT ATTEMPT TO RECAP NEEDLES! Have the participant hold the gauze pad firmly for one to two minutes to prevent bruising.

11. If the blood flow stops before collecting all tubes, repeat the venipuncture on the participant beginning with the first unfilled tube**.** Because of the ratio of anticoagulant to blood, Tube #6 must be filled completely to avoid dilution of the plasma. Tube #7 should be filled completely to standardize the blood to liquid preservative/anticoagulant ratio. Tube #8 should be filled completely to maximize the RNA yield. This tube only collects 2.5 mL of blood. As always, the tourniquet must never be on for longer than two minutes.

F. Overcoming problems.

If a blood sample is not forthcoming, the following manipulations may be helpful:

1. If there is a sucking sound, turn needle slightly or lift the holder to move the bevel away from the wall of the vein.

2. If no blood appears, move needle slightly in hope of entering vein. Do not probe. If unsuccessful, release tourniquet and remove needle. A second attempt can be made on the other arm. The same technician should not attempt a venipuncture more than twice (once in each arm). If a third attempt is necessary, a different phlebotomist should attempt the venipuncture.

3. Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than two minutes at a time.

If a complete set of eight tubes cannot be drawn with three venipunctures, the study coordinator should assess whether the participant should be rescheduled for a second screening visit. Coordinators should check with their site PI or supervisor. If a second visit is scheduled, the coordinator should remind the participant to fast, starting at midnight and encourage the participant to drink plenty of water during the fast, especially during the morning hours prior to the blood draw. If a complete set of blood tubes was not taken at the initial visit, during the follow-up visit(s), the blood draw should resume with the first tube that was missed. If it is clear that a participant will not be able to provide a full set of blood tubes, coordinators should begin with Tube #1 and collect as many blood tubes as possible, dropping the duplicate RT (#2) and EDTA (#4) tubes as needed.

G. Bandaging the arm.

1. Under normal conditions:

    a) Determine if the participant has allergies to tape/adhesives. If so, use gauze pad and dressing only.
    b) Slip the gauze pad down over the site, continuing mild pressure.
    c) Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
    d) If the participant continues to bleed, apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.

2. Wrap a gauze bandage tightly around the arm over the pad.

3. Tell the participant to leave the bandage on for at least 15 minutes.

H. PRECAUTIONS - When a participant feels/looks faint following the blood drawing.

1. The default is to have the participant remain in the chair. However, if necessary due to serious lightheadedness, have them lie on the floor with their legs elevated. Use of a transfer belt may be indicated in this situation.

2. Take an ampule of smelling salts, crush it, and wave it under the participant's nose for a few seconds.

3. If the participant reports feeling nauseated, provide a basin.

4. Have the participant stay seated until the color returns and they feel better.

5. Have someone stay with the participant to prevent them from falling and injuring themselves if they should faint.

6. Place a cold wet cloth on the back of the participant's neck or on their forehead.

7. Once the episode has passed, some fruit juice may be given to the participant to counteract any possible hypoglycemia due to their pre-clinic visit fast.

8. If the participant continues to feel sick, take a blood pressure and pulse reading. Contact a medical staff member for further direction.

3.5 Blood and Urine Processing Safety Precautions

Universal, OSHA, and all institutional-specific (such as bloodborne pathogen training) precautions and training requirements should be followed when processing blood.

- Gloves must be worn at all times when handling biospecimens.
- Care should be taken to minimize aerosols when opening and handling the blood tubes. For instance, hold a piece of gauze or similar product (i.e., BloodBloc Biohazard wipes) over the stopper while slowly removing it from the tube. In addition, locking centrifuge bucket covers should be used.
- Ideally, the processing of whole blood and all the manipulations would be performed in a class II biological safety cabinet (laminar flow hood) while wearing a lab coat and gloves. Alternatively, to minimize infection risk, the technician can wear a lab coat with the following:
    - a mask in combination with an eye protection device, such as goggles or glasses with solid side shields or a chin-length face shield.
    - a desk-mounted or under-shelf mounted clear plastic shield.
- Place all used vacutainer tubes and blood-contaminated products in biohazard bags for proper institutional-dictated disposal.
- Clean up any spills with an appropriate disinfectant (10% bleach, 70-85% ethanol, or appropriate commercial product such as Decon or DuPont RelyOn).
- **Do not perform any pipetting by mouth.**
- If a bloodborne pathogen contamination occurs from a skin prick to the phlebotomist or other research personnel, allow the wound to bleed freely for a minute and then wash

with copious amounts of soap and water. If contamination occurs with a mucous membrane (eyes, nose, mouth), rinse surface with copious amounts of water. Eyes should be irrigated with the emergency eye wash station for at least 15 minutes. Finally, follow all institution reporting and follow-up requirements (i.e., a visit to your employee health services may be warranted) if a bloodborne pathogen exposure occurs.

- For more detailed safety information see: *(http://www.osha.gov/SLTC/biologicalagents/index.html)*

3.6 Blood Tube Mixing and Storage During Venipuncture

All tubes must be mixed following collection. For tubes containing anticoagulants, mixing is required to optimize coagulation, however, even Tubes #1-2 must be mixed since they contain a clot activator that needs to be mixed with the blood. Gently invert Tubes #1-2 five times, Tubes #3-6 eight times, and Tube #7-8 8-10 times. Eight inversions should take 6-8 seconds. Inversions should be performed on each tube while the next tube is being drawn.

Tubes #1 and #2 (8.5 mL red stoppered serum tubes). Mix five times by complete inversion immediately after the sample is drawn. Let sit vertical in a test tube rack at room temperature (RT) for a minimum of 30 minutes and a maximum of two hours prior to centrifugation and removal and aliquoting of serum. Protect tubes from light by placing a box over the rack until centrifugation. Note tube collection time on the BIO form.

Tubes #3 and #4 (10 mL lavender stoppered EDTA tubes). Invert eight times immediately after the sample is drawn. Centrifugation and aliquoting of plasma should occur within two hours of draw. These tubes should be kept covered at RT prior to centrifugation. Note tube collection time on the BIO form.

Tube #5 (3 mL lavender stoppered tube EDTA tube). Invert eight times immediately after the sample is drawn and prior to transport to clinical center lab for CBC counts with differentials. Note tube collection time on the BIO form. Follow site-specific clinical laboratory procedures for labeling of this tube. Sample can be held at RT up to four hours, after which it should be kept at 4°C. Samples should be transported to the clinical lab as quickly as possible.

Tube #6 (8.5 mL red stoppered P100 plasma tube). Immediately invert eight times after the sample is drawn. Centrifugation and aliquoting of plasma should occur within two hours of draw. These tubes should be kept covered at RT prior to centrifugation. Note tube collection time on the BIO form.

Tube #7 (8.5 mL blue stoppered PAXgene DNA tube). Invert 8-10 times immediately after the sample is drawn and place in a rack with the PAXgene RNA tube #8. Note tube collection time on the BIO form. Freeze this sample as described in section 5.6.

Tube #8 (2.5 mL red stoppered PAXgene RNA tube). Invert gently 8-10 times immediately after the sample is drawn and place in a rack at RT for a minimum of two hours and a maximum of 72 hours. Note tube collection time on the BIO form. After RT incubation, freeze this sample as described in section 5.7.

3.7 Snack

Allow time for the participant to obtain a snack before proceeding to the next SOURCE procedure. When scheduling the study visit, study coordinators should be certain to ask the participant whether they have any dietary restrictions. Please see *MOPs 2 and 5 (Pulmonary Function Testing and Sputum Induction and Processing)* for notes on the amount of time between eating and these procedures. For EBCs, have at least one hour between eating or drinking and the EBC collection.

4 EXHALED BREATH CONDENSATE

Exhaled breath condensate (EBC) will be collected at individual clinical centers during study visits using the commercially available RTube (Respiratory Research, Inc., Charlottesville, VA) – a handheld, self-contained, single-use device. The device consists of a mouthpiece connected to a one-way valve via a T-connector that directs exhaled breath through a condenser tube cooled by a chiller sleeve. Gaseous phase liquid and aerosols in exhaled breath condense into liquid form on the inside surface of the chilled condenser tube and can be extracted using a plunger device.

4.1 Preparation for Biospecimen Collection

Ensure that the aluminum chiller sleeve is appropriately chilled by placing the sleeve inside a plastic bag (to prevent condensation) and placing it into a -20°C freezer overnight. Note that for convenience, chiller sleeves can be stored in the -20°C freezer between participants' visits.

4.2 Collecting EBC

1. Remove the RTube device from its wrapping. Squeeze the tube near bottom valve to ensure that the duck-bill valve opens (as it occasionally will stick during storage).

2. Just before collection, remove the chiller sleeve from freezer and place it inside its insulating cover. Place sleeve with cover over the RTube. Paper towels can be used to minimize contact with the chiller sleeve and can be discarded after collection.

3. Have the participant breathe normally through the mouthpiece attached to the RTube for 10 minutes. Discourage shallow breathing but also be careful that the participant does not hyperventilate, which can lead to lightheadedness. The participant should exhale hard enough to hear breath flowing from the top of the RTube, and cool air should be noted coming from the top of the tube. If there are concerns, pause the collection, and remove the chiller sleeve briefly to ensure that there is condensate collecting on the inside of the RTube.

4. Participants should spit out saliva that accumulates in their mouth during collection. Brief pauses should not interfere with the collection.

5. The chiller sleeve can be wiped down with 70% alcohol or other disinfectant, dried, and returned to the freezer after collection.

4.3 Processing EBC

1. Once the 10-minute collection is complete, slide off the chiller sleeve and insulating cover, and remove the T-connector from the bottom of the tube. However, if there will be a delay between collection and processing, keep the RTube with EBC chilled (e.g., leave the chiller sleeve in place).

2. EBC can be stored for longer duration in the RTube by placing one of the supplied caps on the end opposite the valve and freezing the whole device. However, this process has caused loss of sample and is not recommended, if avoidable.

3. Place the RTube over the plunger and slowly push the tube all the way down to the base of the plunger. This maneuver will advance the valve and O-ring assembly to the top of the tube, collecting the condensate at the top of the RTube device.

4. Remove the collected EBC using a standard micropipette. If sufficient volume is available, divide EBC into up to four 500 µL aliquots, noting volume collected in each aliquot. If more than 2 mL is collected, discard the excess volume.

5. Freeze all EBC aliquots at -80°C pending shipment to the Alexis Biospecimen Repository. Place aliquots into box 2 of 2 for the participant according to the box map in section 5.8.

5 BLOOD PROCESSING

5.1 Operating the Centrifuge

Refer to the Centrifuge Operating Manual for specific operating and balancing instructions. Always use the Relative Centrifugal Force (RCF) or x *g* values in this MOP and not Revolutions Per Minute (RPM) when centrifuging blood samples. If your centrifuge does not display RCF values, consult the manual for a conversion chart or use the following formula:

> RCF = 1.12r (RPM/1000), where r = radius in millimeters (measure radius from center of rotor pin to middle of swinging bucket or center of fixed angle rotor) and RPM = revolutions per minute.

If a clinical center's centrifuge is not capable of creating a certain RCF, increase the centrifugation time until the RCF*minutes total the equivalent amount it would have spun at the higher RCF value. If, for example, the maximum force is 2000 RCF rather than 3000 RCF, then increase the time from 30 to 45 minutes. To balance the centrifuge, place tubes of the same size and with equal volume of blood (or blank tube with water), as determined visually, in opposite positions in the bucket/holders.

All centrifugations should be performed in a centrifuge fitted with a swinging bucket or 45-degree fixed angle rotor. A 33-degree fixed angle rotor will not provide efficient separation of the plasma in the P100 tubes.

5.2 Stage One: Immediate Processing and Centrifugation

NOTE: Process blood samples from only one participant at a time.

Immediately, or as soon as possible after completion of venipuncture, begin centrifugation of tubes for plasma and serum extraction in the following order. All centrifugations will be conducted at room temperature (RT). Do not use a mechanical brake to slow the rotor after centrifugation.

Record on the BIO form the time at which these tubes began to spin. *NOTE: For all the following centrifugation steps, if a swinging bucket rotor is used, bucket covers must be used when centrifuging whole blood.* Note that the process of centrifugation does NOT necessarily begin with Tubes #1 and #2.

1. Place Tube #5 in appropriate storage per on-site clinical lab requirements in preparation for CBC analysis.

2. Centrifuge Tubes #3 and #4 at 1100-1300 RCF for 10 minutes at RT in a swinging bucket rotor (or 15 minutes in a fixed angle), balancing the centrifuge as appropriate. Remove plasma from these tubes as described in section 5.3.1 (this removal can be performed while Tubes #1 and #2 are spinning).

3. After a minimum incubation of 30 minutes and a maximum of two hours upright at RT to allow the blood to clot, centrifuge Tubes #1 and #2 for 10 minutes at 1100-1300 RCF at RT in a swinging bucket rotor (or 15 minutes in a fixed angle), balancing the centrifuge as appropriate. Alternatively, if Tubes #1 and #2 have already incubated at RT for 30 minutes, they can be spun along with Tubes #3 and #4.

4. Centrifuge Tube #6 for 15-20 minutes at 2500 RCF at RT. Alternatively, if the centrifuge does not reach 2500 RCF, spin for 30 minutes at 1100-1600 RCF. If a fixed angle centrifuge is used, a 45-degree angle rotor is needed.

    - **Use appropriately sized tube holders and cushions to hold 16 x 100 mm tubes with hemoguard closures**. If this instruction is not followed, P100 tube may shatter upon centrifugation or may sink to the bottom of the tube holder, requiring removal with forceps, or the hemoguard closure may become dislodged. If you are unsure what is needed for a particular centrifuge, contact your local Becton-Dickinson representative for assistance.

5. Incubate Tubes #7 and #8 in an upright position at RT for a minimum of two hours and a maximum of 72 hours.

5.3 Stage Two: Processing of Blood Tubes

NOTE: Process blood from only one participant at a time. In addition, open and process only one type of blood tube at a time.

Stage two begins immediately or as soon as possible after each centrifugation. Working in a biosafety cabinet is recommended, but at the very least, face protection, gloves, and lab coat must be worn during blood processing. All other rules regarding the safe blood specimen handling as described in section 3.5 must also be observed.

5.3.1 Removal of plasma from Tubes #3 and #4

- Remove blood tubes from the centrifuge carefully, so as not to disturb cell layers, and place in a test tube rack in direct proximity to sample aliquoting Rack 2 (section 2.2.5).

- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., LT01PL or LT02PL).**

- Remove screwcaps from the aliquot tubes as each type of blood tube is processed.

- Slowly, so as not to disturb the cell layers or cause aerosols, remove blood tube stopper.

- Carefully and slowly remove plasma using a sterile, 2 mL serological pipette and controller. An automatic pipette can also be used.

    - Do not disturb the white blood cells layer, also called the buffy coat, which forms as a thin layer between the upper plasma layer and the lower layer of packed red blood cells. Stop collecting the plasma within ½" to ¾" above the surface of the buffy coat.

    - If some of the buffy coat is accidentally aspirated while removing the plasma, re-centrifuge the tube using the initial processing conditions. Indicate in either Item 12 or 13 of the BIO form that the tube was re-centrifuged.

    - If lipids (oily/fatty layer) are floating on top of plasma, do not collect this layer, and place tip of pipette below this surface before aspirating off plasma. Indicate the presence of lipids on top of the plasma on the BIO form.

- Directly transfer 150 µL of plasma into each aliquot #1-14. Any remaining plasma should be added to the 14th aliquot, and the final aliquot volume should be noted on the BIO form.

    - Note on the BIO form if less than 150 µL of plasma is placed in any aliquot.

    - If unable to fill all 14 aliquots with 150 µL, then any remaining plasma should be added to the last aliquot already containing 150 µL of plasma. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.

- Recap the aliquot sample tubes.

- Place aliquots into box 1 of 2 for the participant according to the box map in section 5.8.

    - Keep this box on dry ice until all aliquots are added and then transfer it to -80°C for temporary storage prior to batch shipment to the Alexis Biospecimen Repository. Alternatively, after each blood tube is processed, temporarily place aliquot Rack 2 at -80°C until other aliquots are processed. After completion of all processing, aliquots for each individual participant will be consolidated into two 2", 9x9 sectioned freezer boxes.

- Dispose of the empty Tubes #3 and #4 in biohazard waste as specified by individual institution.

5.3.2 Removal of serum from Tubes #1 and #2

- Remove blood tubes from the centrifuge carefully, so as not to disturb cell layers, and place in a test tube rack in direct proximity to sample aliquoting Rack 1 (section 2.2.5).

- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., RT01SER or RT02SER).**

- Remove screwcaps from the aliquot tubes as each type of blood tube is processed.

- Slowly, so as not to disturb the cell layers or cause aerosols, remove the blood tube stopper.

- Carefully and slowly remove serum using a sterile, 2 mL serological pipette and controller. An automatic pipette can also be used.

    - Stop collecting the serum within ¼" above the surface of the clot.

- Directly transfer 150 µL of serum into each aliquot #1-14. Any remaining serum should be added to the 14th aliquot, and the final aliquot volume should be noted on the BIO form.

    - Note on the BIO form if less than 150 µL of serum is placed in any aliquot.

    - If unable to fill all 14 aliquots with 150 µL, then any remaining serum should be added to the last aliquot already containing 150 µL of serum. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.

- Recap the aliquot sample tubes.

- Place aliquots into box 1 of 2 for the participant according to the box map in section 5.8.

- Keep this box on dry ice until all aliquots are added and then transfer to -80°C for temporary storage prior to batch shipment to the Alexis Biospecimen Repository. Alternatively, after blood tubes are processed, place aliquot Rack 1 temporarily at -80°C until other aliquots are processed. After completion of all processing, aliquots for each individual participant will be consolidated into two 2", 9x9 sectioned freezer boxes.

- Dispose of the empty Tubes #1 and #2 in biohazard waste as specified by individual institution.

5.3.3 Removal of plasma from Tube #6

- Remove blood tube from the centrifuge carefully and place in a test tube rack in direct proximity to sample aliquoting Rack 5 (section 2.2.5).

- **Confirm that the label on the blood tube corresponds to the labels on the aliquoting tubes, both for participant ID and specimen type (i.e., P100PL).**

- Remove screwcaps from the aliquot tubes.

- Slowly, so as not to cause aerosols, remove blood tube stopper.

- Carefully remove plasma using a sterile, 2 mL serological pipette and controller. An automatic pipette can also be used.

    - Stop collecting the plasma within ¼" above the surface of the tube's floating mechanical separator.

- Directly transfer 150 µL of plasma into each aliquot #1-14. Any remaining plasma should be added to the 14th aliquot, and the final aliquot volume should be noted on the BIO form.

    - Note on the BIO form if less than 150 µL of plasma is placed in any aliquot.

    - If unable to fill all 14 aliquots with 150 µL, then any remaining plasma should be added to the last aliquot already containing 150 µL of plasma. The total number of aliquots and the volume of the final aliquot should be noted on the BIO form.

- Recap the aliquot sample tubes.

- Place aliquots into box 2 of 2 for the participant according to the box map in section 5.8.

    - Keep this box on dry ice until all aliquots are added and then transfer to -80°C for temporary storage prior to batch shipment to the Alexis Biospecimen Repository. Alternatively, after blood tube is processed, place aliquot Rack 5 temporarily at -80°C until other aliquots are processed. After completion of all processing,

aliquots for each individual participant will be consolidated into two 2", 9x9 sectioned freezer boxes.

- Dispose of the empty Tube #6 in biohazard waste as specified by individual institution.

5.4 Urine Processing

- Remove specimen cup from the refrigerator and place in direct proximity to Rack 3 (section 2.2.5) containing the intermediate processing tube and sample aliquoting Rack 4 (section 2.2.5).

- **Confirm that the label on the specimen cup corresponds to the labels on the intermediate processing tube and the aliquoting tubes, for participant ID and specimen type (i.e., UR and PUR).**

- Gently invert urine specimen cup eight times.

- Remove screwcaps from the aliquot tubes.

- Remove 10 mL of urine from the specimen cup using a sterile, 10 mL serological pipette and controller.

- Directly transfer 1 mL of urine into each <u>non-preserved</u> aliquot #1-10.

- Remove cap from the 15 mL centrifuge tube containing the ascorbic acid preservative.

- Using a sterile, 10 mL serological pipette and controller, transfer 10 mL of urine from the specimen cup into the preservative-containing intermediate tube.

- Gently invert this tube until the preservative is dissolved.

- Remove 10 mL of the preserved urine from the intermediate tube using a sterile, 10 mL serological pipette and controller.

- Directly transfer 1 mL of the preserved urine into each <u>preserved</u> aliquot #1-10.

- Recap the aliquot sample tubes.

- Place aliquots into box 2 of 2 for the participant according to the box map in section 5.8.

    - Keep this box on dry ice until all aliquots are added and then transfer to -80°C for temporary storage prior to batch shipment to the Alexis Biospecimen Repository. Alternatively, after blood tube is processed, place aliquot Rack 4 temporarily at -80°C until other aliquots are processed. After completion of all processing, aliquots for each individual participant will be consolidated into two 2", 9x9 sectioned freezer boxes.

- Dispose of excess urine and specimen cup in biohazard waste as specified by institutional institution.

- Note any comments about urine collection and processing on the BIO form (i.e., if two voids were necessary, if less than 20 mL was collected indicate total volume, if there was blood in the urine, etc.).

5.5 Transfer of Tube #5 to On-site Clinical Lab

Relabel blood tube as necessary and transfer to on-site clinical lab per institution policy for CBC and differentials.

5.6 Final Processing of Tube #7

Place tubes from the individual participants horizontally directly into a -80°C freezer into the current "PXGR – StorDNA" (non-sectioned) 2" box. Keep adding tubes to this box as additional participants are seen until the box is filled (24 tubes should fit) or until your next shipment to the Alexis Biospecimen Repository. Label these boxes starting with a new box number (i.e., 2, 3, etc.) each time you either fill a box or ship a box to the Alexis Biospecimen Repository. These boxes will be shipped on dry ice along with the frozen aliquot boxes to the Alexis Biospecimen Repository.

5.7 Final Processing of Tube #8

Place tubes from the individual participants horizontally directly into a -80°C freezer into the current "PXGR – StorRNA" (non-sectioned) 2" box. Keep adding tubes to this box as additional participants are seen until the box is filled (24 tubes should fit) or until your next shipment to the Alexis Biospecimen Repository. Label these boxes starting with a new box number (i.e., 2, 3, etc.) each time you either fill a box or ship a box to the Alexis Biospecimen Repository. These boxes will be shipped on dry ice along with the frozen aliquot boxes to the Alexis Biospecimen Repository.

5.8 Aliquot Boxes

Aliquots should be placed in the 2", 9x9 sectioned freezer boxes according to the following box maps. During transfer of samples from the aliquoting racks, the boxes should be kept on dry ice. These boxes should be prelabeled with the Lab ID and the designation box 1 of 2 (1/2) or box 2 of 2 (2/2) on both the top and bottom of each box. The order below reflects placement of the aliquots into the boxes based on the original aliquoting rack order, skipping one space between each aliquot type. Frozen aliquots will be shipped to the Alexis Biospecimen Repository.

Box 1

Tube #1 RT Serum aliquot 1	Tube #1 RT Serum aliquot 2	Tube #1 RT Serum aliquot 3	Tube #1 RT Serum aliquot 4	Tube #1 RT Serum aliquot 5	Tube #1 RT Serum aliquot 6	Tube #1 RT Serum aliquot 7	Tube #1 RT Serum aliquot 8	Tube #1 RT Serum aliquot 9
Tube #1 RT Serum aliquot 10	Tube #1 RT Serum aliquot 11	Tube #1 RT Serum aliquot 12	Tube #1 RT Serum aliquot 13	Tube #1 RT Serum aliquot 14		Tube #2 RT Serum aliquot 1	Tube #2 RT Serum aliquot 2	Tube #2 RT Serum aliquot 3
Tube #2 RT Serum aliquot 4	Tube #2 RT Serum aliquot 5	Tube #2 RT Serum aliquot 6	Tube #2 RT Serum aliquot 7	Tube #2 RT Serum aliquot 8	Tube #2 RT Serum aliquot 9	Tube #2 RT Serum aliquot 10	Tube #2 RT Serum aliquot 11	Tube #2 RT Serum aliquot 12
Tube #2 RT Serum aliquot 13	Tube #2 RT Serum aliquot 14							
Tube #3 LT Plasma aliquot 1	Tube #3 LT Plasma aliquot 2	Tube #3 LT Plasma aliquot 3	Tube #3 LT Plasma aliquot 4	Tube #3 LT Plasma aliquot 5	Tube #3 LT Plasma aliquot 6	Tube #3 LT Plasma aliquot 7	Tube #3 LT Plasma aliquot 8	Tube #3 LT Plasma aliquot 9
Tube #3 LT Plasma aliquot 10	Tube #3 LT Plasma aliquot 11	Tube #3 LT Plasma aliquot 12	Tube #3 LT Plasma aliquot 13	Tube #3 LT Plasma aliquot 14		Tube #4 LT Plasma aliquot 1	Tube #4 LT Plasma aliquot 2	Tube #4 LT Plasma aliquot 3
Tube #4 LT Plasma aliquot 4	Tube #4 LT Plasma aliquot 5	Tube #4 LT Plasma aliquot 6	Tube #4 LT Plasma aliquot 7	Tube #4 LT Plasma aliquot 8	Tube #4 LT Plasma aliquot 9	Tube #4 LT Plasma aliquot 10	Tube #4 LT Plasma aliquot 11	Tube #4 LT Plasma aliquot 12
Tube #4 LT Plasma aliquot 13	Tube #4 LT Plasma aliquot 14							

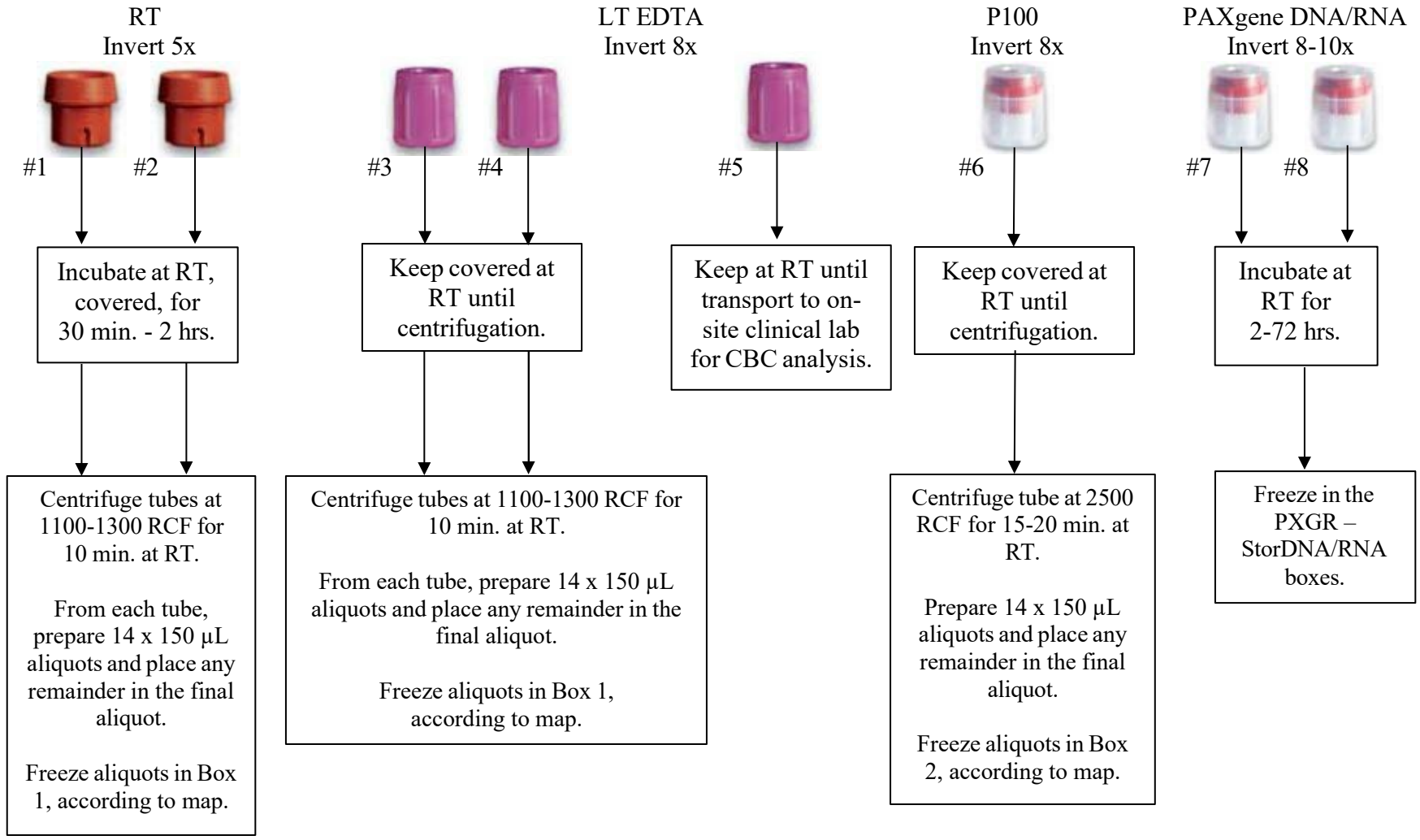
Box 2

Urine unpreserved (UR) aliquot 1	Urine unpreserved (UR) aliquot 2	Urine un-preserved (UR) aliquot 3	Urine unpreserved (UR) aliquot 4	Urine unpreserved (UR) aliquot 5	Urine unpreserved (UR) aliquot 6	Urine unpreserved (UR) aliquot 7	Urine unpreserved (UR) aliquot 8	Urine unpreserved (UR) aliquot 9
Urine unpreserved (UR) aliquot 10		Urine preserved (PUR) aliquot 1	Urine preserved (PUR) aliquot 2	Urine preserved (PUR) aliquot 3	Urine preserved (PUR) aliquot 4	Urine preserved (PUR) aliquot 5	Urine preserved (PUR) aliquot 6	Urine preserved (PUR) aliquot 7
Urine preserved (PUR) aliquot 8	Urine preserved (PUR) aliquot 9	Urine preserved (PUR) aliquot 10		Tube #6 P100 Plasma aliquot 1	Tube #6 P100 Plasma aliquot 2	Tube #6 P100 Plasma aliquot 3	Tube #6 P100 Plasma aliquot 4	Tube #6 P100 Plasma aliquot 5
Tube #6 P100 Plasma aliquot 6	Tube #6 P100 Plasma aliquot 7	Tube #6 P100 Plasma aliquot 8	Tube #6 P100 Plasma aliquot 9	Tube #6 P100 Plasma aliquot 10	Tube #6 P100 Plasma aliquot 11	Tube #6 P100 Plasma aliquot 12	Tube #6 P100 Plasma aliquot 13	Tube #6 P100 Plasma aliquot 14

Sputum MICRO 1	Sputum MICRO 2		Sputum NUC 1	Sputum NUC 2	Sputum NUC 3	Sputum NUC 4		Sputum DPBS Zymo
Sputum CYT 1	Sputum CYT 2	Sputum CYT 3	Sputum CYT 4		EBC aliquot 1	EBC aliquot 2	EBC aliquot 3	EBC aliquot 4

6 OVERVIEW OF BIOSPECIMEN COLLECTION

Blood Tube Processing Flow Chart



7 PACKAGING AND SHIPPING

7.1 Storage, Packaging, and Shipping (For Frozen Biospecimens, including Nasal Swabs)

During the packing process, the aliquot and blood tube boxes should be kept on dry ice at all times.

Ship frozen biospecimens on Mondays – Wednesdays only. Notify the Alexis Biospecimen Repository, using all the emails listed below, at least 48 hours in advance to schedule a shipment. **In this email, please attach all Excel Shipping Manifest Reports run and downloaded in CDART as appropriate (see Appendix 4).**

Determine the number of 2" frozen boxes to be shipped and gather the appropriate number of shipping containers. Each shipping container consists of an inner Styrofoam container and an outer cardboard box. Approximately 12-16 x 2" boxes should fit per container, along with 20-24 lbs. of dry ice.

The total weight of the shipping container should not exceed the overall weight of the shipping container as noted on the bottom of the outside cardboard box (for most standard size shippers this is typically 50 lbs.). The Alexis Biospecimen Repository can send back empty 2" boxes and shipping containers in good condition via FedEx Ground.

7.1.1 Packaging frozen biospecimens for shipment

- Package samples in the early afternoon the day of shipment.
- Put some dry ice in a tray or other temporary container for preparation of the frozen aliquot boxes.
- Use a rubber band to securely close each individual box. Then rubber band together boxes 1 and 2 for each participant.
- Place a piece of absorbent material in the bottom of the inner Styrofoam container. (Alternatively, a piece of absorbent material can be wrapped around each set of boxes.)
- Place some dry ice in the bottom of the inner Styrofoam container (enough to completely cover the bottom).
- Begin layering boxes and dry ice in the container. Be sure that boxes are surrounded by dry ice and that all air pockets contain dry ice.
    - Approximately 12-16 boxes will fit into a single shipping container. The total amount of dry ice should be approximately 20-24 lbs.
- Place Styrofoam top onto the inner Styrofoam container.

- *NOTE: DO NOT tape down the lid of the inner Styrofoam cooler. When shipping frozen samples on dry ice, the package must allow for venting of carbon dioxide gas during transport.*

- Place the Alexis Biospecimen Repository address and Visit Shipping Manifest Report into a plastic folder or bag and tape to top of Styrofoam container.

    - The Visit Shipping Manifest Report can be run in CDART and printed. It should list the boxes being shipped inside each container (i.e., Lab ID 123456 Box 1 and 2, Lab ID 888862 Box 1 and 2, PAXgene Box 1 with 21 samples, etc.).

- Seal the outer cardboard box securely with strapping tape.

- Affix a Biological Substance Category B (UN3373) label and a completed Dry Ice label (UN 1845) to the outside of the cardboard box. Please note these labels are not provided by the GIC.

- Affix the completed FedEx air bill (see the example on the next page for specifics) to the following address to the top of the carboard box. A shipment label can also be created online at Fedex.com using the same information as below (contact the GIC for the login information).

    - Section 1 – **Site address and telephone number**; record the **SOURCE GIC's FedEx account number** on the appropriate line (contact the GIC for this information)
    - Section 2 – **SOURCE GIC internal billing reference** (see Memo 4.2 or contact the GIC for this information)
        - For paper labels, record this number in Section 2.
        - For online labels, record this number in Section 4.
    - Section 3 – **Alexis Biospecimen Repository address** (below):

      Attn: Heather Wells
      UNC CEMALB
      511 EPA Building
      104 Mason Farm Road
      Chapel Hill, NC 27599-7406
      (919) 966-9356

    - Section 4 – **FedEx Priority Overnight**
    - Section 5 – **Other**
    - Section 6 – **Yes/shipper's declaration not required** and **Dry Ice**; record the estimated number of pounds of dry ice
    - Section 7 – **Third Party**

- Use your institution's FedEx shipping procedures or call 1-800-GO-FEDEX for pickup.

- **Send an e-mail message containing the date of shipment, tracking number(s), and Excel manifests to the addresses below.**

  Heather Wells at heather_seaman@med.unc.edu
  Neil Alexis at neil_alexis@med.unc.edu
  SOURCE GIC at source@unc.edu

7.2 Storage, Packaging, and Shipping (For 4°C Temperature Sputum Specimen for Mucin Analysis)

NOTE: Quarterly shipments are possible if not many participants were recruited during the month.

Ship biospecimens on Mondays – Wednesdays only. Notify the Alexis Biospecimen Repository, using all the emails listed below, at least 48 hours in advance to schedule a shipment. **In this email, please attach all Excel Shipping Manifest Reports run and downloaded in CDART as appropriate (see Appendix 4).** Determine the number of 2" refrigerated boxes to be shipped that month and gather the appropriate number of shipping containers (most likely one). Each shipping container consists of an inner Styrofoam container and an outer cardboard box. Use the smallest shipping container possible. The Alexis Biospecimen Repository can send back empty 2" boxes and shipping containers in good condition via FedEx Ground.

7.2.1 Packaging samples for 4°C shipping

- Place a piece of absorbent material in the bottom of the inner Styrofoam container. (Alternatively, a piece of absorbent material can be wrapped around each box.)

- Place a frozen gel pack on bottom of the inner Styrofoam container.
- Use a rubber band to securely close each individual box.
- Place in the box in the container and place another frozen gel pack on top of the box.
- Place packing material generously around gel packs and box to prevent movement.
- Place the Alexis Biospecimen Repository address and Visit Shipping Manifest Report into a plastic folder or bag and tape to top of Styrofoam container.
    - The Visit Shipping Manifest Report can be run in CDART and printed. It should list the boxes being shipped inside each container.
- Seal the outer cardboard box securely with strapping tape. (Do not seal the inner Styrofoam container.)
- Affix a Biological Substance Category B (UN3373) label to the outside of the cardboard box. Please note these labels are not provided by the GIC.
- Affix the completed FedEx air bill (see the example in 7.1.1 for specifics) to the following address to the top of the cardboard box. A shipment label can also be created online at Fedex.com, using the same information as below (contact the GIC for the login information).
    - Section 1 – **Site address and telephone number**; record the **SOURCE GIC's FedEx account number** on the appropriate line (contact the GIC for this information)
    - Section 2 – **SOURCE GIC internal billing reference** (see Memo 4.2 or contact the GIC for this information)
        - For paper labels, record this number in Section 2.
        - For online labels, record this number in Section 4.
    - Section 3 – **Alexis Biospecimen Repository address** (below):

      Attn: Heather Wells
      UNC CEMALB
      511 EPA Building
      104 Mason Farm Road
      Chapel Hill, NC 27599-7406
      (919) 966-9356

    - Section 4 – **FedEx <u>Priority Overnight</u>**
    - Section 5 – **Other**
    - Section 6 – **No**
    - Section 7 – **Third Party**

- Use your institution's FedEx shipping procedures or call 1-800-GO-FEDEX for pickup.

- **Send an e-mail message containing the date of shipment, tracking number(s), and Excel manifests to the addresses below.**

  Heather Wells at heather_seaman@med.unc.edu
  Neil Alexis at neil_alexis@med.unc.edu
  SOURCE GIC at source@unc.edu

8 GENERAL QUALITY CONTROL

General Good Laboratory Practice Checklist:

1. All staff collecting, processing, and shipping samples must be certified on the SOURCE MOP. In addition, they should comply with all additional Institutional and Government regulations for working with human blood specimens.

2. For collecting and processing samples, follow procedures outlined in this MOP.

3. Process all blood specimens within two hours of collection, except the PAXgene which must remain at room temperature for a minimum of two hours and a maximum of 72 hours before freezing.

4. Maintain a daily log of freezer, refrigerator, and room temperatures.

5. Pipettes should be calibrated and certified every six months.

6. Centrifuge speeds should be certified yearly by a certified technician using a tachometer.

7. All biological safety cabinets should be certified yearly by a licensed technician.

9 TRAINING PROCEDURES

This study will not provide general phlebotomy training, which must be provided by the clinical centers. This MOP will serve as the official training guide for all other aspects of biospecimen collection and processing. A central training will be performed via Zoom, and at this time, all sites will need to have present representatives to become certified on the procedures outlined in this MOP. Once certified, these individuals can train and certify new clinical site staff, as necessary. Annual re-certification will be required of all current staff.

10 APPENDICES

Appendix 1. Aliquots to be Collected

1) **Serum from Tube #1 (8.5 mL red stoppered serum tube)**
   a. 13 x 150 µL aliquots
   b. 1 aliquot with remaining serum volume

2) **Serum from Tube #2 (8.5 mL red stoppered serum tube)**
   a. 13 x 150 µL aliquots
   b. 1 aliquot with remaining serum volume

3) **Plasma from Tube #3 (10 mL lavender stoppered EDTA tube)**
   a. 13 x 150 µL aliquots
   b. 1 aliquot with remaining plasma volume

4) **Plasma from Tube #4 (10 mL lavender stoppered EDTA tube)**
   a. 13 x 150 µL aliquots
   b. 1 aliquot with remaining plasma volume

5) **Plasma from Tube #6 (8.5 mL red stoppered P100 plasma tube)**
   a. 13 x 150 µL aliquots
   b. 1 aliquot with remaining plasma volume

6) **Urine from unpreserved urine specimen cup**
   a. 10 x 1000 µL aliquots

7) **Urine from preserved urine tube**
   a. 10 x 1000 µL aliquots

Appendix 2. Biospecimen Label ID Form (LAB)

See study website: https://www2.cscc.unc.edu/spiromics/

Login to secure site.
Study Documents → Data Collection Forms and QxQs → SOURCE → Biospecimen Label ID Form (LAB)

Or find the form in the electronic data management system.

Appendix 3. Partial Participant Sample Set Biospecimen Collection Procedure

If a complete set of blood tubes was not taken at the initial visit, during the follow-up visit(s), the blood draw should resume with the first tube missed. A follow-up visit to complete the blood draws should be scheduled within a month of the first visit.

Appendix 4. Excel Shipping Forms

Utilize the "Lab Shipment" forms generated by the electronic Data Management System under Reports. A list of these reports and detailed instructions on how to produce them can be found in *MOP 6 – Data Management System*.

See section 7 to review instructions on completing the site-level forms for PAXgene DNA and RNA samples (using the DNA and RNA forms), mucin samples (using the MUC form), nasal swab samples (using the NSB form), sputum slides (using the SLI form), and sputolysin samples (using the SLY form).

Appendix A in *MOP 6 – Data Management System* gives instructions on how to create the Visit Shipping Manifest Reports that will need to be emailed to the Alexis Biospecimen Repository and/or included in the boxes with each shipment of samples.

Appendix 5. Equipment and Supplies

Supplies to be provided by the Central Laboratory:

Blood and Urine Collection Supplies

Tube Name Abbreviation	Tube Color	Size	Anticoagulant	Tube Type	Closure
10 mL LT	lavender	10 mL / 16x100 mm	K_2EDTA – 15% sol./vol.	plastic	hemoguard
3 mL LT	lavender	3 mL / 13x75 mm	K_2EDTA – 7.2 mg	plastic	hemoguard
10 mL RT	red	10 mL / 16x100 mm	silica clot activator	plastic	conventional
P100	red	8.5 mL / 16x100 mm	K_2EDTA – 7.2 mg / protein stabilizers	plastic	hemoguard
PAXgene DNA	blue	8.5 mL / 16x100 mm	2 mL K_2EDTA and DNA stabilizers	plastic (treat like glass once frozen)	hemoguard
PAXgene RNA	red / orange	8.5 mL / 16x100 mm / 2.5 mL blood vol.	preservatives and RNA stabilizers	plastic (treat like glass once frozen)	hemoguard
Urine specimen cup	orange	60 mL		plastic, sterile	screw cap

Blood and Urine Processing Supplies

Type	Product	Description
Tubes	1.7 mL screwcap tubes	For final plasma, serum, urine, sputum, and EBC aliquots
	15 mL and 50 mL conical tubes	For intermediate processing steps
Labels	2D barcoded labels	Labels for blood collection tubes, specimen aliquots, and freezer boxes
	2" boxes with 9x9 dividers	For storage of final aliquots in -80°C and 4°C
Storage and Shipping	Insulated shipping containers	Inner Styrofoam container with outer cardboard container for shipping biospecimens to the Alexis Biospecimen Repository

	Gel packs	For 4°C shipments
Other Processing	Pipette tips and serological pipettes	For processing plasma, serum, urine, sputum, and EBC
Chemicals	Ascorbic Acid	For preserved urine samples

Equipment purchased and maintained by Field Centers

- Tabletop centrifuge with swinging buckets and capable of producing 3000 RCF (x *g*)
- Freezer capable of maintaining -70 to -80°C with a minimum of 5 ft^3 storage
- Refrigerator capable of maintaining 4°C for storing urine prior to processing and 3 mL EDTA tubes prior to transfer to clinical lab

Appendix 6. Clean Catch Urine Instructions — Female and Male Participants

INSTRUCTIONS FOR FEMALE PARTICIPANTS

1. Wash hands with soap and warm water.

2. Place clean paper towel on counter.

3. Open the urine cup and place the lid and cup face up on the clean paper towel being careful not to touch the rim or inside of the lid or cup (to prevent contaminants from entering the clean field).

4. Spread the labia (folds of skin) apart with one hand and wipe with the towelette provided. Wipe from front to back.

5. Continue holding the labia apart. As you start to urinate, allow a small amount of urine to fall into the toilet bowl (this clears the urethra of contaminants). Do not touch the inside of the cup.

6. After the urine stream is well established, urinate into the cup. Once an adequate amount of urine fills the cup (the cup only needs to be half-full), remove the cup from the urine stream.

7. Pass the remaining urine into the toilet.

8. Screw the lid on the cup tightly (do not touch the inside of the cup or lid).

9. Wash your hands with soap and warm water.

10. Record the time of the urine collection on the label on the cup with the pen provided.

11. Place the cup in the biospecimen bag provided.***

12. Give the cup to the study coordinator/technician.

*** Alternately, “leave specimen in bathroom and notify the technician that the specimen is waiting” or “place specimen in wall cabinets.”

INSTRUCTIONS FOR MALE PARTICIPANTS

1. Wash hands with soap and warm water.

2. Place clean paper towel on counter.

3. Open the urine cup and place the lid and cup face up on the clean paper towel being careful not to touch the rim or inside of the lid or cup (to prevent contaminants from entering the clean field).

4. If uncircumcised, retract foreskin.

5. Wipe the end of penis with towelette provided. As you start to urinate, allow a small amount of urine to fall into the toilet bowl (this clears the urethra of contaminants). Do not touch the inside of the cup.

6. After the urine stream is well established, urinate into the cup. Once an adequate amount of urine fills the cup (the cup only needs to be half-full), remove the cup from the urine stream.

7. Pass the remaining urine into the toilet.

8. Screw the lid on the cup tightly (do not touch the inside of the cup or lid).

9. Wash your hands with soap and warm water.

10. Record the time of the urine collection on the label on the cup with the pen provided.

11. Place the cup in the biospecimen bag provided.***

12. Give the cup to the study coordinator/technician.

*** Alternately, “leave specimen in bathroom and notify the technician that the specimen is waiting” or “place specimen in wall cabinets.”

Appendix 7. Biospecimen Collection Preparation List

SOURCE BIOSPECIMEN COLLECTION
PREPARATION CHECKLIST

Urine Collection:

- ☐ urine specimen collection cup
- ☐ towelette

Blood Collection Tray:

- ☐ test tube rack that holds at least eight blood collection tubes
- ☐ 12-inch blood collection set (21-gauge butterfly needle with Luer adapter and plastic tube guide) ****mandatory for use with P100 plasma collection tubes**
- ☐ alcohol wipes
- ☐ gauze sponges
- ☐ tourniquet
- ☐ adhesive bandages
- ☐ GIC-provided labels (for eight blood tubes and urine cup)

Appendix 8. Participant Lab ID Tracking Sheet

SOURCE
SPIROMICS Study of Early COPD Progression

Participant ID:

Visit	Date	Lab ID

Appendix 9. Label Descriptions

No.	Sample Type Description	Label	Container Type
0	Scanning label		N/A
1	Red Top 1, Tube 1	RT01	vacutainer blood tube
2	Red Top 2, Tube 2	RT02	vacutainer blood tube
3	Lavender Top 1, Tube 3	LT01	vacutainer blood tube
4	Lavender Top 2, Tube 4	LT02	vacutainer blood tube
5	Lavender Top CBC, Tube 5	LT03_CBC	vacutainer blood tube
6	P100, Tube 6	P100	vacutainer blood tube
7	PAXgene DNA, Tube 7	PXGDN	vacutainer blood tube
8	PAXgene RNA, Tube 8	PXGRN	vacutainer blood tube
9	Urine collection cup	UR	collection cup, not final aliquot
10	Preserved Urine 15 mL conical	PUR	intermediate processing, not final aliquot
11	Spontaneous Sputum collection cup	SPU_cupSP	collection cup, not final aliquot
12	Induced Sputum collection cup	SPU_cupIN	collection cup, not final aliquot
13	EBC collection vessel	EBC	collection vessel, not final aliquot
14	Red Top 1, Tube 1, Aliquot 1	RT01SER01	1.7 mL screwcap tube
15	Red Top 1, Tube 1, Aliquot 2	RT01SER02	1.7 mL screwcap tube
16	Red Top 1, Tube 1, Aliquot 3	RT01SER03	1.7 mL screwcap tube
17	Red Top 1, Tube 1, Aliquot 4	RT01SER04	1.7 mL screwcap tube
18	Red Top 1, Tube 1, Aliquot 5	RT01SER05	1.7 mL screwcap tube
19	Red Top 1, Tube 1, Aliquot 6	RT01SER06	1.7 mL screwcap tube
20	Red Top 1, Tube 1, Aliquot 7	RT01SER07	1.7 mL screwcap tube
21	Red Top 1, Tube 1, Aliquot 8	RT01SER08	1.7 mL screwcap tube
22	Red Top 1, Tube 1, Aliquot 9	RT01SER09	1.7 mL screwcap tube
23	Red Top 1, Tube 1, Aliquot 10	RT01SER10	1.7 mL screwcap tube
24	Red Top 1, Tube 1, Aliquot 11	RT01SER11	1.7 mL screwcap tube
25	Red Top 1, Tube 1, Aliquot 12	RT01SER12	1.7 mL screwcap tube
26	Red Top 1, Tube 1, Aliquot 13	RT01SER13	1.7 mL screwcap tube
27	Red Top 1, Tube 1, Aliquot 14	RT01SER14	1.7 mL screwcap tube
28	Red Top 2, Tube 2, Aliquot 1	RT02SER01	1.7 mL screwcap tube
29	Red Top 2, Tube 2, Aliquot 2	RT02SER02	1.7 mL screwcap tube
30	Red Top 2, Tube 2, Aliquot 3	RT02SER03	1.7 mL screwcap tube
31	Red Top 2, Tube 2, Aliquot 4	RT02SER04	1.7 mL screwcap tube
32	Red Top 2, Tube 2, Aliquot 5	RT02SER05	1.7 mL screwcap tube
33	Red Top 2, Tube 2, Aliquot 6	RT02SER06	1.7 mL screwcap tube
34	Red Top 2, Tube 2, Aliquot 7	RT02SER07	1.7 mL screwcap tube
35	Red Top 2, Tube 2, Aliquot 8	RT02SER08	1.7 mL screwcap tube
36	Red Top 2, Tube 2, Aliquot 9	RT02SER09	1.7 mL screwcap tube
37	Red Top 2, Tube 2, Aliquot 10	RT02SER10	1.7 mL screwcap tube

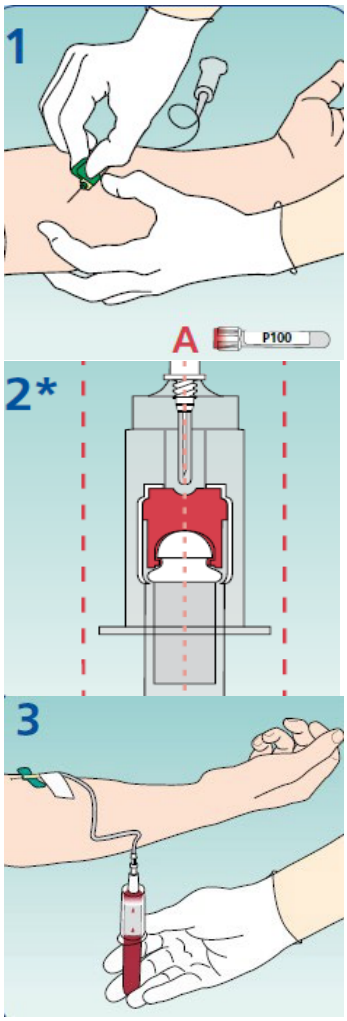
38	Red Top 2, Tube 2, Aliquot 11	RT02SER11	1.7 mL screwcap tube
39	Red Top 2, Tube 2, Aliquot 12	RT02SER12	1.7 mL screwcap tube
40	Red Top 2, Tube 2, Aliquot 13	RT02SER13	1.7 mL screwcap tube
41	Red Top 2, Tube 2, Aliquot 14	RT02SER14	1.7 mL screwcap tube
42	Lavender Top 1, Tube 3, Aliquot 1	LT01PL01	1.7 mL screwcap tube
43	Lavender Top 1, Tube 3, Aliquot 2	LT01PL02	1.7 mL screwcap tube
44	Lavender Top 1, Tube 3, Aliquot 3	LT01PL03	1.7 mL screwcap tube
45	Lavender Top 1, Tube 3, Aliquot 4	LT01PL04	1.7 mL screwcap tube
46	Lavender Top 1, Tube 3, Aliquot 5	LT01PL05	1.7 mL screwcap tube
47	Lavender Top 1, Tube 3, Aliquot 6	LT01PL06	1.7 mL screwcap tube
48	Lavender Top 1, Tube 3, Aliquot 7	LT01PL07	1.7 mL screwcap tube
49	Lavender Top 1, Tube 3, Aliquot 8	LT01PL08	1.7 mL screwcap tube
50	Lavender Top 1, Tube 3, Aliquot 9	LT01PL09	1.7 mL screwcap tube
51	Lavender Top 1, Tube 3, Aliquot 10	LT01PL10	1.7 mL screwcap tube
52	Lavender Top 1, Tube 3, Aliquot 11	LT01PL11	1.7 mL screwcap tube
53	Lavender Top 1, Tube 3, Aliquot 12	LT01PL12	1.7 mL screwcap tube
54	Lavender Top 1, Tube 3, Aliquot 13	LT01PL13	1.7 mL screwcap tube
55	Lavender Top 1, Tube 3, Aliquot 14	LT01PL14	1.7 mL screwcap tube
56	Lavender Top 2, Tube 4, Aliquot 1	LT02PL01	1.7 mL screwcap tube
57	Lavender Top 2, Tube 4, Aliquot 2	LT02PL02	1.7 mL screwcap tube
58	Lavender Top 2, Tube 4, Aliquot 3	LT02PL03	1.7 mL screwcap tube
59	Lavender Top 2, Tube 4, Aliquot 4	LT02PL04	1.7 mL screwcap tube
60	Lavender Top 2, Tube 4, Aliquot 5	LT02PL05	1.7 mL screwcap tube
61	Lavender Top 2, Tube 4, Aliquot 6	LT02PL06	1.7 mL screwcap tube
62	Lavender Top 2, Tube 4, Aliquot 7	LT02PL07	1.7 mL screwcap tube
63	Lavender Top 2, Tube 4, Aliquot 8	LT02PL08	1.7 mL screwcap tube
64	Lavender Top 2, Tube 4, Aliquot 9	LT02PL09	1.7 mL screwcap tube
65	Lavender Top 2, Tube 4, Aliquot 10	LT02PL10	1.7 mL screwcap tube
66	Lavender Top 2, Tube 4, Aliquot 11	LT02PL11	1.7 mL screwcap tube
67	Lavender Top 2, Tube 4, Aliquot 12	LT02PL12	1.7 mL screwcap tube
68	Lavender Top 2, Tube 4, Aliquot 13	LT02PL13	1.7 mL screwcap tube
69	Lavender Top 2, Tube 4, Aliquot 14	LT02PL14	1.7 mL screwcap tube
70	P100, Tube 6, Aliquot 1	P100PL01	1.7 mL screwcap tube
71	P100, Tube 6, Aliquot 2	P100PL02	1.7 mL screwcap tube
72	P100, Tube 6, Aliquot 3	P100PL03	1.7 mL screwcap tube
73	P100, Tube 6, Aliquot 4	P100PL04	1.7 mL screwcap tube
74	P100, Tube 6, Aliquot 5	P100PL05	1.7 mL screwcap tube
75	P100, Tube 6, Aliquot 6	P100PL06	1.7 mL screwcap tube
76	P100, Tube 6, Aliquot 7	P100PL07	1.7 mL screwcap tube
77	P100, Tube 6, Aliquot 8	P100PL08	1.7 mL screwcap tube
78	P100, Tube 6, Aliquot 9	P100PL09	1.7 mL screwcap tube

79	P100, Tube 6, Aliquot 10	P100PL10	1.7 mL screwcap tube
80	P100, Tube 6, Aliquot 11	P100PL11	1.7 mL screwcap tube
81	P100, Tube 6, Aliquot 12	P100PL12	1.7 mL screwcap tube
82	P100, Tube 6, Aliquot 13	P100PL13	1.7 mL screwcap tube
83	P100, Tube 6, Aliquot 14	P100PL14	1.7 mL screwcap tube
84	Urine, Unpreserved, Aliquot 1	UR01	1.7 mL screwcap tube
85	Urine, Unpreserved, Aliquot 2	UR02	1.7 mL screwcap tube
86	Urine, Unpreserved, Aliquot 3	UR03	1.7 mL screwcap tube
87	Urine, Unpreserved, Aliquot 4	UR04	1.7 mL screwcap tube
88	Urine, Unpreserved, Aliquot 5	UR05	1.7 mL screwcap tube
89	Urine, Unpreserved, Aliquot 6	UR06	1.7 mL screwcap tube
90	Urine, Unpreserved, Aliquot 7	UR07	1.7 mL screwcap tube
91	Urine, Unpreserved, Aliquot 8	UR08	1.7 mL screwcap tube
92	Urine, Unpreserved, Aliquot 9	UR09	1.7 mL screwcap tube
93	Urine, Unpreserved, Aliquot 10	UR10	1.7 mL screwcap tube
94	Urine, Preserved, Aliquot 1	PUR01	1.7 mL screwcap tube
95	Urine, Preserved, Aliquot 2	PUR02	1.7 mL screwcap tube
96	Urine, Preserved, Aliquot 3	PUR03	1.7 mL screwcap tube
97	Urine, Preserved, Aliquot 4	PUR04	1.7 mL screwcap tube
98	Urine, Preserved, Aliquot 5	PUR05	1.7 mL screwcap tube
99	Urine, Preserved, Aliquot 6	PUR06	1.7 mL screwcap tube
100	Urine, Preserved, Aliquot 7	PUR07	1.7 mL screwcap tube
101	Urine, Preserved, Aliquot 8	PUR08	1.7 mL screwcap tube
102	Urine, Preserved, Aliquot 9	PUR09	1.7 mL screwcap tube
103	Urine, Preserved, Aliquot 10	PUR10	1.7 mL screwcap tube
104	Sputum Petri Dish	SPU_pet	intermediate processing, not final aliquot
105	Spontaneous Sputum weighing 50 mL conical	SPU_SP_W	intermediate processing, not final aliquot
106	Induced Sputum weighing 50 mL conical	SPU_IN_W	intermediate processing, not final aliquot
107	Sputum aliquot for mucins	SPUmucin	1.7 mL screwcap tube
108	Sputum aliquot 1 for microbiology	SPUmicro01	1.7 mL screwcap tube
109	Sputum aliquot 2 for microbiology	SPUmicro02	1.7 mL screwcap tube
110	Sputum aliquot 1 for nucleotides	SPUnuc01	1.7 mL screwcap tube
111	Sputum aliquot 2 for nucleotides	SPUnuc02	1.7 mL screwcap tube
112	Sputum aliquot 3 for nucleotides	SPUnuc03	1.7 mL screwcap tube
113	Sputum aliquot 4 for nucleotides	SPUnuc04	1.7 mL screwcap tube
114	Sputum aliquot 1 for cytokines	SPUcyt01	1.7 mL screwcap tube
115	Sputum aliquot 2 for cytokines	SPUcyt02	1.7 mL screwcap tube
116	Sputum aliquot 3 for cytokines	SPUcyt03	1.7 mL screwcap tube
117	Sputum aliquot 4 for cytokines	SPUcyt04	1.7 mL screwcap tube
118	Sputum DPBS_Zymo aliquot (MOP 5 - 7.1.4.4a/b)	SPU_DPBS_Zymo	1.7 mL screwcap tube
119	Sputolysin filtrate 50 mL conical	SPU_slysin	intermediate processing, not final aliquot

120	Sputolysin aliquot 1	slysin01	Nalgene 15 mL cryovial
121	Sputolysin aliquot 2	slysin02	Nalgene 15 mL cryovial
122	Sputum cell count (MOP 5 - 7.1.5.1)	SPU_count	intermediate processing, not final aliquot
123	Sputum slide 1	SPUslide01	glass slide
124	Sputum slide 2	SPUslide02	glass slide
125	Sputum slide 3	SPUslide03	glass slide
126	Sputum slide 4	SPUslide04	glass slide
127	EBC Aliquot 1	EBC01	1.7 mL screwcap tube
128	EBC Aliquot 2	EBC02	1.7 mL screwcap tube
129	EBC Aliquot 3	EBC03	1.7 mL screwcap tube
130	EBC Aliquot 4	EBC04	1.7 mL screwcap tube
131	Freezer box 1 of 2 top	Box 01 of 02	box for aliquot storage and shipment
132	Freezer box 1 of 2 bottom	Box 01 of 02	box for aliquot storage and shipment
133	Freezer box 2 of 2 top	Box 02 of 02	box for aliquot storage and shipment
134	Freezer box 2 of 2 bottom	Box 02 of 02	box for aliquot storage and shipment
135	Sputum refrigerated storage top	SPU-Stor	box for aliquot storage and shipment
136	Sputum refrigerated storage bottom	SPU-Stor	box for aliquot storage and shipment
137	Sputolysin 3x3 storage top	Slysin-Stor	box for aliquot storage and shipment
138	Sputolysin 3x3 storage bottom	Slysin-Stor	box for aliquot storage and shipment
139	PAXgene DNA storage top	PXGR-StorDNA	box for blood tube storage and shipment
140	PAXgene DNA storage bottom	PXGR-StorDNA	box for blood tube storage and shipment
141	PAXgene RNA storage top	PXGR-StorRNA	box for blood tube storage and shipment
142	PAXgene RNA storage bottom	PXGR-StorRNA	box for blood tube storage and shipment
143	Misc	MISC	
144	Misc	MISC	
145	Misc	MISC	
146	Misc	MISC	
147	Misc	MISC	
148	Misc	MISC	
149	Misc	MISC	
150	Misc	MISC	
151	Misc	MISC	
152	Misc	MISC	
153	Misc	MISC	
154	Misc	MISC	
155	Misc	MISC	
156	Misc	MISC	
157	Misc	MISC	
158	Misc	MISC	
159	Misc	MISC	
160	Misc	MISC	

161	Misc	MISC	
162	Misc	MISC	

Appendix 10. Tube Drawing Instructions



BD P100 Tube Drawing Instructions

1. With a blood collection set, perform venipuncture using your institution's recommended standard procedure for phlebotomy.

2. **Center the P100 tubes in the single use holder and push tube onto non-participant needle in one swift movement.** The non-participant needle must penetrate the tube stopper and the mechanical separator in the center.

   **NOTE: If the needle penetrates the stopper of mechanical separator in the sidewall, this could result in premature vacuum loss and/or premature separation of the mechanical separator. If vacuum loss or premature separation of the mechanical separator occurs, discard the tube and redraw the specimen with a new tube.*

3. Hold the P100 tube vertically, below the blood donor's arm, during blood collection.

PreAnalytix PAXgene RNA Blood Tube Drawing Instructions

Setup:

1a. Ensure that the PAXgene Blood RNA tube (A) is at room temperature.
1b. The PAXgene Blood RNA tube should be the last tube drawn in the phlebotomy procedure.

Venipuncture:

2. With a blood collection set, perform venipuncture using your institution's recommended standard procedure.

Blood Collection:

3a. Hold the PAXgene Blood RNA tube vertically, below the blood donor's arm, during blood collection.
3b. Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.

After Blood Collection:

4a. Gently invert the PAXgene Blood RNA tube 12 times.
4b. Store the PAXgene Blood RNA tube upright at room temperature (18°C-25°C) for a minimum of two hours and a maximum of 72 hours before processing or transferring to freezer (-80°C).

SOURCE
SPIROMICS Study of Early COPD Progression

MOP 5

SPUTUM INDUCTION AND PROCESSING

Version 4.4
October 13, 2023

1 SUMMARY

To safely obtain airway sputum samples with minimum contamination of oral squamous epithelial cells, and to accurately collect and store the sample prior to delivery to laboratory personnel. Sputum induction provides an opportunity to directly measure biomarker activity within the lung.

1.1 Procedure Specific Inclusion/Exclusion Criteria

Inclusion:

- No specific inclusion criteria.

Exclusion:

- Participants with a known intolerance to albuterol or salmeterol.
- Participants with a known history of poor outcomes with sputum induction.
- Participants with a post-bronchodilator $FEV_1 < 35\%$ during screening.
- Participants incapable of performing adequate spirometry.

1.2 Brief Testing Sequence for Each Visit

To conduct a sputum induction, subjects must not eat or drink anything (except water, clear fruit juice, clear carbonated caffeine-free beverages, or mild herbal/caffeine free tea) for at least one hour prior to beginning the induction. Subjects must also have been able to perform three reproducible flow-volume curves during the pulmonary function testing portion of the study visit. Post-bronchodilator values should be used as the sputum induction baseline FEV_1.

Subjects should be coached in the proper technique for saline inhalation, coughing, and nasal/oral/pharyngeal cleansing. The coordinator then conducts the induction processes. Saline concentration levels and length of inhalation are determined by the initial post-bronchodilator spirometry baseline. Sputum samples must be processed without delay after collection.

The sputum sample must be kept on ice during the sputum induction. If the processing unit is not in the same building and/or the specimen cannot be delivered immediately, then prior to transporting to the processing unit, the sputum cup should be placed deep into a lab bucket which is full of ice, and the bucket should be covered with a lid for immediate transport.

Processing should begin without delay following collection. If a delay cannot be avoided, it should be no longer than two hours.

1.3 Measurements

1) Mucin/water content:
    a. Total mucin concentration
    b. MUC5AC and MUC5B contributions to total mucins
    c. Mucus rheologic parameters
    d. Mucin complex discovery proteomics: mass spectrometry
2) Microbiology
    a. Bacteria
    b. Viruses
3) Cytokines
4) Nucleotides/nucleosides
5) Cell numbers

2 EQUIPMENT

2.1 Durable Supplies/Equipment

Devilbiss Ultra-Neb 99 Ultrasonic Nebulizer (or SOURCE-approved equivalent nebulizer) and pulmonary function equipment. The testing must be performed in an area with a sink the subject can expectorate into.

2.1.1 Cleaning

- Nebulizer Unit
- BP monitor and stethoscope
- Worktable
    - Equipment should be wiped down with disinfectant, such as Sani-cloth plus germicidal disposable wipes or other institutional disinfectant.

2.2 Disposable Supplies

Disposable supplies required are (see **Appendix III** for additional supplier information):

- nebulizer cups and lids
- disposable plastic aerosol tubing
- mouthpiece
- tissues
- container with ice for sample storage
- sterile specimen cups with lids

- paper spacer for albuterol puffer
- drinking cup
- sterile syringes
- 3%, 4%, and 5% and 0.9% saline
- Albuterol

3 PERSONNEL

3.1 Qualifications and Training

The individual performing the sputum induction should be familiar with the equipment used and all required steps in the induction procedure. The individual should also be knowledgeable about sample requirements and preparation. Persons performing sputum induction should be proficient in pulmonary function testing and able to administer fast-acting bronchodilators (albuterol) when necessary. Additionally, since many of these subjects will have Chronic Obstructive Lung Disease (COPD) and/or asthma, the technician should be able to recognize and respond to other respiratory problems, which may occur with these study subjects.

Study personnel are either centrally trained, trained by individuals who have participated in central training, trained during monitoring visits, or trained via webcast. All personnel conducting SOURCE sputum induction and processing must be certified and maintain that certification on an annual basis.

4 SUBJECT PREPARATION

4.1 Safety

Always use universal precautions when handling body fluids (wear gloves).

Subjects should never be left unattended once the procedure has started. All subjects will have albuterol (2 puffs) administered at least 165 minutes prior to the procedure. Oxygen and albuterol will be available.

Communicate with the processors and study physician prior to starting.

4.1.1 FEV_1 values

Sputum induction should not be performed if the subject is unable to perform acceptable/valid spirometry trials or if baseline spirometric indicators are below stated acceptable criteria. Participants whose initial baseline post-bronchodilator FEV_1 is less than 35% predicted should not be included in the sputum induction portion of SOURCE. If at any point during the procedure, a participant's FEV_1 decreases by 20% or greater from their post-bronchodilator sputum induction baseline (taken immediately prior to induction), the procedure should be

stopped. *NOTE: Healthy control subjects will not be treated with a bronchodilator prior to sputum induction.*

Immediately prior to sputum induction: Spirometry will be performed (3 trials) using ATS/ERS standards in mind and the best FEV_1 calculated *(NOTE: Sites may also use a peak flow meter or other device capable of assessing FEV_1 in place of a spirometer if a spirometer is not available or to reduce participant fatigue)*. If < 165 minutes have elapsed since the last post-bronchodilator FEV_1, then use the best FEV_1 from these three trials as the pre-sputum induction baseline and calculation of safety values. If > 165 minutes have elapsed since the last post-bronchodilator FEV_1, then redose with two puffs bronchodilator and perform post-bronchodilator spirometry (3 trials). Use the best post bronchodilator FEV_1 from these three more recent trials as the baseline and for calculation of safety values. *NOTE: All spirometry trial values should be reviewed by a qualified healthcare provider (MD, RN, RCP) or knowledgeable PI prior to beginning the actual sputum induction.*

For patients with COPD, depending on the severity of the disease, the procedure will always have the flexibility to administer doses of hypertonic saline less than 3%, 4%, or 5% for periods less than 7 minutes, if necessary.

4.1.2 Previous problems with induction or bronchodilators

Subjects with known intolerance to albuterol or salmeterol, or who have a history of intolerance to sputum induction, should not be included in sputum induction portion of this study.

5 PROCEDURE STEPS

5.1 Nebulizer Preparation

1. The technician should wash their hands prior to preparing the nebulizer, and all procedures should be performed in a clean (not sterile) fashion.

2. Insert the nebulizer chamber into the unit so that the notch at the bottom of the chamber is aligned with the locating rib at the front of the unit. When positioned properly, the fill label on the chamber will be facing you.

3. Insert the RF reflector ring (metal disc with a hole in the middle and a notch on one side) into the nebulizer chamber.

4. Fill the nebulizer chamber with tap water up to the fill line on the side of the chamber. The unit will not function if the water level is too low or too high.

5. Place the disposable nebulizer cup and lid into the chamber. A length of disposable aerosol tubing, two sections or approximately 12”, should be attached to the elbow for the air supply and to the lid on the disposable cup. A second length of tubing, five sections

or approximately 30", should be attached to the other port on the lid of the nebulizer cup. The mouthpiece should be inserted on the other end of the aerosol tubing.

6. Place 30 mL of saline at each concentration into the disposable cup.

7. Turn the unit on with the switch on the lower right-hand side of the cover. Adjust the output to the highest level the subject can tolerate, ideally no less than halfway.

5.2 Saline Preparation

3% saline, 0.9% saline, and 10% saline are available in 15 mL disposable vials.

- To make 4% saline, mix equal parts of 3% saline and 5% saline.
- To make 5% saline, mix 18 mL of 0.9% saline and 15 mL of 10% saline.

Prior to mixing the saline preparations, the technician should use good handwashing technique, and all solutions should be handled in a sterile manner (single patient use saline, mix in sterile containers, use new syringe for each subject).

In some cases, the site pharmacy will prepare the saline concentrations. In these instances, it is acceptable for the pharmacy to use bulk volumes of saline to make the appropriate concentrations.

Instructions on how to use 7% to make 5% are as follows:

1. To make 100 mL of 5%: 71 mL 7% + 29 mL sterile water
2. To make 50 mL of 5%: 35.5 mL of 7% + 14.5 mL sterile water

Once the 5% has been made up, equal volumes of 3% and 5% make 4%. If a different volume is required, follow the same proportions (ratios) as per 100 mL or 50 mL formulas.

The following Amazon link can be used to purchase 7% saline:
https://www.amazon.com/RSV-Hypertonic-Saline-7-BRONCHIOLITIS/dp/B079N1DN1J/ref=sr_1_1_s_it?s=hpc&ie=UTF8&qid=1532976112&sr=1-1&keywords=rsv+7%25

5.3 Subject Preparation

The patient should be seated in a non-rolling chair. Subjects should have last eaten one hour prior to sputum induction. Once one hour has elapsed, all subjects will lightly brush/scrape their tongue 3-5 times. Following this, subjects will thoroughly gargle and rinse their mouths with water, then discard all contents from their mouth into the sink. Once this process is complete, sputum induction may begin. The coordinator conducting the sputum induction should confirm in the DMS that a complete medical history and current health status assessments were

completed during the Fasting Block (i.e., first block) of the study visit. The coordinator should repeat the vital signs assessment prior to sputum induction (heart rate, respiratory rate, temperature, and blood pressure) should be obtained prior to spirometry. Spirometry should not be conducted if the participant is currently or was recently ill *(NOTE: Participant visit should be rescheduled if they are currently or recently ill. Please see MOP 1 – Clinical Center Procedures)*.

For the actual induction, instruct the subject to relax and to inhale through their mouth and exhale through the nose when breathing the saline. The subject should be told to breathe semi-deep tidal breaths but not to hyperventilate. Instruct the subject to expectorate any saliva salt water into a separate waste cup (i.e., not to swallow any buildup of this fluid). This prevents any nausea or discomfort from the saline and helps to preserve a more acceptable specimen. Describe the three-step cleansing technique to the subject prior to starting the induction.

Subject should have fasted for one hour prior to the procedure except for clear liquids, which are allowed *(see above)*. No caffeine should have been consumed within the past 12 hours. However, participants may have clear fruit juice (e.g., apple or cranberry juice), clear carbonated beverages without caffeine (e.g., caffeine free seven-up or ginger ale), or mild herbal/caffeine free tea. These restrictions have implications for timing of induction for coordinators. Coordinators should plan to conduct inductions after PFTs and CT scans are completed and at least one hour after lunch and/or snack have been given to the participant.

Prior to beginning the induction procedure, if a subject is able to produce a spontaneous sputum sample, you may collect this sample in a separate specimen cup labeled spontaneous sample (i.e., SPU_cupSP label). Do not aggressively urge the subject to produce a spontaneous sputum sample – only acquire it if it is readily available following your inquiry.

5.4 Induction Procedure

The induction procedure is dependent on the pre-sputum induction baseline FEV_1 obtained from the subject.

5.4.1 If the pre-sputum induction baseline FEV_1 is greater than or equal to 50% predicted, then:

NOTE: Complete the Induced Sputum Worksheet (ISW) in CDART as you perform the following procedures.

Put 15 mL of 3% saline into the disposable nebulizer. Turn the unit on and start the timer for seven minutes. Ensure that the subject is comfortable and has the mouthpiece properly in their mouth. Adjust the output. If the subject has an urge to cough, they may do so, without scraping, and expectorate the sample into the sterile cup labeled with the subject number (labeled SPU_cupIN).

At the end of the two minutes, stop the timer, turn off the nebulizer, and have the subject come off the mouthpiece. Be careful not to spill or drain the saliva into the nebulizer – the saliva can build up in the tubing.

Perform spirometry. It is acceptable to only obtain one effort at this point – if it is technically acceptable and if it falls into the required range. This is to avoid subject fatigue. If there is any question about the quality of the effort, then allow the subject another minute or so of rest and repeat.

If the FEV_1 has not fallen 10% or more, restart the timer and the nebulizer, and continue until the seven minutes have elapsed. Once the inhalation period is complete, turn off the nebulizer, and perform the three-step cleansing routine, as follows: 1) Bring the subject to the sink and have them rinse mouth, gargle thoroughly, and then spit into the sink; 2) Have the subject clear their throat 2-3 times (i.e., scrape the back of the throat and roof of mouth *(demonstrate)*) and again expectorate this into the sink; and 3) Have the subject blow their nose and discard tissue.

Finally, have the subject give a good cough effort from ONLY the chest (huffy type chest cough) with no coughing from the throat and without scraping the back of the throat, expectorate the sputum into the cup. **DO NOT HAWK OR SCRAPE** when producing the sample. Passively and quietly (no noise) move the sample from the back of the throat to the mouth for final expectoration into the cup. Once the subject can no longer bring up sputum, repeat spirometry with only one good effort.

If the FEV_1 falls less than 10% from the post-bronchodilator FEV_1 baseline, dispose of the remaining saline, and put 15 mL (or total amount of saline when mixed) of 4% saline into the cup. Repeat the procedure for two minutes, then stop the clock and repeat the spirometry. If the FEV_1 fall is still less than 10%, continue with the 4% saline until the 7-minute mark. If the FEV_1 fall is greater than 10% but less than 20%, do not continue with 4% but rather continue with 3% or less (0.9%, if the FEV_1 fall is 16-19%) until the 7-minute mark. Repeat the cleansing and cough steps as above followed by spirometry.

Again, if the FEV_1 falls less than 10%, continue to the final inhalation period with 15 mL of 5% saline for two minutes, then stop the clock. Repeat the spirometry. If the FEV_1 drops less than 10%, continue to completion, and repeat the three-step cleansing routine followed by chest cough, then final spirometry. If the FEV_1 drop is 10-19%, do not continue with 5% but rather give 4% (or 3%, if the FEV_1 is 16-19%) instead of 5%; then repeat cleansing and cough and final spirometry.

5.4.2 If the pre-sputum induction baseline FEV_1 is less than 50% but greater than or equal to 35% predicted, then:

NOTE: Complete the Induced Sputum Worksheet (ISP) in CDART as you perform the following procedures.

Put 15 mL of 0.9% saline into the disposable nebulizer. Turn the unit on and start the timer for seven minutes. Ensure that the subject is comfortable and has the mouthpiece properly in their

mouth. Adjust the output. If the subject has an urge to cough, they may do so, without scraping, and expectorate the sample into the sterile cup labeled with the subject number (labeled SPU_cupIN).

At the end of one minute, stop the timer, turn off the nebulizer, and have the subject come off the mouthpiece. Be careful not to spill or drain into the nebulizer the saliva which typically builds up in the tubing.

Perform spirometry. If the effort is technically acceptable and it falls into the required range, obtain only one effort at this point. This is to not fatigue the subject. If there is any question about the quality of the effort, then allow the subject another minute or so of rest and repeat. If the fall in FEV_1 is less than 20%, restart the timer and the nebulizer, and continue until two minutes have elapsed.

Repeat spirometry. Again, if the fall in FEV_1 is less than 20%, continue the induction until five minutes have elapsed. Repeat spirometry. If the fall in FEV_1 is still less than 20%, continue until the full seven minutes have passed. Bring the subject to the sink and have them perform the three-step cleansing routine as described above: 1) To rinse mouth and gargle thoroughly and then spit into the sink; 2) The subject should be told to clear their throat (i.e., scrape the back of the throat and roof of mouth *(demonstrate)*) and again expectorate this into the sink; 3) The subject then blows their nose and discard tissue. Finally, have the subject give a good huffy type chest cough effort from the chest and without scraping the throat, expectorate the sputum into the cup. **DO NOT HAWK OR SCRAPE** when producing the sample. Passively and silently move the sample from the back of the throat to the mouth for expectoration into the sample cup.

Repeat spirometry. If the fall in FEV_1 is less than 10%, dispose of the remaining saline and put 15 mL (or total amount of saline when mixed) of 3% saline into the cup. If the fall in FEV_1 is between 10%-19%, add a new 15 mL of 0.9% saline. With either 3% or 0.9% in the cup, repeat the same procedure as above for another seven minutes (i.e., performing FEV_1 checks at 1, 2, and 5 minutes). If the fall in FEV_1 is less than 20% at 1, 2, and 5 minutes, continue the procedure until the 7-minute mark, then perform the cleansing steps and cough attempts. Following the last cough attempt, perform spirometry.

If the fall in FEV_1 is less than 10% and you had 3% saline in the cup for the previous inhalation, put 15 mL of 4% saline in the cup and repeat all spirometry steps (FEV_1 checks at 1, 2, and 5 minutes); cleansing and cough attempts as per the previous inhalation period. If you had 0.9% saline in the cup for the previous inhalations, put 15 mL of 3% saline in the cup and repeat all spirometry steps (FEV_1 checks at 1, 2, and 5 minutes); cleansing and cough attempts as per the previous inhalation period.

If the fall in FEV_1 is greater than 10% but less than 20%, repeat the final inhalation period with the same (or lesser) saline concentration that was previously used performing FEV_1 checks at 1, 2, and 5 minutes, cleansing steps, and cough attempts.

After the final cough attempt, perform the final spirometry check prior to discharge. FEV_1 should return to within 10% of pre-bronchodilator baseline prior to discharge.

5.5 Important Notes

If the spirometry drops between 10%-19% of post-bronchodilator baseline, you may continue the test at the same saline concentration or a lower saline concentration, but never increase the concentration of saline – lesser concentrations should be considered if the FEV_1 fall is 16-19%.

The test is always terminated immediately when the FEV_1 drops by 20% or more or if the subject becomes distressed and requests that the test be terminated. Once a test is terminated, it may not be restarted under any circumstances. Always be prepared to administer a dose of albuterol if necessary. If a second dose of albuterol is given, perform spirometry after 10 minutes. The subject should not be discharged unless the FEV_1 is within 10% of pre-bronchodilator baseline. Prior to discharge, assess the subject (lung sounds) and assess vital signs. If vital signs are outside the normal range, contact the physician in charge immediately.

The sputum sample should be kept on ice throughout the induction procedure. The subject may take the cup out of the ice bucket to expectorate into, but it should go back on ice immediately afterwards. A second specimen cup labeled "waste" should be used during the procedure to capture saliva and spit that may build up in the subjects' mouth. This waste material should not be expectorated into the "sample" specimen cup (labeled SPU_cupIN).

Unless safety reasons dictate, complete the entirety of the procedure. Do not terminate the procedure early based on the volume of sample in the specimen cup.

Deliver the sample to the processing lab without delay.

5.6 Recordkeeping

Spirometry data should be recorded on the worksheet for each induction. A qualified healthcare provider should review and sign all worksheets.

5.7 Cleaning and Infection Control

Use a new disposable nebulizer cup for each subject each day. Wipe the exterior of the unit with alcohol or another surface disinfecting solution. Change the disposable aerosol tubing between subjects. Remove the nebulizer from the unit and drain it each day.

6 RISKS TO HUMAN SUBJECTS/SAFETY ISSUES

6.1 Possible Adverse Events

Risks with hypertonic saline: Inhalation of hypertonic saline for sputum induction may result in wheezing, coughing, or chest tightness, particularly in susceptible individuals such as those with asthma. Asthmatic subjects in our study will be pre-medicated with albuterol in order to

minimize this risk. Spirometry will be evaluated for all subjects before induction as well as at prescribed intervals during each of the three levels of hypertonic saline. Subjects may also experience transient throat irritation during the hypertonic saline inhalation, but this generally resolves post-induction when the subject is provided with a snack and juice or water.

Risks with albuterol: Risks associated with albuterol use in this study are minimized by carefully observing subjects with serious concomitant illnesses or risk factors for chronic illness, including but not limited to individuals with cardiovascular disease, diabetes, hypertension, active arrhythmias or thyroid disease, glaucoma, and all individuals older than 50 years. Study subjects may most commonly notice a short-lived increase in heart rate and mild tremor as a result of albuterol use.

6.2 Procedure Termination

The procedure is terminated: 1) if the FEV_1 falls by > 20% at any time point, 2) if the subject requests that the procedure be stopped, or 3) after three 7-minute inhalation periods have been completed.

6.3 Central Quality Assurance (GIC and/or Reading Center)

6.3.1 Site Visits

6.3.2 Training/retraining

6.3.3 Equipment validation

6.3.4 Procedure verification

7 SPUTUM PROCESSING, STORAGE, AND SHIPPING

7.1 Sample Processing

Sputum samples will be processed according to the initial weight of the induced sample (see 7.1.1) and whether a spontaneous sample was acquired prior to induction. See **Appendix 9 in *MOP 4 – Biospecimen Collection and Processing*** for specific labels and descriptions for sputum processing. There are five condition options to consider based on these two parameters:

Table 1: Criteria for Selecting Condition and the Steps for Processing each Condition			
Condition	**Induced sputum sample weight is:**	**Spontaneous sample?**	**Complete steps:**
Condition 1	> 1 g	No	7.1.2-7.1.6
Condition 2	0.5 – 1 g	No	7.1.2-7.1.6
Condition 3	< 0.5 g	No	7.1.2-7.1.3
Condition 4	< 0.5 g	Yes	Induced sample: 7.1.2-7.1.3 Spontaneous sample: 7.1.4-7.1.6
Condition 5	None	Yes	Use either Condition A, B, or C (below) depending on the sample weight
IF Condition 5:			
Condition A		> 1 g	7.1.2-7.1.6
Condition B		0.5 – 1 g	7.1.2-7.1.6
Condition C		< 0.5 g	7.1.2-7.1.3

Indicate which condition you are using in Question 6a on the Sputum Processing Worksheet (SPW).

In situations where only 0.2 g of sputum with heavy salivary contamination is collected, devote the sample to the first mucin endpoint.

7.1.1 Weighing sputum sample

NOTE: The labels to be used in the following steps include – SPU_pet, SPU_SP_W or SPU_IN_W.

1) Pour entire sample into Petri dish and record color (clear, yellow, green) and characteristics (visible plugs, aggregates, foamy, viscous) – do not spend more than a minute or two on this.
2) Weigh an empty 50 mL tube with lid and record weight on it.
3) Zero the balance.
4) Pour sample into the tube and measure the total weight in grams.
5) Put sample on ice. Steps 7.1.2 through 7.1.6 draw off this sample.

7.1.2 Processing whole sample for mucins

NOTE: The labels to be used in the following steps include – SPUmucin.

1) Weigh an empty micro centrifuge tube.
2) Zero the balance.
3) Measure 0.250 g of whole sputum sample (if there is a small amount of sputum, you can go as little as 0.100 g).
4) Record weight of whole sputum.

5) Add 0.50 mL of 6M guanidine reduction buffer. Mix by tipping the tube back and forth manually (NO vortexing, a rotator mixer can be used if necessary and available) until sample is reasonably homogenized.
6) Store sample at 2-8°C in box labeled "SOURCE Mucin."

7.1.3 Processing microbiology sample

NOTE: The labels to be used in the following steps include – SPUmicro01, SPUmicro02.

1) Weigh an empty micro centrifuge tube.
2) Zero the balance.
3) From remaining sample in 7.1.1 above, measure ~0.500 g of whole sputum sample and record the weight (where there is < 0.500 g available, don't use less than 0.250 g).
4) Add an equal volume (~500 µL) of Zymo Research RNA/DNA Shield to the sample and mix.
5) Divide the sample into two equal aliquots, store at -80°C, and ship on dry ice.

7.1.4 Processing total remaining whole sample for cytokines, nucleotides, cell counts, and cytospins

NOTE: The labels to be used in the following steps include – SPUnuc01, SPUnuc02, SPUnuc03, SPUnuc04, SPUcyt01, SPUcyt02, SPUcyt03, SPUcyt04, SPU_DPBS_Zymo, SPU_slysin, slysin01, slysin02.

1) Weigh an empty 50 mL conical test tube.
2) Zero the balance.
3) After separating any clear runny saliva from the more viscous "main" sample, add the remaining "main" sample from 7.1.1 above to the 50 mL conical test tube and record weight in grams.
4) i. Add 8x sample weight of cold EDTA-DPBS buffer solution, homogenize for 15 minutes on a rotating tumbler then; ii. spin at 790 g at 10 minutes then remove half the volume of supernatant; iii spin this removed volume at 1500 g for 10 minutes and collect the supernatant of this sample and store at -80ºC as follows:

    a. If the sample volume is greater than 8.6 mL, obtain four 1000 µL aliquots for nucleotides, four 1000 µL aliquots for cytokines. Of the remaining sample, take 600 µL and mix it 1:1 with Zymo Research RNA/DNA shield. Apply the label called "SPU_DPBS_Zymo" to the aliquot containing the Zymo Research RNA/DNA shield.
    b. If the sample volume is less than 8.6 mL, start by getting one nucleotide sample between 200-500 µL and one cytokine sample at 200 µL. Mix one of the next cytokine aliquots 1:1 with Zymo Research RNA/DNA shield and apply the label called "SPU_DPBS_Zymo" to this aliquot.

c. If there is sample leftover after that, then continue alternating between nucleotide and cytokine aliquots (i.e., 200-500 µL for nucleotides, 200 µL for cytokines) until finished.
d. Store all supernatant samples immediately in a -80ºC freezer.

5) To the remaining "main" sample, add a volume of 0.2% DTT (diluted with 1mM EDTA) that is equal to the same volume you previously removed from the "main" sample (i.e., half the volume of DPBS you added to initial weight of sample. For example, if initial volume of DPBS added to the sample = 8 mL, you need to add 4 mL of 0.2% DTT as follows: 0.8 mL DTT from stock vial (1%) + 3.2 mL of cold DPBS = 4 mL of 0.2% DTT).
6) Pipet the sample up and down with P1000 pipette to break up any clumps (this is especially necessary for very viscous/thick samples), then homogenize the sample for 15 minutes on a rotating tumbler. Following homogenization, filter the sample through a pre-wetted 48-53 µm nylon mesh filter into a new 50 mL tube.
7) Spin the sample filtrate at 790 g for 10 minutes. Remove and store the entire volume of the DTT containing supernatant at -80ºC in the two tubes provided. The two tubes will either be:

a. 2 x 1.5 mL screwcap tubes, if the volume is < 2.6 mL (= 2 aliquots of 1.3 mL each), or
b. 2 x 15 mL Nalgene tubes, if the volume is > 2.6 mL.

8) Resuspend the cell pellet in 0.25 – 2 mL of cold DPBS depending on the thickness of the pellet ("thin" pellets require less volume; "thick" pellets require more volume).

7.1.5 Cell Counts

NOTE: The labels to be used in the following steps include – SPU_count.

1) From last step in 7.1.4 above, combine 10 µL of resuspended sample with 10 µL of trypan blue stain.
2) Place 10 µL of mixture on the hemacytometer.
3) Count live (clear) cells and dead (blue) cells in each of the four corner quadrants. *(NOTE: Include bronchial epithelial cells (BECs) but exclude RBCs. Enumerate squamous epithelial cells in each quadrant but do not include them in the total live/dead cell count.)*
4) Total Cell Count (TCC) in the sample = [{sum of 4 grids/4}x 2 x 10^4] x Volume of Sample.
5) Determine number of cells/mg (= TCC/weight of processed sample from 7.1.5 (1) above).
6) % Viability = (live cells/total cells) x 100.

7.1.6 Cytospins

NOTE: The labels to be used in the following steps include – SPUslide01, SPUslide02, SPUslide03, SPUslide04.

1) From 7.1.5 above, adjust the sample by removing (too dilute) or adding (too concentrated) DPBS to give a final concentration of 0.5 – 1.0 x 10^6 cells/mL; mix before loading the cytospins.
2) Make four slides (if possible, you must make at least one slide): use 60 µL for the 1st slide, 70 µL for the 2nd, 80 µL for the 3rd, and 90 µL for the 4th (single cytofunnel recommended). *NOTE: These volumes are based on the Shandon Cytospin IV model. If you are using a different cytospin instrument, refer to the manufacturer's instructions on appropriate sample concentrations and volumes and spin speed to use.*
3) Spin for six minutes at 450 rpm, then remove slides and allow to air dry.
4) Fix all four slides with 95% ethanol for 10 minutes, then allow to air dry. Cells should be fixed for 10 minutes.
5) Do NOT mount with Cytoseal or coverslip.
6) Ship all slides to the reading center. Store all slides in a SOURCE box at room temperature.
7) Centrifuge the remaining sputum sample at 790 g for 10 minutes; then remove and discard the entire supernatant volume. Add 1 mL of Zymo Research RNA/DNA Shield reagent to the cell pellet, then store at -80°C.

7.2 Sample Storage and Shipping

Sputum specimen storage and shipping-

Sputum samples will be stored and shipped in different types of boxes depending on how the given sample should be shipped (i.e., according to differences in temperature):

- Sputum samples that should be stored at -80°C (with the exception of the Sputolysin/DPBS samples) will be shipped in **Box 2 (see MOP 4)**, along with urine and plasma aliquots collected for the given participant. Aliquots should be placed in the 2 x 2", 9x9 sectioned freezer boxes in the order outlined below.

Box 2

Urine un-preserved (UR) aliquot 1	Urine un-preserved (UR) aliquot 2	Urine un-preserved (UR) aliquot 3	Urine un-preserved (UR) aliquot 4	Urine un-preserved (UR) aliquot 5	Urine un-preserved (UR) aliquot 6	Urine un-preserved (UR) aliquot 7	Urine un-preserved (UR) aliquot 8	Urine un-preserved (UR) aliquot 9
Urine un-preserved (UR) aliquot 10		Urine preserved (PUR) aliquot 1	Urine preserved (PUR) aliquot 2	Urine preserved (PUR) aliquot 3	Urine preserved (PUR) aliquot 4	Urine preserved (PUR) aliquot 5	Urine preserved (PUR) aliquot 6	Urine preserved (PUR) aliquot 7
Urine preserved (PUR) aliquot 8	Urine preserved (PUR) aliquot 9	Urine preserved (PUR) aliquot 10		Tube #6 P100 Plasma aliquot 1	Tube #6 P100 Plasma aliquot 2	Tube #6 P100 Plasma aliquot 3	Tube #6 P100 Plasma aliquot 4	Tube #6 P100 Plasma aliquot 5
Tube #6 P100 Plasma aliquot 6	Tube #6 P100 Plasma aliquot 7	Tube #6 P100 Plasma aliquot 8	Tube #6 P100 Plasma aliquot 9	Tube #6 P100 Plasma aliquot 10	Tube #6 P100 Plasma aliquot 11	Tube #6 P100 Plasma aliquot 12	Tube #6 P100 Plasma aliquot 13	Tube #6 P100 Plasma aliquot 14
Microbiology Aliquot 1 (MICZ) See 7.1.3	Microbiology Aliquot 2 (MICZ) See 7.1.3		Nucleotide Aliquot 1 (NUC) See 7.1.4.4a	Nucleotide Aliquot 2 (NUC) See 7.1.4.4a	Nucleotide Aliquot 3 (NUC) See 7.1.4.4a	Nucleotide Aliquot 4 (NUC) See 7.1.4.4a	Cytokine Aliquot 1 (CYT) See 7.1.4.4a	Cytokine Aliquot 2 (CYT) See 7.1.4.4a
Cytokine Aliquot 3 (CYT) See 7.1.4.4a	Cytokine Aliquot 4 (CYT) See 7.1.4.4a	DPBS Aliquot mixed with Zymo Shield (CYTZ) See 7.1.4.4a			EBC aliquot 1	EBC aliquot 2	EBC aliquot 3	EBC aliquot 4

- Sputolysin/DPBS samples (see 7.1.4.7; recorded in questions 10c1/10c2 on the Sputum Processing Worksheet form) are to be stored at -80°C and shipped in a 3x3 box. Aliquots should be placed in 3x3 sectioned freezer boxes in the order outlined below.

Sputolysin Box

Participant 1 - Sputolysin/DPBS aliquot 1	Participant 1 - Sputolysin/DPBS aliquot 2	Participant 2 - Sputolysin/DPBS aliquot 1
Participant 2 - Sputolysin/DPBS aliquot 2	Participant 3 - Sputolysin/DPBS aliquot 1	Participant 3 - Sputolysin/DPBS aliquot 2
Participant 4 - Sputolysin/DPBS aliquot 1	Participant 4 - Sputolysin/DPBS aliquot 2	

- Mucin samples (section 7.1.2) are to be stored at 4°C and shipped in a 9x9 sectioned box. Samples should be placed in the box as follows (no more than 24 participants' samples should be placed in a given box):

Mucin Samples Box

Participant 1 Mucin Sample	Participant 2 Mucin Sample	Participant 3 Mucin Sample	Participant 4 Mucin Sample	Participant 5 Mucin Sample	Participant 6 Mucin Sample	Participant 7 Mucin Sample	Participant 8 Mucin Sample	Participant 9 Mucin Sample
Participant 10 Mucin Sample	Participant 11 Mucin Sample	Participant 12 Mucin Sample	Participant 13 Mucin Sample	Participant 14 Mucin Sample	Participant 15 Mucin Sample	Participant 16 Mucin Sample	Participant 17 Mucin Sample	Participant 18 Mucin Sample
Participant 19 Mucin Sample	Participant 20 Mucin Sample	Participant 21 Mucin Sample	Participant 22 Mucin Sample	Participant 23 Mucin Sample	Participant 24 Mucin Sample			

Slides

Storage: Store slides in covered slide box at room temperature.

Shipping: Send all slides to the slide reading core lab; ensure all slides are fixed but unstained and do NOT have a cover slip. See below **Appendix II** for specific shipping instructions and diagrams. Shipping address is below:

Dr. Neil Alexis/Ms. Heather Wells;
US EPA Human Studies Facility,
UNC Chapel Hill
104 Mason Farm Rd.
511 EPA Building
Chapel Hill NC, 27599
919-966-9356

8 REFERENCES

1. Pin, I; Use of Induced Sputum cell counts to Investigate Airway Inflammation in Asthma. Thorax 1992; 47: 25-29
2. De la Fuentes et al. Safety of Sputum Induction in Severe Asthma. Am. J. Respir Crit Care Med. 1998; 157(4): 1127
3. DeVilbiss ULTRA-NEB□99 Ultrasonic Nebulizer Instruction Guide

9 APPENDICES

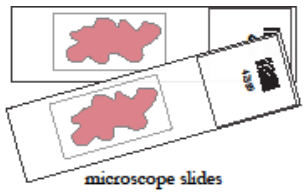
Appendix I. EDTA Formulation

- The molecular weight of EDTA is 372.
- Weigh 0.186g of solid EDTA and add it to 500 mL of 1X PBS buffer to make 1mM EDTA solution.
- Mix solution until homogenized.

Appendix II. Instructions for Creating a Shipping Manifest and Packaging and Shipping of Sputum Slides

If slides are created for a SOURCE participant, please enter the participant's Label ID into the SLI form, under the site-level ID (see Section 7.2 in *MOP 6 – Data Management System*). Print this shipping form/manifest and include it in the package that will be sent to the Sputum Reading Center.

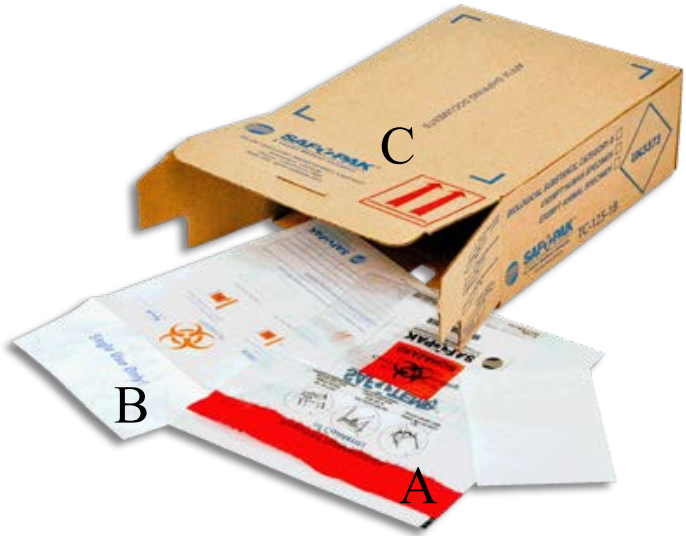
Microscope Slide Shipping Protocol



Place slide(s) in a plastic slide carrier

Securely wrap slide carrier in bubble wrap

Place bubble-wrapped slides in biohazard bag and seal (**A**), then place in secondary biohazard envelope (**B**)



Place envelope in appropriately labeled cardboard box (Biological Substance, Category B, UN 3373, and Exempt Human/Animal Specimen Markings) (**C**)

A + B + C is the Saf-T-Pak S250 Shipping Kit (Recommended)
To order: Fisher Scientific Catalog Number: NC9202961

Appendix III. Sputum Induction and Processing Disposable Supply Information

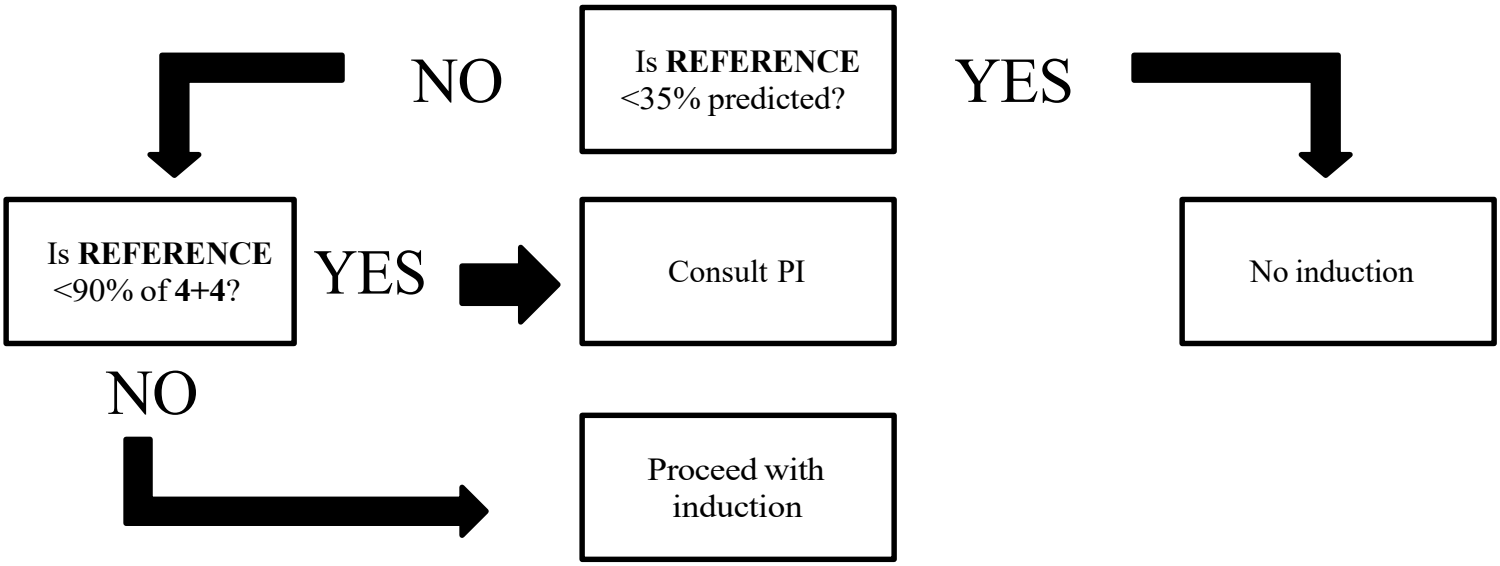
Sputum Induction Disposable Supplies	**UNC BioSpecimen Processing Facility (BSP) supplies**	**Site responsibility**
Nebulizer cups and lids		X
Disposable plastic aerosol tubing		X
Mouthpiece		X
Tissues	X	
Container with ice for sample storage		X
Sterile specimen cups with lids	X	
Paper spacer for albuterol puffer	X	
Drinking cup	X	
Sterile syringes		X
0.9%, 3%, 4%, and 5% saline	X (*can supply 0.9%, 3%, and 7%, if needed, from which 4% and 5% can be made*)	X
Albuterol		X
Sputum Processing Disposable Supplies	**UNC BioSpecimen Processing Facility (BSP) supplies**	**Site responsibility**
Petri dish	X	
50 mL conical	X	
Microcentrifuge tubes	X	
6M guanidine reduction buffer	X	
5” x 5” x 2” boxes	X	
Zymo Research DNA/RNA Shield	X	
DPBS (w/o Ca/Mg)	X	
Sputolysin (DTT in phosphate buffer)	X	
EDTA	X	
Various serological and micropipette tips	X	
15 mL Nalgene tubes	X	
Hematocytometer		X
Slides	X	
Cytofunnels	X	
95% ethanol	X	
Slide box	X	

SPIROMICS Sputum Induction Procedural Flow Chart

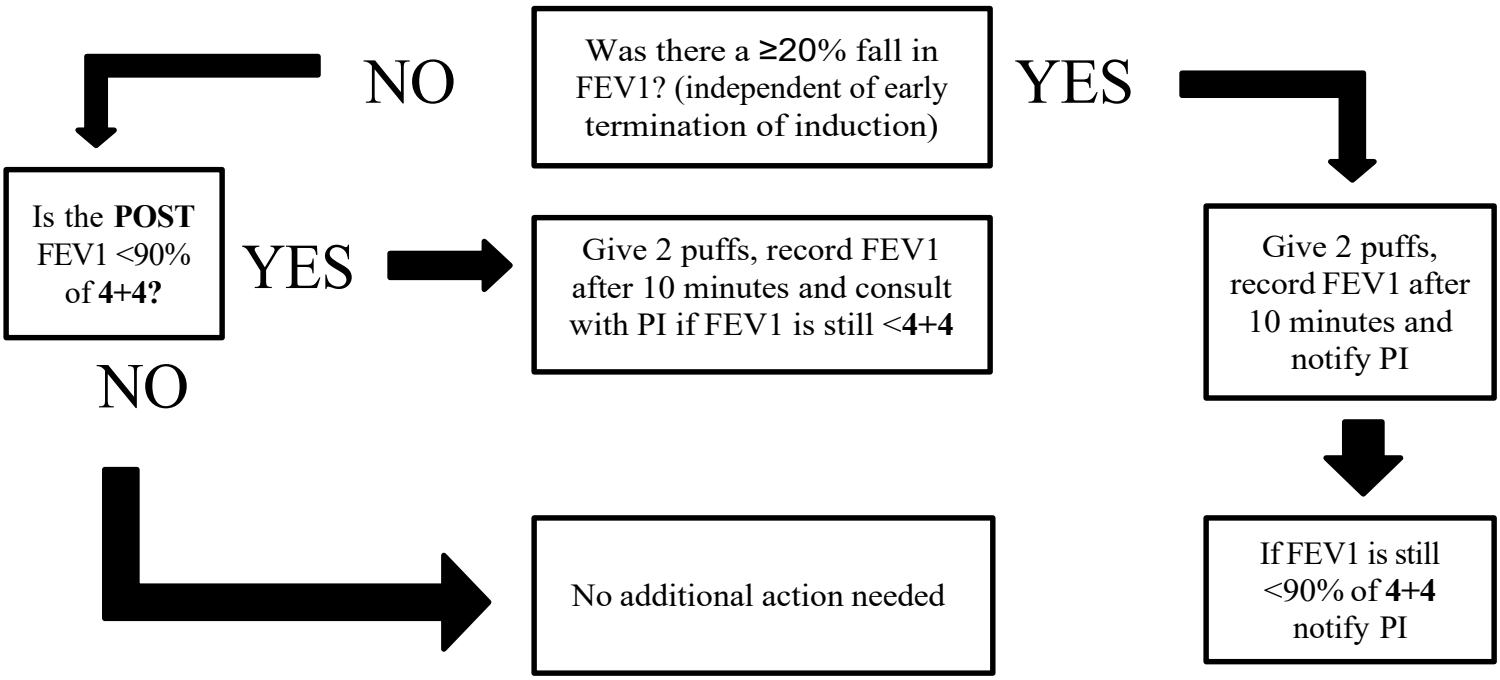
Legend:

4+4 = Nadir of pre-BD FEV1 and post-4+4 FEV1
REFERENCE = FEV1 value used to determine 10% and 20% values to observe falls in FEV1 during sputum induction
POST = Immediate post-induction FEV1

PRE INDUCTION



POST INDUCTION



Sputum Induction/ Processing Webinar

https://www.youtube.com/watch?v=_bxbypN9IQo

Sputum Processing Training Video

https://www.youtube.com/watch?v=d-hEgDcOIGk

Core CT Mini Data Dictionary and Category Codes (see below)

Row #	CTCategoryLabel	CTCategoryCode	CORE 7 CT MINI Variable Name (GIC)	CORE 7 CT MINI Variable Description	Units	Primary Dataset Source (GIC)	CORE 6.3 CT Variable Name (VIDA)	Old CORE CT Variable Name (VIDA)
1	Lung Volumes (Inspiration)	1	both_total_volume_cm3_INSP	Total volume of both lungs at TLC on inspiratory scan	ml	Core6_3_ct_master_sp1_inv_221028	both_total_volume_cm3	BOTH_TOTAL_VOLUME_CM3_INSP
2		1	both_air_volume_cm3_INSP	Air volume of both lungs at TLC on inspiratory scan. Air volume and tissue volume should add up to total volume	ml	Core6_3_ct_master_sp1_inv_221028	both_air_volume_cm3	BOTH_AIR_VOLUME_CM3_INSP
3		1	both_tissue_volume_cm3_INSP	Tissue volume of both lungs at TLC on inspiratory scan. Tissue volume includes vessel volume	ml	Core6_3_ct_master_sp1_inv_221028	both_tissue_volume_cm3	BOTH_TISSUE_VOLUME_CM3_INSP
4		1	both_vessel_volume_cm3_INSP	Vessel volume of both lungs at TLC on inspiratory scan. Also included in tissue volume	ml	Core6_3_ct_master_sp1_inv_221028	both_vessel_volume_cm3	BOTH_VESSEL_VOLUME_CM3_INSP
5	Lung Volumes (Expiration)	2	both_total_volume_cm3_EXP	Total volume of both lungs at RV on expiratory scan	ml	Core6_3_ct_master_sp1_inv_221028	both_total_volume_cm3	BOTH_TOTAL_VOLUME_CM3_EXP
6		2	both_air_volume_cm3_EXP	Air volume of both lungs at RV on expiratory scan. Air volume and tissue volume should add up to total volume	ml	Core6_3_ct_master_sp1_inv_221028	both_air_volume_cm3	BOTH_AIR_VOLUME_CM3_EXP
7		2	both_tissue_volume_cm3_EXP	Tissue volume of both lungs at RV on expiratory scan. Tissue volume includes vessel volume	ml	Core6_3_ct_master_sp1_inv_221028	both_tissue_volume_cm3	BOTH_TISSUE_VOLUME_CM3_EXP
8		2	both_vessel_volume_cm3_EXP	Vessel volume of both lungs at RV on expiratory scan. Also included in tissue volume	ml	Core6_3_ct_master_sp1_inv_221028	both_vessel_volume_cm3	BOTH_VESSEL_VOLUME_CM3_EXP
9	Lung Density	3	both_mean_hu_INSP	Mean lung density of both lungs at TLC on inspiratory scan	HU	Core6_3_ct_master_sp1_inv_221028	both_mean_hu	BOTH_MEAN_HU_INSP
10		3	both_std_dev_hu_INSP	SD density of both lungs at TLC on inspiratory scan	HU	Core6_3_ct_master_sp1_inv_221028	both_std_dev_hu	BOTH_STD_DEV_HU_INSP
11		3	both_mean_hu_EXP	Mean density of both lungs at RV on expiratory scan	HU	Core6_3_ct_master_sp1_inv_221028	both_mean_hu	BOTH_MEAN_HU_EXP
12		3	both_std_dev_hu_EXP	SD density of both lungs at RV on expiratory scan	HU	Core6_3_ct_master_sp1_inv_221028	both_std_dev_hu	BOTH_STD_DEV_HU_EXP
13	Airway Geometry	4	whole_tree_all_INSP	Pi10 for all airways. Inspiratory scan favored to avoid airway deformation during exhalation.	mm	Core6_3_ct_master_sp1_inv_221028	whole_tree_all	WHOLE_TREE_ALL_INSP
14		4	whole_tree_leq20_INSP	Pi10 for airways with inner perimeter ≤20 mm. Considered more reliable when trachea is excluded.	mm	Core6_3_ct_master_sp1_inv_221028	whole_tree_leq20	WHOLE_TREE_LEQ20_INSP
15		4	rb1_avginnerarea	Mean segmental lumen area RB1	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb1_avginnerarea	
16		4	rb1_stddevinnerarea	SD segmental lumen size RB1	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb1_stddevinnerarea	
17		4	rb1_avgavgwallthickness	Mean segmental wall thickness RB1	mm	Core6_3_ct_airmea_sp1_inv_221028	rb1_avgavgwallthickness	
18		4	rb4.5_avginnerarea	Mean segmental lumen area RB4.5	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb4.5_avginnerarea	
19		4	rb4.5_stddevinnerarea	SD segmental lumen size RB4.5	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb4.5_stddevinnerarea	
20		4	rb4.5_avgavgwallthickness	Mean segmental wall thickness RB4.5	mm	Core6_3_ct_airmea_sp1_inv_221028	rb4.5_avgavgwallthickness	
21		4	rb10_avginnerarea	Mean segmental lumen area RB10	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb10_avginnerarea	
22		4	rb10_stddevinnerarea	SD segmental lumen size RB10	mm^2	Core6_3_ct_airmea_sp1_inv_221028	rb10_stddevinnerarea	
23		4	rb10_avgavgwallthickness	Mean segmental wall thickness RB10	mm	Core6_3_ct_airmea_sp1_inv_221028	rb10_avgavgwallthickness	
24		4	lb1_avginnerarea	Mean segmental lumen area LB1	mm^2	Core6_3_ct_airmea_sp1_inv_221028	lb1_avginnerarea	
25		4	lb1_stddevinnerarea	SD segmental lumen size LB1	mm^2	Core6_3_ct_airmea_sp1_inv_221028	lb1_stddevinnerarea	
26		4	lb1_avgavgwallthickness	Mean segmental wall thickness LB1	mm	Core6_3_ct_airmea_sp1_inv_221028	lb1_avgavgwallthickness	
27		4	lb10_avginnerarea	Mean segmental lumen area LB10	mm^2	Core6_3_ct_airmea_sp1_inv_221028	lb10_avginnerarea	
28		4	lb10_stddevinnerarea	SD segmental lumen size LB10	mm^2	Core6_3_ct_airmea_sp1_inv_221028	lb10_stddevinnerarea	
29		4	lb10_avgavgwallthickness	Mean segmental wall thickness LB10	mm	Core6_3_ct_airmea_sp1_inv_221028	lb10_avgavgwallthickness	
30	Dysanapsis	5	airway_to_lung_ratio_V1	Measure of airway diameter in relation to total lung size	ratio	ms159_dysanapsis_210527		
31	Texture	6	BOTH_GROUND_GLASS	AMFM ground glass opacity (% of TLC)	%	core6_3_ct_txture_sp1_inv_221028	BOTH_GROUND_GLASS	
32		6	BOTH_GROUND_GLASS_RETICULAR	AMFM ground glass reticular (% of TLC)	%	core6_3_ct_txture_sp1_inv_221028	BOTH_GROUND_GLASS_RETICULAR	
33		6	BOTH_HONEYCOMB	AMFM honeycombing (% of TLC)	%	core6_3_ct_txture_sp1_inv_221028	BOTH_HONEYCOMB	
34		6	BOTH_BRONCHOVASCULAR	AMFM bronchovascular bundles (% of TLC)	%	core6_3_ct_txture_sp1_inv_221028	BOTH_BRONCHOVASCULAR	
35		6	both_pct_between_250_600_INSP	Fibrosis	%	Core6_3_ct_master_sp1_inv_221028	both_pct_between_250_600	both_pct_between_250_600
36	Heterogeneity of Ventilation	7	dpm_both_Jacobian_Mean	Mean volume change between inflation and deflation (units?)	ratio	Core6_3_ct_master_sp1_inv_221028	dpm_both_Jacobian_Mean	DPM_BOTH_JACOBIAN_MEAN
37		7	dpm_both_Jacobian_StdDev	SD volume change between inflation and deflation (units?)	ratio	Core6_3_ct_master_sp1_inv_221028	dpm_both_Jacobian_StdDev	DPM_BOTH_JACOBIAN_STDDEV
38		7	dpm_both_ADI_Mean	Mean Anisotropic Deformation Index: unevenness of volume/shape change (units?)	ratio	Core6_3_ct_master_sp1_inv_221028	dpm_both_ADI_Mean	DPM_BOTH_ADI_MEAN
39		7	dpm_both_ADI_StdDev	SD Anisotropic Deformation Index: unevenness of volume/shape change (units?)	ratio	Core6_3_ct_master_sp1_inv_221028	dpm_both_ADI_StdDev	DPM_BOTH_ADI_STDDEV
40	Air Trapping	8	BOTH_PCT_BELOW_EQUAL_856_EXP	Percentage of lung with density ≤-856 HU (%). Air trapping is evaluated on expiratory scans (RV)	%	Core6_3_ct_master_sp1_inv_221028	BOTH_PCT_BELOW_EQUAL_856	BOTH_PCT_BELOW_EQUAL_856
41		8	dpm_both_DPM_AirTrap_pct_EXP	Percentage of lung with air trapping. DPM uses both TLC and RV scans to derive % of RV volume	%	Core6_3_ct_master_sp1_inv_221028	dpm_both_DPM_AirTrap_pct	DPM_BOTH_DPM_AIRTRAP_PCT
42		8	PRM_V1_PERCENT_FSAD_TOTAL	PRM fSAD uses both TLC and RV scans to derive % of RV volume	%	prm_v1_dataset_20171117		TOTAL_FUNCTIONAL_V5
43	Emphysema	9	BOTH_PCT_BELOW_EQUAL_950_INSP	Percentage of lung with density ≤-950 HU (%). Emphysema is assessed on inspiratory scans (TLC)	%	Core6_3_ct_master_sp1_inv_221028	BOTH_PCT_BELOW_EQUAL_950	BOTH_PCT_BELOW_EQUAL_950
44		9	both_hu_at_percentile_15_INSP	Lung density below which 15% of all voxel densities lie (HU), Inspiration	HU	Core6_3_ct_master_sp1_inv_221028	both_hu_at_percentile_15	
45		9	dpm_both_DPM_Emphysema_pct_EXP	Percentage of lung with emphysema. Uses both TLC and RV scans to derive % of RV volume	%	Core6_3_ct_master_sp1_inv_221028	dpm_both_DPM_Emphysema_pct	DPM_BOTH_DPM_EMPHYSEMA_PCT
46		9	PRM_V1_PERCENT_EMPHYSEMA_TOTAL	PRM emphysema uses both TLC and RV scans to derive % of RV volume	%	prm_v1_dataset_20171117		TOTAL_PERSISTENT_V5

Category Code	Category Label
1	Lung volumes on inspiration
2	Lung volumes on expiration
3	Lung density
4	Airway Geometry
5	Dysanapsis
6	Texture
7	Heterogeneity of ventilation
8	Air trapping
9	Emphysema