Manual of Procedures

Lung Tissue Research Consortium (LTRC)

National Heart, Lung, and Blood Institute

Version 3.0

November 8, 2017

The Manual of Operating Procedures (MOP) is to be used as a reference document for NHLBI policies and procedures as they apply to the LTRC study. All staff members participating in the conduct of this study at participating institutions should have access to the MOP and be familiar with its contents. The current version of the MOP and archived versions are posted to the LTRC website:

www.ltrcpublic.com

TABLE OF CONTENTS

1. ROLES AND RESPONSIBILITIES

(See Appendix B for contact information.)

Table 1: Roles and Responsibilities

If You Have Questions Regarding	Study Contact
Site Training (CT)	Brian Bartholmai (Mayo)
Site Contracts or Payments	Lisa Viviano (NHLBI)
Regulatory Documentation	ltrcdm@emmes.com
ICF Review	ltrcdm@emmes.com
Study Materials	ltrcdm@emmes.com
Website (Access and Content)	ltrcdm@emmes.com
Lab Supplies/Resupply	Kathy Matzen (Tissue Core Laboratory)
Barcode Labels	Kathy Matzen (Tissue Core Laboratory)
Advantage eClinical[SM] System	ltrcdm@emmes.com
Data Queries	ltrcdm@emmes.com
Site Monitoring	ltrcdm@emmes.com
Any Other Study Questions	ltrcdm@emmes.com

2. LIST OF ABBREVIATIONS

Table 2: List of Abbreviations

ABBREVIATION	FULL NAME OR TITLE
AIP	Acute Interstitial Pneumonia
ABG	Arterial Blood Gases
ACR	American College of Radiology
AE	Adverse Event
ALARA	As Low As Reasonably Possible
ATS	American Thoracic Society
BD	Bronchodilator
CC	Clinical Center
CL	Core Laboratory
CLIA	Clinical Laboratory Improvement Amendments
COPD	Chronic Obstructive Pulmonary Disease
eCRF	Electronic case report form
CT	Computed Tomography
1-DGel	1-Dimensional Gel
2-D Gel	2-Dimensional Gel
DCC	Data Coordinating Center
DLCO	Diffusing Capacity of Carbon Monoxide
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
ETC	External Tissue Contributor
FEV	Forced Expired Volume
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescent in Situ Hybridization
FRC	Functional Residual Capacity
FTP	File Transmission Protocol
FVC	Forced Vital Capacity
G_{aw}	Airway Conductance
HIPAA	Health Insurance Portability and Accountability Act of 1996
H&E	Hematoxylin & Eosin
HOPE	Hepes-Glutamic acid buffer mediated Organic

ABBREVIATION	FULL NAME OR TITLE
HRCT	High Resolution CT Scan
IAC	Image Acquisition Computer
IHC	Immunohistochemistry
IIP	Idiopathic Interstitial Pneumonias
ILD	Interstitial Lung Disease
IPF	Ideopathic Pulmonary Fibrosis
IRB	Institutional Review Board
ISH	In Situ Hybridization
LCM	Laser Capture Microdissection
LTRC	Lung Tissue Research Consortium
LVRS	Lung Volume Reduction Surgery
MOP	Manual of Procedures
MW	Maximum Voluntary Ventilation
NCHS	National Center for Health Statistics
NDI	National Death Index
NETT	National Ephysema Treatment Trial
NHLBI	National Heart, Lung, & Blood Institute
NSIP	Non-specific Interstitial Pneumonia
OCT	Optimal Cutting Temperature Compound
OSMB	Observational Study Monitoring Board
PACS	Picture Archive Communication System
PCR	Polymerase Chain Reaction
PFT	Pulmonary Function Test
PI	Principal Investigator
PVC	Premature Ventricular Complexes
PRC	Protocol Review Committee
QC	Quality Control
QT-PCR	Quantitative Polymerase Chain Reaction Solvent Protection Effect
R_{aw}	Airway Resistance
RCL	Radiographic Core Laboratory
RV	Residual Volume

ABBREVIATION	FULL NAME OR TITLE
SELDI	Surface Enhanced Laser Desorption/mediated dUTP-fluorescence nick end labeling ionization
SLB	Surgical Lung Biopsy
TCL	Tissue Core Laboratory
TLC	Total Lung Capacity
TMA	Tissue Microarray
TUIMEL	Terminal deoxynucleotidyl transferase (TdT)
UIP	Usual Interstitial Pneumonia
VATS	Video Assisted Thorascopic Lung Biopsy

3. PROTOCOL SYNOPSIS

Table 3: Protocol Synopsis

PROTOCOL TITLE	LUNG TISSUE RESEARCH CONSORTIUM
Objective	Primary: To collect lung tissue and blood samples from participants who undergo medically-indicated lung surgery. Secondary: To annotate collected samples with an array of clinical data, limited exposure data, physiologic studies, and radiographic studies.
Study Design	Observational study of adults with and without lung disease.
Study Population	Adults age 21 and older.
Number of Participants	Up to 400.
Number of Centers	4 clinical; 2 core laboratories.
Inclusion	Adults age 21 and older undergoing lung surgery for suspected malignancy or metastases.
Exclusion	Diagnosis of cystic fibrosis or pulmonary hypertension. Any other condition that, in the judgment of the investigator, precludes participation. Failure to obtain written consent.
Study Duration	November 2016 through February 2019.
Participation Duration:	Typically, not more than four weeks from time of consent, depending on participant's health status and schedule for lung surgery.
Safety	All adverse events and serious adverse events occurring within two calendar days following a protocol-mandated procedure will be monitored.

4. OVERVIEW OF THE LUNG TISSUE RESEARCH CONSORTIUM (LTRC)

4.1 Purpose

The National Heart, Lung and Blood Institute (NHLBI) Lung Tissue Research Consortium (LTRC) was originally established with the primary purpose of collecting lung tissue and blood samples and a secondary purpose of collecting clinical data, limited exposure data, physiologic studies, and radiographic studies from participants with chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), other related idiopathic interstitial pneumonias (IIP) and interstitial pneumonias associated with connective tissue diseases who undergo medically-indicated lung resection. Under this contract period, the protocol will be modified slightly to enhance the collection of lung tissue and clinical data from control participants who require lung surgery. Control participants will be individuals with suspected lung cancer or metastatic disease to the lung, who have pulmonary function tests (PFT) showing normal lung function and normal pre-operative appearance of the lungs on CT imaging, except for lesions thought to represent discrete malignancies. For all participants, regardless of disease findings post-enrollment, non-neoplastic tissue will be collected remotely from a nodule or mass. All tissue and blood specimens and clinical data will be banked and stored for distribution to future investigators who have approved study proposals and are investigating the pathogenesis of lung diseases. It is the ultimate goal of this study that information derived from the LTRC will lead to novel interventional treatments for all disease categories under study.

The Manual of Procedures (MOP) documents the procedures to be used to conduct the LTRC. As these procedures are modified, the DCC will prepare and distribute (via the LTRC study website: www.ltrcpublic.com) a new version of the MOP. Additionally, a numbered memo will alert LTRC staff to the new version and which changes occurred. Every participating institution is expected to have several copies of the MOP available for reference.

During the course of using this MOP, LTRC staffs are encouraged to send feedback to the DCC that will improve the document. Every effort will be made to provide procedure instructions that are clear and complete. This will provide the highest quality data for future analysis. When the MOP is revised, sites must replace the old version with the new version posted to the study website. A three-ring binder is recommended for storing the MOP.

5. ELIGIBILITY CRITERIA

5.1 Screening

In order to identify potential LTRC participants, the study coordinator will routinely review patient records, appointment lists and surgical schedules for patients undergoing treatment within their department. Prior to approaching a potential participant, the coordinator should confirm he/she meets the LTRC inclusion and exclusion criteria as specified in the protocol.

The Enrollment Checklist DCF will be used to confirm the participant's eligibility. For ineligible participants or participants refusing to consent, selected information from the Enrollment Checklist DCF will be transferred to the screening log. This spreadsheet is supplied by the DCC and is discussed further in Section 8. This will not include any personal health information or identifiers.

5.1.1 Screening Time Windows

Screening needs to be completed far enough in advance to allow time for the LTRC interviews and procedures to be completed without interfering with the clinical center’s preoperative procedures. The LTRC participant interviews and procedures are expected to take approximately five hours to complete and must be completed prior to surgery (with the exception of the following forms: Demographic Information, Medical History, Family History, Smoking History, Concomitant Therapy, Environmental, Occupational and Environmental Questionnaires, which may be collected after). Each clinical center should determine the sequence and schedule that is efficacious for the institution and the participant.

6. INFORMED CONSENT

6.1 Background

Obtaining informed consent is essential prior to involvement of human participants in research. The purpose of the informed consent process is to protect an individual's rights. The principles guiding this process are those of respect for persons, beneficence and justice. Each center will document the consent process by use of consent forms that have been approved by their local institutional review board (IRB).

6.2 Institutional Review Board (IRB) Process

A template Informed Consent form for LTRC has been developed and is available on the LTRC study website (www.ltrcpublic.com). Clinical centers are expected to use this document for submission to their institutional review boards (IRBs) for approval to participate in the LTRC. Each clinical coordinator must send the final copies of the consent form to be used in their clinical center, with their IRB's seal of approval notice, to the DCC prior to initiating participant activities in the LTRC. DCC staff will compare the local consents to the template consent. Specific local additions to and editing of the templates may be required in individual institutions, but deletion of material and major rewording of text may need to be explained and justified. Once the consent form has been approved by the local IRB, it cannot be changed without resubmitting it for approval. The DCC must likewise be sent a copy of the revised form.

6.3 Consent Administration

The first step of the process is the assessment on the part of the clinical coordinator and/or Principal Investigator of whether the individuals providing consent have the capacity to make this decision. If decisional capacity is impaired, then informed consent cannot be obtained.

The patient is told about the study procedures, what is expected in terms of time commitment, and the risks and benefits of participating in the study. The consent form contains the required information that is to be disclosed. Each portion of the consent form should be explained in detail. When obtaining consent, the Investigator and/or coordinator should explain the following:

- Purpose of the LTRC.
- Procedures: A clear description of the study in terms of the types of measurements that will be taken, the time it will take to make the measurements and the overall length of commitment.
- Benefits associated with participation.
- Risks, discomforts and precautions. This would include radiation exposures and possible effects of the exercise testing.
- Alternatives to participation.
- Confidentiality of information.
- Availability of information: Patients should be told who to contact if they have questions about study procedures (the PI) or questions about their rights as a research participant (the chair of the local IRB).
- The right to withdraw from the study at any time.

After providing information regarding the procedures, risks and benefits, it is important to give the patient an opportunity to ask questions. Sufficient time must be provided to answer all patient questions prior to signing the consent. It is important to ascertain whether the patient understands and appreciates what they are agreeing to do. Not only does this protect their rights, but it also minimizes the risk of the patient dropping out of the study.

Care should be used throughout the recruitment and informed consent process so a patient does not feel coerced into participation. They should be reassured that their future care at the institution will not be affected should they decide not to participate.

The patient must sign the consent form in the presence of LTRC staff indicating their consent to participate in the study. At least one LTRC coordinator or investigator will witness the signature. Additional witnesses may be required by the local IRB. All signatures on the consent form must be in a non-erasable ink. The participant's LTRC ID number should NOT appear on the consent. A copy of the signed consent is given to the participant to keep.

6.4 Time Constraints for Obtaining Consent

While a patient may be screened for the LTRC, no other LTRC interview or procedure may be performed until written consent has been obtained. Consent should be obtained far enough in advance of the surgical procedure to allow for the completion of the LTRC interviews and procedures without interfering with preoperative preparations or unduly stressing the participant physically or mentally.

6.5 LTRC Main Study Consent Handling

The main study consent statement is an "all or none" form. The participant either accepts it in its entirety and signs it, or does not. Neither partial exclusions, nor special exemptions, are allowed in this process. Consenting to the main LTRC study may not commit the participant to consent to a LTRC Protocol CT scan or Genetics Testing. Checkboxes will be used to consent to these procedures.

Signed consent forms are important legal documents. These original forms should be kept in the participant's LTRC file together with his/her other LTRC forms and documents. These forms are not part of the individual's institutional medical records, but part of his/her participation in the LTRC. Consent forms will be examined during site visits by DCC staff.

6.6 Health Insurance Portability and Accountability Act (HIPAA)

Each clinical center is responsible for administering their institution's HIPAA release form. The original form should be kept in the LTRC file, and the participant should be given a copy.

6.7 Surgical Consent

Each clinical center is responsible for administering their institution's Surgical Consent form or consent process. This is separate from the LTRC consent form.

6.8 Multiple Enrollments in LTRC

Signing the LTRC consent covers the administration of the questionnaires by interview, pulmonary function testing, six-minute walk test, blood draw, and CT scan culminating in surgery, at which time tissue collection is expected. If a participant is scheduled for subsequent pulmonary surgeries, the participant will need to sign a new consent form and the cycle is repeated as outlined in the consent. A new signed consent, administration of questionnaires, pulmonary

function testing, six-minute walk test, blood draw, CT scan are expected for each surgery. Refer to the LTRC Advantage eClinical[SM] Forms Instructions for details on re-enrolling a participant in the data system.

7. PARTICIPANT ASSESSMENT PROCEDURES

7.1 Interviews

Advance work by the clinical center staff facilitates completion of interviews and reduces waiting time. To be properly prepared for the participant's visit, it is suggested that the clinical center staff follow the procedures described below.

The Research Coordinator in each clinical center assembles packets so that forms and other materials are readily available. These packets should include a consent form for the potential participant to sign and the following LTRC study forms: Enrollment Checklist and Demographic Information, Medical and Family History Questionnaires, Smoking History, Concomitant Therapy, Symptom Questionnaire, SF-12 Health Survey, St. George Respiratory Questionnaire, Environmental Questionnaire, and Occupational and Environmental Questionnaire. The ID number assigned to the participant should be written on the forms in the header spaces provided, once the participant is enrolled in the DCC Advantage eClinical^{SM} system and obtains the ID number.

At the beginning of the interview, the interviewer determines the potential participant's willingness to participate in the study and, if willing, obtains informed consent and completes the Participant Demographic and Enrollment Checklist forms. This is estimated to take about one-half hour to complete.

The interviewer then administers the history and exposure questionnaires. This is estimated to take about two hours to complete. All interviews will be conducted in a private, quiet office environment that will facilitate the participant's cooperation and help put the participant at ease.

During an interview, use the exact wording of each question. If the participant expresses doubt as to the meaning of the question, repeat it exactly. Emphasizing individual words or phrases often makes the meaning clear. Further explanation may be needed, but do not cross-examine the respondent. When after brief explanation doubt remains as to whether the answer should be "yes" or "no," the answer should be recorded as "no."

No LTRC procedures will be carried out after a participant has his/her surgical procedure. The tests are designed to assess the participant's phenotype prior to surgery. However, completion of specific questionnaires (Demographic Information, Medical History, Family History, Smoking History, Concomitant Therapy, Environmental and Occupational and Environmental Questionnaires) and the collection of historical information from the medical record or by participant interview may be possible after surgery while the participant is still in the hospital up to four weeks' post-surgery. Questionnaires may not be administered by telephone.

7.2 Pulmonary Function Testing

7.2.1 Post Bronchodilator (BD) Testing

- Testing may be performed up to six months prior to surgery.
- ABGs can be performed pre or post BD.
- Spirometry test procedure should adhere to the 2005 ATS/ERS Standard (3). Spirometry is performed post BD. Airflow obstruction is defined as FEV_1 % predicted < 70 and FEV_1/FVC ratio < 0.60. Use of published Lower Limits of Normal for FEV_1/FVC as criteria for obstruction is also acceptable.

- Lung volume procedures should conform to the 2005 ATS/ERS Standard (2). Lung volumes and airway resistance performed only post BD for obstructed/COPD participants.
- Diffusing capacity for carbon monoxide, single-breath technique performed post BD for obstructed/COPD participants. DLCO test performance should adhere to the 2005 ATS/ERS Standard (4).

Note: Prebronchodilator testing = at least four hours following use of short acting bronchodilator and 12 hours since last use of long-acting bronchodilator. Post bronchodilator testing = at least 15 minutes and no longer than one hour after four inhalations of albuterol. Short acting bronchodilators include short acting beta agonists (e.g., albuterol, fenoterol) and parasympatholytic agents (ipratropium bromide). Long-acting bronchodilators include theophylline preparations and salmeterol, and long-acting pill forms of albuterol.

7.2.2 Bronchodilator Administration

- Albuterol used for BD response testing.
- Metered dose inhaler tube spacer. Activate inhaler in air to check for adequacy of operation.
- Instruct participant to blow out to residual volume (RV), then insert tube in participant's mouth.
- Instruct participant to inhale slowly, inhaler activated during inspiration.
- Participant holds breath for ten seconds.
- Wait one minute and repeat (total 2-4 inhalations).
- Retest 15 minutes later.
- For participants, too dyspneic to properly utilize the metered dose inhaler, alternate administration of albuterol solution via compressor-type aerosol nebulization is acceptable. Post-testing should occur no sooner than 15 minutes following completion of nebulization.

7.2.3 Arterial Blood Gas Tensions (pH, PC02, P02, Carboxyhemoglobin, Total Hemoglobin)

- Participant should be off oxygen and resting for at least ten minutes*. During the rest period, participant is seated comfortably and quiet. Isolation from family/friends is not necessary.

  *If participant de-saturates to 87% oxygen or stays at 88% for one full minute, draw the blood gas without waiting the full ten minutes

- Before or after BD, and in any sequence relative to other PFTs at clinical center's discretion.
- Any brand of blood gas analyzer that meets CLIA/JCAHO standards may be used.
- Carboxyhemoglobin is expressed as percent of total hemoglobin.

7.3 Spirometry

7.3.1 Standardization

- Equipment and procedures are based on the 2005 ATS/ERS recommendations for accuracy and precision (3).
- All technicians should be certified pulmonary function technologists (Nat'l Board of Resp. Care) or meet the recommendations for personnel qualifications issued by the ATS/ERS (1). At a minimum, all technicians performing pulmonary function testing must be "certified" as properly trained by the center PI or by the medical director of the pulmonary function laboratory.
- Standing height should be determined by the formula height = arm span /1.06 for participants with deformity of the thoracic cage.

7.3.2 Reference Equations

- The published predicted values of Hankinson et al (7) will be used. The general form of the prediction equation is:
    - Predicted Value = Z + A (Age) + B (Age2) + C (Height2).

Table 4: Values for Reference Equations

Value	Ethnicity	Gender	Z	A	B	C
FVC	Caucasian	Male	-0.1933	0.00064	-0.000269	0.00018642
FVC	Caucasian	Female	-0.356	0.0187	-0.000382	0.00014815
FVC	African American	Male	-0.1517	-0.01821	0	0.00016643
FVC	African American	Female	-0.3039	0.00536	-0.000265	0.00013606
FVC	Mexican American	Male	0.2376	-0.00891	-0.000182	0.00017823
FVC	Mexican American	Female	0.121	0.000307	-0.000237	0.00014246
FEV_1	Caucasian	Male	0.5536	-0.01303	-0.000172	0.00014098
FEV_1	Caucasian	Female	0.4333	-0.00361	-0.000194	0.00011496
FEV_1	African American	Male	0.3411	-0.02309	0	0.00013194
FEV_1	African American	Female	0.3433	-0.01283	-0.000097	0.00010846
FEV_1	Mexican American	Male	0.6306	-0.02928	0	0.00015104
FEV_1	Mexican American	Female	0.4529	-0.01178	-0.000113	0.00012154

Value	Ethnicity	Gender	Z	A	B	C
FEV_4	Caucasian	Male	0.1102			0.00018188
FEV_4	Caucasian	Female	-0.1373	0.01317		0.00014395
FEV_4	African American	Male	-0.0547			0.00016429
FEV_4	African American	Female	-0.1981	0.00047		0.00013497
FEV_4	Mexican American	Male	0.5757			0.0001784
FEV_4	Mexican American	Female	0.2033	0.0002		0.00014106
PEF	Caucasian	Male	1.0523	0.08272		0.00024962
PEF	Caucasian	Female	0.9267	0.0629		0.00018623
PEF	African American	Male	2.2257			0.00027333
PEF	African American	Female	1.3597	0.03458		0.00019746
PEF	Mexican American	Male	0.087	0.0658		0.00030243
PEF	Mexican American	Female	0.2401	0.06174		0.00022203

- Age is in years at last birthday.
- Height is standing height in cm. (Arm span should be done if indicated by laboratory protocol).
- PFTs predicted are in liters.
- Predicted values for Asian-Americans will use a factor of 0.88 applied to the Caucasian predicted value (6). Other ethnic groups will be as for Caucasians.
- A participant's ethnic identity is self-defined.

7.3.3 Participant Preparation

- Participant seated during testing.
- Nose clips worn.
- Participant to loosen restrictive clothing.
- Participants should avoid a heavy meal for at least two hours prior to testing.
- Pre-BD testing: At least four hours since last short-acting BD and at least 12 hours since last long-acting BD.
- Post-BD testing: At least fifteen minutes and no longer than one hour following administration of albuterol.

7.3.4 Forced Vital Capacity (FVC)

- Instruct participant, including demonstration. Emphasize the necessity for deep, full inspiration, a hard and forceful "blast" and a complete expiration for at least six seconds.
- Perform test until ATS acceptability and repeatability criteria are met. The repeatability criteria are 150 ml for FVC and FEV1. For participants with a FVC of 1.0 liter or less, repeatability for both FVC and FEV1 criteria is ±100 ml.
- Acceptability criteria and problems with suggestions on how to fix them.
    - **Test start:** Peak flow rate should be reached within 80 msec and the peak flow should be "sharp" on FV curve. Back extrapolation no more than 150 cc or 5% of the VC (whichever is greater). Trials with excessive back extrapolated volume should be rejected. Participants may need coaching to get this right (e.g., "BLAST" it out).
    - **Cough:** This can cause flow irregularities. Reject test when cough is within the first one second (FEV_1 will not be accurate). Cough in the later part of the VC is not a reason per se to reject the effort. Often cough can be reduced by asking the participant to exhale slightly less forcefully.
    - **Test end:** When the expiratory effort lasts at least six seconds and flow reaches 25 ml/sec for > 1.0 sec. Participants may need coaching to continue the expiratory effort. Participants with severe obstructive lung disease may continue to exhale for ten or more seconds although >15 seconds exhalation time is not generally unnecessary. Occasionally, premature glottis closure causes abrupt test end. Participants may need to relax and try again with slightly less than maximum effort.

7.3.5 Repeatability

- Two criteria are used to determine how well each acceptable effort compares with the largest acceptable effort.
    - **FEV_1:** The second largest FEV_1 should be within 0.15 L of the largest acceptable FEV_1 and 0.1 L when FEV_1 is ≤ 1.0 L.
    - **FVC:** The second largest FVC should be within 0.15 L of the largest acceptable FVC and 0.1 L when FVC is ≤ 1.0 L. At least three acceptable and two repeatable efforts should be obtained. If this cannot be obtained after approximately eight attempts, then the testing should be halted and the participant reported to the coordinator or investigator.

7.3.6 Reporting

- The largest acceptable FEV_1 and FVC are reported. These do not have to be taken from the same maneuver. The ratio of FEV_1/ FVC is calculated as the ratio of the largest FEV_1 and the largest FVC. At least two quality assurance values will be reported as well; the total expiratory time (FET100%) and back extrapolation volume (V_{ext}). FET100% will be reported from the largest FVC, and V_{ext} will be reported from the trial with largest FEV_1. They will not be reported as data, but will serve the PFT center as quality assurance indicators.

7.4 Lung Volume Airway Resistance and Diffusing Capacity Measurements

7.4.1 Lung Volume General Methodology

Lung volumes and airway resistance will be measured using a constant volume plethysmograph (DuBois box). The 2005 ATS/ERS standards for lung volume measurement procedures should be followed (2).

7.4.2 Lung Volume Reference Equations

The equations of Crapo et al. (8) will be used for predicted values. A scaling factor of 0.88 will be used for African-Americans. Asian-Americans will use a scaling factor of 0.94. Other ethnic groups will use the predicted values of Caucasians.

Lung volumes and airway resistance will be measured within one hour post-BD, as indicated. It is often easier for participants with severe airflow obstruction to perform the plethysmographic studies prior to the post BD spirometry.

7.4.3 Procedure for Measurement of Thoracic Gas Volume (TGV) at FRC

Equipment and procedures are based on the 2005 ATS/ERS recommendations for accuracy and precision (1). Prior to closing plethysmograph door, instruct participant on panting technique and linked IC and SVC maneuvers. Also, instruct the participant on the airway resistance test maneuvers. Close the box and allow at least one minute for thermal equilibration. With hose clips in place, and hands on cheeks, the participant will breathe quietly until the FRC baseline is established. This is a critical prerequisite for technically sound FRC determination. After achieving a stable baseline activate the airway shutter at resting end-expiratory level and have the participant pant lightly against this shutter at a rate of about 60 per minute. It is important NOT to pant too quickly, as this will lead to discrepancies between mouth and alveolar pressure in obstructed participants and lead to overestimation of lung volume (9). This is because normally during the panting maneuver against a shutter, it is assumed that there is equilibration between mouth and alveolar pressure (no flow condition). However, with severe airways obstruction, there may be inequality between mouth and alveolar pressure (10, 11), leading to overestimation of lung volume. This is because of the presence of non-homogeneous swings in regional pleural pressures with airway closure and occurrence of airflow in and out of the compliant upper airway during rapid panting.

When the shutter opens, have the participant perform an IC maneuver to TLC followed by a slow expiratory vital capacity (SVC) maneuver to RV (technique 1). For some pulmonary function instrumentation, the participant may need to perform an expiratory reserve volume (ERV) maneuver) to RV following the VTG maneuver and then perform an inspiratory vital capacity (IVC) to TLC for lung volume determination (technique 2). Both techniques are acceptable and preferably should be performed as linked maneuvers. For technique 1, TLC is determined by the following equation for each test trial: TLC = RC- + IC-. For technique 2, TLC is determined by first determining residual volume (RV) for each trial: RV = FRC - ERV. An IVC maneuver is also measured for each trial.

During the panting maneuvers, the technician may have to coach the participant to make more or less vigorous efforts, and to speed up or slow down the panting rate and insure that no bulging of the cheeks or lips occurs during the closed shutter maneuver. At least three efforts should be produced. TLC reproducibility should ideally be within 10% of the mean TLC. VTG (FRC) should be reproducible to within 5% of the mean and IC should be reproducible to within 100 ml of the highest, excluding an outlier value. Most participants can adequately perform these maneuvers,

but the occasional participant will not be able to perform the test with the most common reason being an inability to adequately perform the additional breathing maneuvers following the closed shutter maneuver because of severe dyspnea. In these cases, lung volume determination should become unlinked (i.e., the VTG maneuvers should be performed first and the other lung subdivisions should be performed as part of separately performed IC-SVC maneuver or by the ERV-IVC maneuver). Attention should be paid to establishing a stable resting baseline prior to any independent unlinked IC-SVC or ERV-IVC maneuvers. In all cases, following the efforts, the technologist will review the loops to check and adjust if necessary the machine generated regression lines. The technician will also check to make sure that the switching error is adequately accounted for.

TLC will be reported according to either technique 1 or 2. For both techniques, FRC pleth (VTG) will be reported as the mean of three acceptable maneuvers. For technique 1, TLC is reported as the mean of three acceptable, preferably linked, maneuvers of TLC = FRC + IC. RV is then determined by TLC - VC (largest). For technique 2, TLC first requires determination of residual volume (RV) as FRC (mean) - ERV (mean). TLC is then determined by RV + VC (largest). For unlinked lung volume determination, the individual test components are measured and acceptable values are reported and included in the above equations for TLC and RV.

7.4.4 Procedure for Measurement of Functional Residual Capacity via N2 Washout or Helium Dilution

For participants who are unable to perform lung volume and resistance testing in the body plethysmograph, lung volume may be determined by gas dilution technique. Reasons for a participant not performing body plethysmography include claustrophobia, orthopedic limitation, intravenous access equipment problems, and inability to adequately perform VTG and R_{aw} maneuvers despite repeated efforts or body habitus reasons.

Equipment and procedures for inert gas testing are based on the 2005 ATS/ERS recommendations for accuracy and precision (1). Either nitrogen washout or helium dilution techniques may be used. Participants should be discontinued from supplemental oxygen for at least 15 minutes prior to the test, if possible. This is particularly important for nitrogen washout testing. If participants cannot tolerate or be safely discontinued from supplemental oxygen for this period of time, lung volume testing will not be completed, and this data will be annotated as missing with this reason given.

Care must be given to assure the participant achieves a stable resting breathing pattern prior to "switch-in" to the test circuit. A tight seal must be maintained throughout testing to avoid leaks. Generally, the greater the degree of airway obstruction, the longer the time it will take for either washout or equilibrium conditions to be met. An occasional sigh (at least one per minute) will facilitate distribution of ventilation. In cases of very severe obstruction, it may be useful to set a time limit for test duration, such as 15 minutes, since participants become very uncomfortable on the mouthpiece during prolonged periods of time, and this increases the risk of dry mouth or cough, thus creating a leak. Follow manufacturer instructions for equipment calibration and test performance.

7.4.5 Procedure for Measurement of Airway Resistance (R_{aw}-I and R_{aw}-S)

Typically, Inspiratory Airway Resistance testing is performed immediately following VTG testing with the plethysmograph door closed. With nose clips in place, and hands on cheeks, the participant will breathe quietly until the resting end-expiratory baseline is established.

Following 4-5 successful resistance (open-shutter) loops, the shutter is closed and 2-4 pants against a closed airway are performed as for measurement of TGV. Pant rate should be about 60 per minute during both open and closed maneuvers.

As with the VTG maneuver, the technician may have to coach the participant to make more or less vigorous efforts, and to speed up or slow down the panting rate. The participant should also be instructed not to take in a breath before the open shutter panting portion of the test. The open shutter panting maneuver should occur as close to resting FRC as possible.

At least three efforts should be produced. No reproducibility standards exist, although it is reasonable to obtain results that are ± 10% of the mean l-R_{aw} value. For each effort, the technologist will review the loops to ensure that only the inspiratory limb of the pressure-flow (P/F) loop is included in resistance calculation for obstructed participants. The tangent should be fit over the linear inspiratory or end-inspiratory portion of the loop. Unobstructed participants should have a standard center-fit tangent (R_{aw}-S) over the +0.5 to - 0.5 L/sec flow range.

R_{aw}-I will be reported for obstructed participants and R_{aw}-S. The value will be the mean of three acceptable maneuvers. Conductance (G_{aw}-I or G_{aw}-S) is determined as the reciprocal of R_{aw}. Dividing G_{aw}-I and G_{aw}-S by TGV will yield Specific Conductance, SG_{aw}-I and SG_{aw}-s which will be reported as the mean of three acceptable resistance maneuvers.

7.4.6 Diffusing Capacity (Single Breath) Methodology

A reduction in diffusion capacity for CO is used to determine a diagnosis of emphysema.

DLCO is measured by standard techniques (11) in which a tracer gas, either helium, methane or neon is introduced for the measurement of alveolar volume (VA).

Equipment and procedures are based on the 2005 ATS/ERS recommendations for accuracy and precision (4). Participants will be seated, more than 30 minutes following any exercise, more than two hours following a meal or more than 15 minutes following bronchodilator. Ambient FiO^2 (21%) is for at least five minutes prior to testing. Participants who cannot be safely taken off or tolerate removal of supplemental oxygen should not be tested and this data noted as missing with the reason documented.

Breath-holding time should be 9 -11 seconds, washout volume at least 0.5 L for participants with vital capacity less than two liters, or low VC and 0.75 - 1 liter for a vital capacity greater than two liters.

At least two maneuvers should be performed and should agree within 10% or 3 ml/min/mmHg. A four minute pause should occur between each test. No more than four trials should be attempted.

Breath-hold time is calculated using the method of Jones and Meade (12), where the beginning and end of inspiration is determined from the extrapolation of the best fit linear regression of volume vs. time during inspiration. Breath-hold includes 70% of inspiratory time and 50% of the sample collection time and should be 9-11 seconds.

VA is calculated from simultaneous measurement of single breath tracer gas dilution. Anatomic dead space will be estimated as 2.2 ml/kg (1 ml/lb). The dead space volume of the circuit is supplied by the manufacturer of the equipment.

The mean of two acceptable maneuvers is reported as the data point, both Hgb corrected and uncorrected. Since hemoglobin (Hgb) may vary considerably among individuals in this study, a correction will be made for Hgb in the LTRC database. Both the corrected and uncorrected values will be reported. The Hgb obtained from the CBC will be used.

Hemoglobin correction will be according to the Cotes equation:

DLadj = DLCO * ((10.22 + Hgb)/(1.7*Hgb))

Adjustment for carboxyhemoglobin need not be generally done. CO transfer coefficient will be calculated as DCO/VA.

7.4.7 Diffusing Capacity Reference Equations

The equations used will be of Crapo and Morris (13):

- Males: DLCO (ml/min/mmHg) = 0.416 (Height) - 0.219 (Age) - 26.34.
- Females: DLCO (ml/min/mmHg) = 0.256 (Height) - 0.144 (Age) - 8.36.
- No race/ethnic adjustment is used for DLCO.

7.4.8 Reported Measures

See Pulmonary Function Testing data collection form.

7.5 Six-Minute Walk Test

The 2002 ATS standard should serve as procedural guidelines for the six-minute walk test (5). If any of the following conditions are present, a six-minute walk for LTRC research purposes should not be performed. However, if the treating physician has requested a six-minute walk for clinical care purposes, this information can be recorded on the Six Minute Walk form and the appropriate box checked.

7.5.1 Contraindications

- Resting heart rate > 120 bpm.
- Unstable angina (all participants with a clinical suspicion of angina should undergo appropriate cardiac evaluation prior to walk testing).
- Exercise related syncope.
- Claudication.
- Uncontrolled hypertension (resting SBP > 200 mmHg or resting DBP > 110 mmHg).
- Resting bradycardia (< 50 beats/min), complex ventricular arrhythmia or sustained SVT.
- Need for >5 L/min oxygen flow for exertion to maintain oxygen saturation > 88% at rest.

7.5.2 Facilities/Equipment

- Stopwatch.
- Ruler or measuring tape.
- Portable oxygen delivery setup (nasal cannula).
- Perceived symptom (Borg) scale.
- Sphygmomanometer.
- Worksheet on a clipboard.
- Emergency (resuscitation) equipment.

- Instructions for Participants during six-minute Walk Testing.

7.5.3 Walk Course

The path should be unobstructed, flat, indoors and a minimum of 100 ft. in length. If a public corridor is used, ability to control traffic should be assured.

7.5.4 Procedure

One walk will be performed. Oxygen use during testing will be the nasal cannula flow rate determined during the assessment for oxygen requirement per usual clinical protocol at each clinical center. If formal clinical exercise titration is not available, then the O^2 flow will be set at 2 L/min above resting prescription for COPD participants and 3 L/min above resting prescription in IPF participants. The use of supplemental O^2 flow during testing in participants who do not have an available clinical titration and who do not require supplemental oxygen at rest will be left to the discretion of the PI. The purpose of administering supplemental oxygen during this test is to maintain adequate oxygen saturation. SpO^2, heart rate and blood pressure are not to be monitored during the walk. The six-minute walk will be performed immediately following a ten-minute rest after the oxygen titration assessment. A staff member will carry portable oxygen supply if supplemental oxygen is used by the participant. Borg scale ratings of perceived breathlessness are obtained whether the six-minute walk test terminates normally or abnormally.

7.5.5 Participant Preparation

- Short acting bronchodilator at least 15 minutes and no more than four hours before testing. This step may be omitted if the participant has ILD and does not routinely use a bronchodilator.
- A light meal two to four hours prior to testing is advised; at least two hours must have elapsed since the participant last ate a meal.
- The participant should rest in a sitting position for ten minutes before testing.
- Participant should wear loosely fitting clothing and comfortable walking shoes

7.5.6 Participant Instructions

- Pre-test instructions: It is critical that identical instructions be given to each participant at each test. Say to the participant "You are now going to begin a six-minute walking test." The object of this test is to walk as quickly as you can sustain to cover as much ground as possible in six minutes. You may slow down if necessary. If you stop, we wish you to continue the walk again as soon as possible. Your goal is to walk as fast and as far as you can in six minutes." Then review the course with the participant. Specific instructions on walking the course will differ somewhat between clinical centers because of differences in the layout of each walking course. Showing the participant the modified Borg scale, say "At the end of exercise you will be asked to describe your symptoms. You will be asked to point to the number on the scale which best describes the degree of breathlessness you are experiencing at that time. Zero means no breathlessness, and 10 is the maximum you have ever felt." Finally, say "Wait until I say 'start' before beginning. It is important that you not talk during the test unless you are having a problem. Do you have any questions?"

- Instructions during walking: At the end of each minute, the participant will be given the time elapsed, the time remaining and a standard phrase of encouragement as follows:
    - Minute 1 - "Do your best"
    - Minute 2 - "Try your hardest"
    - Minute 3 - "Keep going"
    - Minute 4 - "Give it your all"
    - Minute 5 - "Walk faster if you can"
- For example, at the end of minute 4, the technician would say: "Four minutes, you have two to go, give it your all."
- Participants should be instructed that they may stop and rest by leaning against a wall if necessary. The participant should be instructed to continue to walk when they feel able. Warn the participant as the end of the test nears and be prepared to have the participant sit if exhausted. Record pulse oximetry, heart rate, end of test Borg scores for breathlessness and leg fatigue, walk distance and note exercise limiting symptoms.
- Participants unable to complete the walk due to disabling dyspnea or for safety reasons should be stopped and given a seat.

7.5.7 Technician Responsibilities

- Carry supplemental oxygen, if used by the participant.
- Begin by saying "Start."
- Provide standard instructions before the test and standard information and encouragement during the walk at set intervals as described in "Participant Instructions."
- Say "Stop" at the end of six minutes.
- Measure the distance walked to the nearest meter or foot.
- Rest periods are not recorded; a test lasts six minutes if the participant is on the course for six minutes; the participant need not walk continuously for six minutes for the walk to be considered to have a normal termination.
- If test terminates normally (i.e., participant is on the course for six minutes, regardless of rest periods), administer Borg scores for perceived breathlessness and muscle fatigue.
- If the test terminates abnormally (i.e., participant leaves the course before six minutes have elapsed), distance walked, time on the course, Borg scales and reason why the test was terminated before six minutes had elapsed are recorded.

7.5.8 Termination Criteria (Abnormal Termination)

- Chest pain suspicious for angina.
- Evolving mental confusion or lack of coordination.
- Evolving lightheadedness.
- Otherwise warranted clinically.

7.5.9 Forms/Data Reporting

The Six-Minute Walk Test data collection form must be completed. The distance walked, time on the course, reason for termination if walk did not last six minutes and Borg scores will be recorded. Number and duration of rest periods are not recorded.

7.6 Radiologic Assessments

7.6.1 Introduction

Before obtaining the LTRC protocol CT, the clinical coordinator and /or CT Tech must verify that the participant has consented to the LTRC protocol CT. This verification must occur by reviewing the LTRC Protocol CT check box on the participant's informed consent form. The LTRC protocol includes volumetric imaging of the entire thorax, with separate acquisitions in supine position in full inspiration, supine position in full expiration and prone position in full inspiration, if the subject is able. In general, images of 1mm or less thickness will be acquired with 50% overlap as a single volumetric acquisition in each phase during a single breath-hold of less than 15 seconds. Multiple reconstructions of the image data will be performed, with specific accommodation for a high-resolution/high-spatial-frequency reconstruction kernel that has density accuracy as well as kernel with lower noise and spatial frequency.

The specific imaging parameters for particular CT scanners utilized to obtain the LTRC Protocol CT are designed to be as consistent as possible between the different scanners at the clinical centers. These protocols include accommodations for technical differences between CT scanner manufacturers, specific CT scanner models and software versions. The specific imaging acquisition parameters for the LTRC Protocol CT will be intended to produce images which are consistent with regard to image noise characteristics, radiation dose, slice thickness, in-plane resolution, reconstruction algorithm consistency and optimal acquisition time.

Image quality consistency between different CT scanners and of each scanner over time will be achieved through an ongoing image quality assessment and scanner qualification process throughout the LTRC effort, also as described in the Radiology Manual (R-MOP, Appendix C).

If the LTRC Protocol CT cannot be acquired, the Radiology Core Laboratory will accept other CT scan images of the chest obtained previously for clinical purposes.

7.6.2 Imaging Protocols

Specific parameters for consistent acquisition of the LTRC Protocol CT will be provided by the Radiology Core Laboratory to each clinical center in the LTRC Radiology Manual of Procedures ("R-MOP") (Appendix C). This R-MOP will include documentation and instructions regarding:

- Specific instructions for compliance with the LTRC CT QA program.
- CT scanner QA procedures (image quality, doses, etc.) with specific steps tailored for each CT scanner device.
- Acquisition parameters for the LTRC CT Protocol, also tailored for each CT scanner device at that clinical center.
- Breathing instructions for participants.
- Instructions for the software utilized to acquire, de-identify and transfer CT scans to the Radiology Core Laboratory.

As CT scanners are updated or new CT scanners are acquired, these devices will be qualified for use in the acquisition of the LTRC Protocol CT. Based on the capabilities of the particular CT device, the particular acquisition parameters will be specified by the Radiology Core Laboratory. A revised MOP will be sent to the clinical center, and Appendix C of this manual will be updated.

7.6.3 Clinical Center Image Storage Procedures

Each clinical center will be required to store the original image data acquired by the LTRC protocols either to a local archive, such as a local PACS archive, or to digital archive media approved by the CT scanner manufacturer for the duration of the LTRC study. This local archival is utilized to minimize the risk of data loss. Prior to image transfer to the RCL, the clinical site study coordinator should request physical media of the LTRC scan (or the appropriate historic scan). If the clinical site is using the LTRC Data Transfer Tool, the image data does not need to be de-identified (see below). If the clinical site is submitting data via physical media, the study coordinator is responsible for ensuring the data is properly de-identified prior to submission.

7.6.4 Image De-Identification and Tracking

A CT dataset that will be utilized for the LTRC must have all personal health information removed before sending to the RCL. The clinical center staff will use a DCC generated LTRC specimen number to de-identify the DICOM image data. This number will also be used to track the new CT Scan. The identifiable data in the DICOM information for each image will be systematically replaced with standard de-identified information as well as the image number and study accession number. The DCC database will store the ID number, specimen number, number of CT series and number of images per series. A new CT shipping notification will be sent to the RCL, so that image analysis planning and initial RCL tracking can begin. Tracking information and study information (series number, image count) will be made available to the RCL by the DCC for later verification when images arrive and are stored at the RCL, and messaging to verify the archive of images will be passed to the DCC.

Electronic transfer of the data using the LTRC Data Transfer Tool is the preferred method of data Transfer. The tool is selected from the LTRC RCL website (http://rportal.mayo.edu/LTRC). The tool only works on Microsoft Windows computers. It is expected that the computer system will have sufficient resources (computational power and memory) to support the de-identification and data transfer process. Additionally, it is expected that the system will have a physical media drive (i.e., a DVD/CD drive) in order to load the data into the tool. The tool will not require installation and will not install any software on the local system. All data is expected to be in the DICOM format. The study coordinator will be required to answer a limited set of questions regarding the image data. Proper operation of the tool is detailed in the instructions provided with the tool and may also be found in Appendix C.

Prior to electronic submission, the data is de-identified using the DICOM recommended Basic Attribute Confidentiality Profile defined in the DICOM Part 15 (ftp://medical.nema.org/medical/dicom/2011/11_15pu.pdf) documentation.

This de-identification process will protect patient information (through de-identification) prior to data transmission. Data are submitted to the RCL using the Secure FTP (https://en.wikipedia.org/wiki/Secure_file_transfer_protocol) protocol. Accordingly, the study coordinator's computer system must be connected to the internet, and it must be able to connect to the RCL through that internet connection.

7.7 Adverse Event Reporting

Any adverse event (even a minor event) that occurs within two calendar days of an LTRC mandated procedure must be reported on the Adverse Event DCF and entered into the Advantage eClinical[SM] data entry system. Serious adverse events (SAEs) that occur within two calendar days of the lung surgery (i.e., if the surgery occurred within two calendar days of an LTRC procedure) must also be reported on the Adverse Event DCF and entered into the Advantage eClinical[SM] data entry system.

AEs related to study procedures will be followed until resolution even if this extends beyond the study-reporting period. AE information will be gained from direct monitoring of the participant as well as from clinician observation, and self-reporting by the study participant. Phone follow up with the participant will occur monthly until resolution. Resolution of an AE is defined as the return to pre-study procedure status or stabilization of the condition with the expectation that it will remain chronic.

The DCC will work with the NHLBI Project Office to summarize these adverse events for presentation to the LTRC OSMB. The clinical centers will report any adverse event to their local IRBs.

7.8 Final Clinical Diagnosis

A final diagnosis of the participant's lung disease will be rendered by the clinical center PI by two months post-surgery. In making this diagnosis, the PI will consider the following:

- CT Scan Report - completed by the RCL.
- Central Pathology Report - completed by the TCL.
- Local Pathology Report LTRC - completed by clinical center staff using information from the local pathologist.
- Participant's medical record.
- Consultation with other physicians involved in the participant's care.
- Additional input from the participant (if needed).

The PI will complete the Clinical Diagnosis DCF. On this form, two primary diagnoses and up to three secondary diagnoses can be reported. The PI will also communicate any new information provided by the RCL and TCL to the participant's primary care physician that would assist in treatment.

7.9 Study Status Form

The Study Status Form will be completed when a participant withdraws from the study.

8. TISSUE COLLECTION

8.1 Tissue Collection Kits Supplied by the TCL

The TCL will send out empty, pre-labeled tissue collection kits to each clinical center. These kits are specific for each surgical procedure: Lobectomy (one lobe).

Each clinical center will receive at least five kits. Each kit will contain individual specimen containers pre-labeled by the TCL with the 6-digit specimen identifier number (supplied by the DCC) and type of fixative/preservation method. The clinical center will be responsible for ensuring that the specimen containers are appropriately labeled with the correct sample location (Right or Left, Upper, Middle or Lower). If there are unused containers, they will be returned to the TCL with the rest of the kit.

The TCL will provide the RNAlater reagent to each clinical center. This can be stored at room temperature and does not have an expiration date. The clinical centers will be responsible for providing fresh 10% neutral buffered formalin, liquid nitrogen and dry ice.

Before using a tissue collection kit, the clinical center should double-check that all the labeled containers and form labels are printed with the same specimen identifier number. Any discrepancies should be reported to the TCL immediately. The specimen number recorded on the Site Specimen Inventory DCF and on the specimen containers must match.

8.2 Materials Provided by the Clinical Center

The clinical center will provide the following materials for LTRC tissue collection:

- Sterile pack consisting of:
    - Small cutting board Forceps, small Scalpels, scissors.
    - Parafilm packing tape.
    - Reagents:
        - 10% NBF
        - Liquid Nitrogen or a dry ice/isopentane slurry
        - Dry Ice

8.3 Tissue Acquisition Procedure

The primary goal of the tissue acquisition procedures is to get the tissue samples into a fixative/preservative within 30 minutes of removal from the participant. The logistics of the local tissue collection procedure need to be worked out at each clinical center with all relevant parties involved and informed. The clinical center will need to arrange for an area near the OR suites for sterile field set-up and have adequate room for all needed supplies. When an enrolled subject undergoes the surgical procedure, the Operating Room (OR) staff should be notified ahead of time.

The amount of tissue available for the LTRC will depend upon the clinical circumstances and should be determined by an appropriate pathologist (or their designee) at the clinical center. This is generally amounts to < 10% of the overall specimen.

8.4 Tissue Preparation for Lobectomies

The following procedures should be completed within 30 minutes of removal of the specimen from the participant:

a. When there is more than one lobe, procure tissue from one lobe at a time.

b. A representative sample should be removed from each lobe.

c. The tissues should be cut to the appropriate size and placed in each fixative in the following order and general quantities:

- 45% of the sample should be Flash frozen.
- 45% of the sample should be placed in RNAlater.
- 10% of the sample should be placed in Formalin.

d. Specimens that are too small to provide samples for all fixatives should be subdivided into the appropriate size and placed in fixative in the following order of priority: Flash frozen, RNAlater and Formalin.

e. Samples that have, or are suspected to have a nodule, should be handled by a pathologist. No portion of the nodule should be submitted to the TCL.

8.4.1 Fixatives

- **Flash Frozen Tissue:** Place the specimen into the vial labeled "frozen" and place the vial into liquid N2 or use a dry ice/isopentane slurry. Once the sample is completely frozen, transfer the vial to a container filled with dry ice or a -80°C freezer.
- **RNAlater:** Fill the 25 ml tube labeled “RNAlater”-labeled with enough fixative to cover the tissue, making sure it is totally immersed in the fixative. (As a general rule, the final fixative volume should be approximately 5-10x the volume of the specimen.)
- **Ten Percent Neutral Buffered Formalin:** Fill the "Formalin"-labeled vial with enough fixative to cover the tissue, making sure it is totally immersed in the fixative. (As a general rule, the final fixative volume should be approximately 5-10x the volume of the specimen.)

8.5 Shipping Instructions for Tissue Specimens

Specimens should be packed and shipped in the pre-labeled foam shipper boxes supplied by the TCL the day the tissue is collected, unless the shipment will be delayed by a weekend or holiday. The DCC will create a manifest each time a clinical center ships specimens to the TCL. A copy of the manifest will be provided to the clinical center and the TCL. Refer to the section below for shipping instructions.

8.5.1 Tissue Packing Instructions

- **Frozen Specimens:** Place layer of dry ice pellets on the bottom of one of the Styrofoam shipping boxes. Place the samples inside the larger Whirlpak bag. Put another layer of dry ice on top. Five pounds of dry ice is required to keep the specimens frozen. Fill in the extra space with paper to keep the ice from shifting and damaging the frozen tissue.

- **Wet-fixed Specimens**: Place specimen containers containing liquid fixatives into an absorbent pouch inside the supplied re-sealable bag. These bags go into the second Styrofoam box elevated off of the bottom of the Styrofoam box, along with two pre-frozen gel packs.
- Place the Styrofoam box inside the cardboard box.
- Attach shipping labels to the box. Do not allow any labels to overlap. The placement of labels is as follows: Class 9 Dry Ice label placed on front of box in lower left corner; Exempt Human Specimen label in the right corner.
- Call the TCL at (303) 398-1449 to notify that a shipment will be sent via FedEx or UPS and provide them with the tracking number, and add the following email address to the email notification section of the UPS/FedEx shipping manifest section on their website: ParrJ@njhealth.org.
- Airbill and shipping list should be placed in plastic sleeve and attached to the top of the box.
- Ship the specimens to:

  Jane Parr
  Professional Research Assistant
  National Jewish
  1400 Jackson St.
  D502
  Denver, CO 80206
  Tel: 303-398-1449

8.6 Collection of Blood

Before collecting blood in the PAXgene tube, the clinical coordinator must verify whether or not the participant consented to genetic testing. This verification must occur by reviewing the Genetic Testing check box on the participant's informed consent form. A peripheral blood sample of 20.5 ml should be obtained for shipment to the TCL. The pre-labeled vacutainers included in the collection kit from the TCL should be used. The PAXgene tube should be filled last since it has the potential to lose its vacuum. The tubes included are:

- One blue-top Qiagen 'PAXgene' DNA tube (8.5 ml).
- One red/grey top SST tube (8 ml).
- One green/gray top PST tube (8 ml). In addition, the following materials are included for processing the samples:
  - One red-labeled 8 ml plastic tube for the separated serum.
  - One green-labeled 8 ml plastic tube for the separated plasma.

The following procedures should be used to process the samples prior to storage:

- The red/gray top SST Vacutainer will be filled, gently inverted 5 times, allowed to clot upright for 30 minutes, then centrifuged for 15 minutes at 1100 x G. Remove the supernatant layer into the red-labeled serum 8 ml plastic tube, then freeze at -20° C until shipping.

- The green/gray top PST Vacutainer will be filled and gently inverted 8 times to mix with anticoagulant. Plasma will be processed immediately. After centrifuging at 1100 x G for 10 minutes, the top layer (plasma) will be removed to the green-labeled plasma 8 ml plastic tube, taking care not to disturb the barrier. Plasma must be clear before freezing at -20° C; no cells or debris should be present.
- For DNA collection, the blue top 'PAXgene' DNA tube will be filled, frozen at -20° C, then stored at -20° C until ready to ship. It should be frozen upright in a wire test tube holder. Do not freeze it in a styrofoam tray, as this may cause the tubes to crack.

The clinical center will be responsible for storing these samples under appropriate conditions until shipment to the TCL. All tubes should be stored at -20° C.

Specimens should be shipped in the pre-labeled foam shipper boxes supplied by the TCL the day the samples are collected unless the shipment will be delayed by a weekend or holiday. The DCC will create a manifest each time a clinical center ships specimens to the TCL. A copy of the manifest will be provided to the clinical center and the TCL. Refer to the section below for shipping instructions.

An additional blood sample (25 ml) will be collected to perform specific laboratory tests designed to assist in phenotyping the participant. This sample will be analyzed locally at the clinical center laboratory. These tests are: White blood cell count, hemoglobin, hematocrit, platelet count and alpha antitrypsin.

8.7 Shipping Instructions for Blood

The blood samples are to be shipped to the TCL in the containers supplied by the LTRC. These specimens will be shipped using dry ice to keep all specimens frozen. Refer to the tables below for detailed instructions.

8.7.1 Materials Needed for Packing Blood Shipment

NOTE: Items (a-c and e) supplied by TCL; Items (d, f, and g) supplied by clinical center:

a. Styrofoam shipping container in cardboard box.

b. Pre-labeled blue top 'PAXgene' DNA tube; red labeled plastic serum tube; green labeled plastic plasma tube.

c. Labels: Exempt Human Specimen label and Class 9 Dry Ice label.

d. Dry ice.

e. One absorbent bag for each tube, along with one Biohazard bag (all tubes in their individual absorbent bags can go in the one Biohazard bag) for secondary containment.

f. Packing tape.

g. Protective material (bubble-wrap, foam peanuts, etc.).

8.7.2 Blood Packing Instructions

- Place the Qiagen blue top tube, the serum tube and the plasma tube into individual absorbent pouches. Do not send the spun down plasma and serum vacutainer tubes that the serum and plasma have been removed from. Put the 3 pouches into the Biohazard bag. Seal the bag. Put the shipping manifest in the pocket on the back of the bag.
- Place the sealed bags into the Styrofoam box.

- Place dry ice on top of the sealed bag.
- Any empty spaces in the Styrofoam box may be filled with newspaper or bubble-wrap. Replace the Styrofoam cover for the container. Place the Styrofoam box inside the cardboard box. Seal the outer box with packing tape. Attach shipping labels to the box. Do not allow any labels to overlap! Airbill should be placed in plastic sleeve and attached to the top of the box.

8.8 TCL Diagnostic Slides

The TCL will make H & E and pentachrome stained slides from the formalin-fixed specimens submitted to them. If there is not enough tissue to send formalin fixative to the TCL, then the clinical center should provide two (2) blank formalin slides from their surgical pathology department from each lobe to be used for a diagnosis. If possible, for lobectomies, ask for a block that does not contain the tumor.

8.9 Specimen Storage

All tissues will be stored in the TCL in the proper storage facilities, 4°C, -20°C, or -80°C or liquid nitrogen, depending on the tissue and fixative/preservative requirements. Frozen tissue specimens will be processed on dry ice and stored in aliquots at -80°C so that the entire aliquot can be sent to an investigator without thawing. Slides of paraffin-embedded samples will be cut "as needed." All freezers/refrigerators are backed up with an alternate electrical source to prevent warming in the case the main electrical source at the TCL in interrupted.

Table 5 summarizes the storage conditions at the TCL for each specimen type:

Table 5: Archival Storage Conditions

Paraffin Blocks	Room Temperature
Frozen blocks/tissue	-80°C
RNAIater tissue	-20°C
Slides	-20°C or -80°C
Blood - DNA tubes	-80°C
Blood – Isolated DNA	-20°C
Blood - plasma and serum	-80°C

9. COMPLETION OF DATA COLLECTION FORMS

9.1 Identification Numbers and Codes

9.1.1 General Guidelines

Each form has certain key items at the top which uniquely identify that form. These items must be filled in on all forms. These items are the participant's identification (ID) number, visit number and the date of the assessment. If this information is not completed correctly, the form cannot be processed by the DCC.

Each participant is assigned a unique participant ID number at the time the participant is enrolled into the DCC Advantage eClinical[SM] data entry system. This ID number consists of seven digits; the first three digits represent the clinical center number (assigned by the DCC before participant enrollment begins), while the next four digits identify the participant. Once an ID number is assigned by the Advantage eClinical[SM] system, it cannot be changed. The ID number must be used on all of a participant's LTRC forms and correspondence.

9.1.2 Clinic ID

The DCC has assigned the following clinical center ID numbers:

001: Mayo Clinic Rochester

003: University of Michigan

004: University of Pittsburgh

008: Temple University

This three digit code comprises the first three digits of the participant ID number.

9.1.3 Specimen ID

Each core laboratory specimen (tissue and blood) will be labeled by a 6-digit specimen ID number which is independent of the participant ID number. These specimen ID numbers will be generated by the DCC.

10. LTRC STUDY WEBSITE

10.1 Overview

The LTRC study website will serve as the primary portal for all study-related materials, Advantage eClinical[SM] data system access, and event management among other items, and is available for use by all LTRC clinical centers, the Project Office and Core Laboratory staff. The website is supported by current versions of Google Chrome, Mozilla Firefox, and Microsoft Internet Explorer, though performance is typically optimized by using Chrome or Firefox. The LTRC website is accessed at www.ltrcpublic.com and contains publicly-available content as well as content accessible only to users with a username and password. Contact the DCC for a username and password if you have not been assigned one. For all other questions about the website, contact the LTRC DCC at ltrcdcc@emmes.com.

10.2 Contents of Study Website

The public pages of the website contain basic study primer information including a summary of the LTRC, specimen collection protocols, clinical site and core laboratory descriptions, publications, announcements, and information regarding how to request specimens and data from the LTRC.

Additional content is available to authenticated users after logging in with a username and password. This content includes:

- Detailed study rosters for each clinical center, core laboratory, NHLBI and the DCC.
- Calendar tool for organization of study-related conference calls and meetings, agendas and associated minutes.
- Extensive document library containing study-related documents, forms, reports and communications.
- A study annex (e.g., homepage) which serves as a one-stop shop for pertinent study information, including accrual and other study reports, study protocol and MOP, data collection forms, system training requirements, forms instructions and system user guides and substantive study communications.

The private section of the website also hosts access to the Advantage eClinical[SM] data entry system used to enter all study data; however, users of the system are able to bookmark the eClinical URL separately, so that log-in to the study website is not needed each time data entry is performed.

11. MONITORING STUDY PROGRESS AND DATA QUALITY

11.1 Introduction

A primary concern of the DCC staff will be to assure the timely collection of complete and accurate data. There are two major steps associated with insuring that accurate and complete data will be collected: 1) all persons associated with data collection should be properly trained and familiarized with the tasks they are to perform; and 2) performance of the required procedures should be monitored and large deviations from study norms investigated. Monitoring reports on clinical center performance of recruitment, adherence to Protocol and performance of follow-up procedures will be prepared at regular intervals.

11.2 Participant Screening, Tracking and Deactivation Log

An Excel Spreadsheet has been designed by the DCC to record the screening of study participants at each clinical center. No personal health information will be included in this log. A sequence number (1, 2, 3,) will uniquely identify each study participant. If the participant is enrolled into LTRC, the LTRC participant ID will be included in the log. If the participant is a screen failure, the participant ID column will be left blank. The spreadsheet will contain the following columns:

- Patient Screening ID (1, 2, 3, etc., or other scheme as determined by site).
- Diagnosis (e.g., COPD, IPF).
- Screening Date.
- Participant Consented (Yes/No).
- Date of Consent (if applicable).
- LTRC Participant ID Number (if applicable).
- Reason for No Consent (if applicable).

These logs are to be sent monthly from the clinical centers to the DCC by email. The DCC will use these spreadsheets to tabulate the total number of participants screened for the LTRC and the number of refusals and ineligibles. LTRC screening activity will be periodically reviewed to determine if changes are needed in LTRC procedures to better attract and retain participants.

A template Excel spreadsheet is available for download from the LTRC study website (www.ltrcpublic.com) under Resources > LTRC > Data Collection.

11.3 Summary Reports

Summary reports will be programmed by the DCC and posted to the LTRC study website (www.ltrcpublic.com). The reports will refresh nightly and will reflect data entered into the Advantage eClinical^{SM} database as of the previous day. The following reports will be included:

- Accrual by gender, racial/ethnic background, and disease state at each clinical center and over the entire study.
- The percent of recruitment goals achieved for each clinical center and over the entire study.
- Missing forms, missing values and outstanding data quality checks.
- Adverse events.

- Core Laboratory performance (number of specimens or radiographs received, number processed).

Careful review of these reports during the study by DCC staff will ensure the timely resolution of any data entry or data quality problems at the clinical centers. These report may also be reviewed by the LTRC OSMB at three-month intervals.

11.4 Site Monitoring

Monitoring is considered a continuous, ongoing and multifaceted process. This process may include on site and centralized monitoring by LTRC DCC staff, NHLBI staff and local IRBs. For the purposes of this document, centralized monitoring is defined as any activity of study data review that is not performed on site (i.e., is performed remotely). Centralized monitoring may include but is not limited to: Front end range checks in the Advantage eClinical^{SM} system, Web-based data quality reports, Automated Email alerts and Metrics reporting. To maintain data quality, all identified issues of concern will be communicated to the study teams via web reports (as described above), immediate automated email alerts, and during regularly-scheduled review calls or as needed.

On-site monitoring visits and events are conducted to ensure required human participant protection, study procedures, laboratory and data collection processes are of high quality and meet NHLBI requirements. Site visits are conducted by an authorized representative of the LTRC DCC to inspect study data, participants' medical records and eCRFs in accordance with ICH guidelines, GCP and respective local or government regulations and guidelines.

The LTRC DCC staff responsible for site monitoring will contact participating sites to facilitate their conduct. The DCC monitor will send the clinical center a letter /email confirming any agenda items and visit dates as applicable.

11.5 Site Visits

Annual site visits to each of the clinical centers will be conducted during the course of participant recruitment. Plans for site visits will be provided to the LTRC Steering Committee and NHLBI Project Officer on a schedule agreed to with the NHLBI Project Office. The site visit teams will consist of a core group of professionals working on LTRC at the DCC as well as a representative from NHLBI if needed.

A report summarizing the findings of the site (audit) visit will be sent to the clinical center Principal Investigator and the NHLBI Project Officer.

The components of the site visit will include the following activities:

- Participant Recruitment - The clinical center's methods for identifying and recruiting eligible participants will be reviewed.
- Clinical Center Operations - The methods of scheduling and record keeping will be reviewed and study files examined.
- Review of Medical Records and Study Forms - Selected study forms for a sample of study participants will be reviewed. The data on the forms for these study participants will be compared against listings of data in the DCC database.
- Consent forms will be reviewed for 10% of the study participants.
- Retraining and reinforcement of data collection methods - to be individualized to suit the needs identified for each clinical center.

A site visit report will be prepared within four weeks of the visit and distributed to the clinical center. The clinical center team will be allowed two weeks to respond to the site visit recommendations and findings. The final report, which includes the clinical center responses, will be submitted to the NHLBI Project Officer within four weeks of the visit.

12. DATA MANAGEMENT

The source document is defined as the first place that data are recorded (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, participants' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical study).

The LTRC DCC will provide data collection forms (DCFs) derived from the eCRFs that may be used optionally as source documentation, when data is not otherwise 'sourced' in the participants medical records. These DCFs are not required to be used and should be used only as follows:

- To augment data that are already being captured elsewhere in clinic notes. For example: If the site consistently misses collecting ethnicity (Hispanic, non-Hispanic), the DCF could capture this detail.
- For recording study-specific specialized testing or specimen collection, where the DCF would capture required data and not impact clinical care.
- To document Investigator confirmation of eligibility with an eligibility checklist.
- As a supplement to the medical record to capture additional study-specific information; for example, while the AE DCF should not be the sole source of AE data, it can capture additional information.

Blank DCFs are posted to the study website. Sites are encouraged to use the DCFs as is; if any modifications are made to the posted forms by the site, it is the site's responsibility to ensure that all required data elements are collected. In some instances, staff might need documentation from their own or other institutions (e.g., laboratory reports or a hospital report for an SAE). In this case, please request a certified copy of the record from the institution.

To qualify as a certified copy, a document must have been photocopied directly from the original; each page of the copy must be signed and dated by the individual making the copy, attesting that the document is a duplicate of the original. In cases where the sending institution does NOT certify the copy prior to sending it, the receiving site should verify that the copy is unaltered as received by signing or initialing a dated statement indicating that it is unaltered as received. The statement may be in the form of a stamp, as long as it is accompanied by an original signature or initials (of receiver) and date. When the copy has been made by the site, the clinical coordinator or applicable staff member must mark the document "Certified by: _____," sign it, and date the copy.

Data should be handled in accordance with ICH E6 GCP, US federal regulations, local/institutional regulations (if applicable) and instructions from LTRC for the protection of confidentiality of participants. All DCFs should be completed by the clinician (or other appropriate study personnel) and at a minimum must be signed by the person collecting the data for that form. (Note: The determination of causality and severity of AEs, Eligibility and Medical History requires the signature or co-signature of a licensed clinician [i.e., medical doctor, nurse practitioner or physician's assistant] who is licensed to diagnose and is listed on the site delegation log.) A qualified study team member, as delegated by the PI on the site delegation log to oversee such tasks, may sign the Enrollment, Medical History and AE DCFs signifying their review and confirmation.

Data entries into paper/hardcopy source documents should be made in permanent ink. Corrections will be done according to GCP guidelines, correction or modification should be made with a single line through the entry and the change initialed and dated. Original entries should remain legible (i.e., they should never be erased or covered with correction fluid to cover the original entry). Late entries should be initialed and dated at the time entered. Source documents for participants who sign the Informed Consent document but are not enrolled must be retained following the same guidelines as other study source documents. Forms should NEVER be back- or post-dated.

12.1 Electronic Case Report Forms (eCRFS)

Data will be entered electronically over the Internet by site study staff into the Advantage eClinical[SM] data system, developed and maintained by The Emmes Corporation. Instructions for use of the system and completion of the data screens (eCRFs) for each study are included in the AdvantageEDC[SM] User's Guide, which is posted to the data system's main menu (and on the study website) and the study-specific Forms Instructions, which are posted on the study website.

Note: Study staff are responsible for reading and understanding these guides prior to gaining access to the data system.

The clinical center is expected to enroll all participants into Advantage eClinical[SM] and enter data within 72 business hours of enrollment or each subsequent study visit/procedure/contact. Corrections to eCRFs will be performed using the system, which will track modifications in the audit history. The audit history contains the date, time, field name and username of the individual who modified the study data.

12.2 Form Submission Schedule

Advantage eClinical[SM] includes a report which indicates the current status of forms submitted for each participant. In addition, a Forms Submission Schedule is posted for each protocol on the study website within the Forms Instructions and with the DCF packet.

12.3 Missing Forms Report

The Missing Forms Report accessed from the Advantage eClinical[SM] Dashboard provides a listing of missing forms, by participant. Forms listed on the Missing Forms Report should be entered into Advantage eClinical[SM] as soon as possible. A missing form will continue to be requested until the form is completed or until an exception is granted and entered into the missing forms exception file. When a form is missing and will never be submitted (e.g., a participant misses a visit or a required study procedure was not performed), the clinical center should request an exception for the missing form(s). If a participant terminates the study before all study visits are completed, a Study Status eCRF must be completed in Advantage eClinical[SM]. Completion of this eCRF will remove the requirements for submitting subsequent eCRFs. Clinical centers are required to review these reports for all study participants on a regular basis.

12.4 Missing Values Report

The Missing Values Report posted in Advantage eClinical[SM] provides a listing of missing required values for each participant. Similar to the Missing Forms Report, this program will identify the missing values by Participant ID, Visit Day, form and data element. Missing values will continue to be reported until completed or until an exception is granted by the LTRC DCC.

12.5 Data Review and Quality

The clinical center PI is responsible for ensuring that all study personnel are compliant with study procedures and local policies at the participating center. The PI must spend adequate time observing study procedures and regularly reviewing and training staff on various aspects of the study to address issues that may arise at the center or to be preemptively avoided based on information acquired through sponsor communications/meetings. Participating clinical centers are responsible for reporting problems to the NHLBI and LTRC DCC that could affect the quality of the data or study operation. The PI will be copied on all substantive site communications, and is encouraged to utilize study provided website materials, reports and data to assist in investigational oversight.

12.5.1 Data Query Reports

Data query reports, containing results from programmatic data anomaly checks, will be provided to centers twice monthly or as needed for data quality during the course of the study. The reports should be reviewed and responded to by the site within five business days. Data corrections entered on the specific form in question or an explanation provided as to why the data are correct need to be indicated on the report. A signed report by the clinical center should be scanned as a PDF and distributed via email to the LTRC DCC staff as indicated on the form header. In addition, the LTRC DCC may identify specific issues and send periodic manual query reports to the clinical center via email.

12.5.2 Web-based Data Quality Reports

The LTRC DCC will provide a number of quality assurance reports to ensure that study data are clean and complete. On a nightly basis, data summarized in the reports are automatically updated to ensure currency of reports. The following are examples of web reports that will be provided. This is not an all-inclusive list.

- Accrual
- AE and SAE Listings
- Sample Collection
- Enrollment Listing
- Protocol Deviations Listing
- Delinquent forms and data

13. CERTIFICATION

13.1 Overview

One of the quality assurance strategies used in the LTRC is certification of clinical center staff. Certification is required before any LTRC visits or data collection may occur. A clinical center may collect information on a prospective participant's eligibility prior to certification, but that participant may not begin any screening examinations, sign any consent statements, or complete any LTRC forms until the clinical center has been certified.

The primary purpose of certification is to help assure consistent conduct of the LTRC over time and within and across clinical centers. The conduct of procedures should be similar across participants and clinical centers. LTRC-specific procedures for data collection and specific items of equipment may be required as LTRC procedures and calibration requirements for equipment may vary from the usual practice of a participating clinical center.

Certification is also a managerial tool for the study. It identifies the staff and clinical centers that carry out LTRC procedures and identifies to staff that they and their clinical center are a part of the LTRC. It also provides a mechanism for tracking who collected key items of data or made key decisions. The process of obtaining certification may help a clinical center prepare for LTRC activities by presenting the training, facility and equipment needs in an organized fashion and requiring acquisition or completion of these items before LTRC activities may be started.

The DCC will organize a training session prior to the start of recruitment and will schedule on-going in-person sessions and conference calls as needed during the course of the LTRC.

13.2 LTRC Certification Examination

The LTRC Certification Examination is designed to assess if the staff member understands the overall scope of the LTRC. All staff members must pass this test prior to performing any LTRC procedure. The test is an open-book, multiple choice test based on the LTRC Protocol. Grading is on a Pass/Fail basis with 80% correct answers considered passing.

The test questions were compiled by the DCC. The test is administered after the completion of an LTRC training session, one-on-one training session, or review of training slides. The DCC is responsible for distributing, collecting and grading the tests and informing the staff member of their grade. Staff members who do not pass the test may re-take the test after further review of the training materials.

13.3 Clinical Center Staff Certification

13.3.1 General Certification Procedures

The purpose of staff certification is to identify to the DCC who is responsible for the overall conduct of data collection procedures, who is carrying out selected data collection procedures, and who is responsible for key aspects of eligibility decisions. Certification also identifies to the staff member that he/she is a part of LTRC. Certifying staff also helps to assure that the staffs have acquired a basic level of training in the LTRC protocol. All personnel who request certification must agree to abide by the design tenets of the study.

The certification program includes only a subset of the personnel involved in the LTRC. For example, staff who schedule participants and some staff who assist in testing, surgery, nursing or other aspects of care for LTRC participants do not need to be certified, but are important to the success of LTRC.

For the majority of LTRC staff, the following minimum requirements must be met:

- Read the Protocol, Consent Form and MOP.
- Attend an LTRC training session or receive one-on-one training from an LTRC certified staff member.
- Be included on the Site Delegation of Authority Log.
- Pass the LTRC Certification Examination.
- Submit requested materials to the Data Coordination Center (DCC).

LTRC staff functions requiring certification are:

- Pulmonary physician(s).
- Thoracic surgeon(s).
- Clinic coordinator(s).
- Data entry personnel.
- Radiology Supervisor.
- Lead Tissue Technician(s).

Specific functions may have additional requirements – (e.g., additional reading assignments or practice with procedures). Backup personnel and personnel joining the LTRC subsequent to the LTRC extension will be trained by LTRC certified personnel at the clinical center.

A staff member may be certified for more than one function, and more than one staff member may be certified for the same function. It is recommended that at least two staff members be certified for each function so that there is back-up in case of illness, during vacations or other absence.

13.3.2 Pulmonary Physician

The function of the pulmonary physician in LTRC is to participate in screening participants (review of records for referred participants, completion of exams and review of screening tests), determine eligibility (jointly with the thoracic surgeon), discuss with the participant the impact of his/her participation in LTRC on participation in a lung transplantation program (if applicable) and conduct visits on LTRC participants. The pulmonary physician is also a LTRC study physician (as is the thoracic surgeon), and hence may sign LTRC consent statements. The pulmonary physician is responsible for any necessary communications with the participant's private physician regarding the participant's enrollment in LTRC.

It is not required to submit proof of training or experience to be certified. Rather, the clinical center Principal Investigator and Co-Principal Investigators should be listed on the Site Delegation of Authority Log sent to the DCC from the clinical center. This checklist will document that these physicians are qualified to perform their LTRC duties and are able to monitor other physicians who may work with LTRC participants.

Summary of requirements for certification:

- Attend an LTRC training session or receive one-on-one training from a previously certified Principal Investigator or Co-Principal Investigator.
- Read the LTRC Protocol, Consent Form and MOP.

- Pass the LTRC Certification Examination.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.3.3 Thoracic Surgeon

Clinical centers should certify any thoracic surgeon who receives funding as an LTRC Investigator. Some clinical centers may not have thoracic surgeons funded by the LTRC.

It is not required to submit proof of training or experience to be certified. Rather, any thoracic surgeon should be listed on the Site Delegation of Authority Log sent to the DCC from the clinical center Principal Investigator.

Summary of requirements for certification:

- Attend an LTRC training session or receive one-on-one training from a previously certified study coordinator or Principal Investigator.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.3.4 Study Coordinator

The function of the study coordinator is to oversee, organize, and coordinate the visit and data collection activities for LTRC. The study coordinator is also responsible for review of all data collection forms for consistency, completeness and accuracy prior to data entry. The study coordinator will also oversee and supervise the recruitment of participants, assuring that all eligibility requirements are met.

Each clinical center must certify a principal study coordinator. Additional study coordinators may be certified at a clinical center. The principal study coordinator is to be the primary liaison for the DCC on LTRC issues at that site. In addition, the principal study coordinator is responsible for training other clinic coordinators at that site.

Summary of Requirements for certification:

- Attend an LTRC training session or receive one-on-one training from a previously certified study coordinator or Principal Investigator.
- Read the LTRC Protocol, Consent Form and MOP.
- Pass the LTRC Certification Examination.
- Successfully complete the Advantage eClinical^{SM} training quizzes and practicum.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.3.5 Data Entry Personnel

Some clinical center staff may be designated just to perform data entry and query resolution. These staffs typically have no contact with participants and thus just need basic training on the protocol, as well as training on the Advantage eClinical^{SM} data entry system. Summary of Requirements for Certification:

- Read the LTRC Protocol, Consent Form and MOP.
- Attend an LTRC training session or receive individual training from a DCC staff or Lead Coordinator at the clinical center.
- Pass the LTRC Certification Examination.
- Successfully complete the Advantage eClinical[SM] training quizzes and practicum.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.3.6 Radiology Supervisor and Lead Radiology Technicians

As part of the training of clinical center radiology supervisors and lead technologists and validation of clinical center staff for involvement in the LTRC, the QA and Training Technologists from the RCL will formally assess the competency and experience of the clinical center staff. Written documentation of the particular skills needed to acquire images by the LTRC MOP and a general assessment of knowledge will be obtained by the Radiology Center QA and Training Technologist during this site visit. A sample documentation form and basic CT knowledge survey is included in within the Radiology Manual (Appendix C). Supervisors and lead technologists who have completed the LTRC training program will sign a written attestation that they understand the materials describing the acquisition parameters and image quality requirements of the exams for which they will be responsible in the study.

On-site evaluation will be supplemented by ongoing data monitoring to ensure that the clinical center staffs are properly trained and consistently following LTRC protocols.

Summary of Requirements for Certification:

- Read the LTRC Radiology Manual (Appendix C).
- Pass the RCL Certification Examination.
- Complete the CT Knowledge Survey and Documentation Form (Appendix C) and submit this form to the RCL.
- Included on the Site Delegation of Authority Log.
- Complete an LTRC training session led by the RCL or another LTRC certified radiologist.
- Submit these materials to the DCC.

13.3.7 Pulmonary Function Technician

Individual certification of Pulmonary Function Technicians will be required. The clinical center Principal Investigator should document their local Pulmonary Function laboratory's past NETT experience and other certification on the Staff Delegation of Authority Log.

Summary of Requirements for Certification:

- Read the LTRC Protocol, Consent Form and MOP.
- Attend LTRC training session or receive one-on-one training from a certified technician.
- Included on the Staff Delegation of Authority Log.
- Submit these materials to the DCC.

13.3.8 Tissue Technicians

The Tissue Core Laboratory (TCL) will sponsor training for the LTRC tissue technicians from each clinical center. If a tissue technician joins the TCL staff during the LTRC, the TCL is available to assist with arrangements for training at the clinical centers. After training, the TCL will test the tissue technician before certification.

Summary of Requirements for Certification:

- Read the LTRC Protocol, Consent Form and MOP.
- Receive training from a certified tissue technician.
- Pass the TCL Certification Examination.
- Pass the LTRC Certification Examination.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.4 Core Laboratory Staff

13.4.1 Radiologists

All of the LTRC CT studies will be reviewed by Board certified radiologists with additional experience or training in thoracic radiology. Specifically, these radiologists must have a valid, active state medical license and documented training in the physics of diagnostic radiology and radiation safety, as evidenced by completion of an accredited diagnostic radiology residency or 80 hours of documented, relevant classroom instruction. This training should include instruction in radiation monitoring requirements and the hazards of radiation exposure as well as the physical principles of CT, CT artifacts, technical parameters for multi-slice CT examinations (exposure factors, detector configuration, table speed, field of view, reconstruction kernels, etc.) and digital image processing. These radiologists should have documented involvement with the supervision or performance, review and interpretation of at least 300 chest CT examinations in the past three years or completion of an ACGME accredited radiology residency within the preceding 24 months. Experience in interpretation of high-resolution CT (HRCT) of the chest is desirable. Qualified radiologists must participate in continuing medical education in accordance with the American College of Radiology (ACR) Standard, which recommends 150 hours of Category 1 (minimum of 60 hours) or Category 2 (maximum of 90 hours) over three years. This should include credits in thoracic imaging and chest CT.

All interpreting radiologists at the RCL must complete a basic LTRC certification before they can be eligible for reporting. The Mayo Clinic Radiology Department maintains a research database of proven cases, including many cases of specific pulmonary pathology to be studied within the LTRC. To increase the consistency of analysis and confirm the diagnostic skills of the reporting radiologists, a group of 20 biopsy-proven cases of specific interstitial lung diseases and 20 cases of emphysema will be utilized to verify consensus terminology and classification of abnormalities. The LTRC radiologists will review these cases and the specific features to be reported to the DCC will be noted by each radiologist. Significant deviation from consensus standards may be grounds for additional training or exclusion from the pool of radiologists qualified to interpret LTRC studies.

Summary of requirements for certification:

- Attend an LTRC training session or receive one-on-one training from a previously certified core lab radiologist.

- Read the LTRC Protocol and MOP.
- Pass the LTRC Certification Examination.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

13.4.2 Tissue Processing Technician

The Tissue Core Laboratory (TCL) will be responsible for the fixation, processing and storage of tissue before it is shipped to authorized investigators. The TCL will also cut sections and stain the slides with hematoxylin & eosin (H&E) and pentachrome for the TCL pathologist's assessment and diagnosis. Lung tissue must also be tested for RNA quality prior to storage. In order to perform these tasks, the Tissue Processing Technicians should be either professional research assistants with experience in molecular techniques, tissue processing and basic histotechnology or qualified histotechnologists.

Summary of requirements for certification:

- Attend an LTRC training session or receive one-on-one training from a previously certified tissue processing technician or Principal Investigator.
- Read the LTRC Protocol and MOP.
- Included on the Site Delegation of Authority Log.
- Submit these materials to the DCC.

14. TISSUE CORE LABORATORY PROCEDURES

14.1 Completing Fixation Process and Processing to Paraffin or Freezing

For formalin fixation to paraffin, the tissue is submitted to the Histology Laboratory at National Jewish Health for automatic processing. The TCL histotechnologist then embeds the tissue in paraffin and prepares slides as needed. H & E and pentachrome staining are performed on sections from a tissue block from each lobe submitted for analysis and classification of disease by the pathologist.

The RNAlater tissue is aliquoted into separate tubes and frozen, and the frozen tissue is cut into smaller pieces and aliquoted into frozen tubes to prevent thawing.

14.2 Sectioning Tissue (Microtome)

All slides are sectioned as authorized investigators make requests. Paraffin-embedded tissue is sectioned on a microtome at 4-5 um and placed on superfrost plus slides for immunohistochemical work.

14.3 Storage of Tissue

All tissues will be stored in the principal investigator's laboratory or freezer farm in the proper storage facilities, Room temperature, 4°C, -20°C or -80°C, depending on the tissue requirements. Backup generator power is available.

Although most storage formats are in tissue or block form, if storage of slides is needed prior to shipping to individual investigators, the slides will be cut and then stored at 4°C.

14.4 Blood Processing and Storage

A peripheral blood sample of 20.5 ml should be obtained from every participant. The blood samples are divided at the clinical centers as follows:

- One blue top Qiagen 'PAXgene' DNA tube (8.5 mL).
- One red/gray top SST tube (8 mL) for serum.
- One green/gray top PST tube (8 mL) for plasma.

The PAXgene Blood DNA specimens are processed at the TCL according to the manufacturer's kit instructions. DNA is split into 5 microgram aliquots and stored at -20°C.

14.5 Tissue Analysis

Each specimen is sectioned and stained with H & E and pentachrome (Movat's stain) for diagnostic, light-microscopic, and histopathologic analyses by the TCL pathologist, and a completed Central Pathology Form submitted to the DCC.

14.6 Methods of Assessing and Reporting Technical Quality of Specimen Preparation and Storage

Initial assessment will be performed upon receipt of tissues. All tissue will be assessed for morphology using H & E.

All specimens must be carefully inventoried and stored at appropriate temperatures. Temperature of freezers will be continuously monitored through a computerized alarm system. Back-up

generator supply is available for all frozen and refrigerated material. A bar-coding system is linked directly to the database to minimize manual entry of specimen tracking data.

The TCL will assess the RNA integrity of representative RNAlater samples as appropriate.

The ultimate quality assurance mechanism for the laboratory is feedback from users. The TCL will query all users concerning the appropriateness and quality of the material supplied. Feedback will be logged and, if negative, appropriate corrective measures taken as possible.

14.7 Preparation of Specimens in Formats Suitable for Distribution to Researchers

With a well-characterized, well-preserved selection of tissue for processing and distribution to researchers, the TCL will be able to provide multiple formats suitable for further study. As previously outlined, these will include:

- Microtome sectioning of paraffin-embedded tissue for morphometric analysis, immunohistochemistry, immunofluorescence, special stains and laser capture microdissection (LCM).
- Frozen tissue for protein and molecular work.
- RNAlater preserved tissue for protein and molecular work.
- Plasma and serum from peripheral blood specimens.
- DNA from peripheral blood specimens.

Requests from investigators for tissue are first reviewed by the LTRC Protocol Review Committee (PRC) who will send their recommendation to the NHLBI Project Office. If the request is approved by the Project Office, the DCC will be notified and in turn notify the TCL of the approved request including details of the number and types of specimens and the name and address of the requesting investigator. Specimens will then be sent to individual investigators using the appropriate shipping method. No protected health information (PHI) will be included.

15. RADIOLOGY CORE LABORATORY

15.1 Processing

Upon arrival at the Radiology Core Laboratory (RCL) at the Mayo Clinic in Rochester, MN, LTRC media will be handled by a study coordinator. Specifically, the RCL coordinator loads the CT data from the media to a dedicated LTRC research computer, where the DICOM images would be transferred to the LTRC Mayo Institutional Digital Image Archive (MIDIA) storage pool. The successful archive report is reviewed to verify the receipt of images through the LTRC tracking database hosted at the DCC through a web-based interface. The verified study information and image count by the RCL staff will be relayed to the DCC such that any discrepancies between the LTRC CT Shipping Form and the final archived data at the RCL can be resolved.

In the event that the tracking information hosted in the DCC indicates errors or incomplete information was received at the RCL, the LTRC Radiology staff will contact the DCC staff or the appropriate clinical center coordinator to determine if a problem had occurred. For example, errors might include failure to send DICOM images, errors in media creation, incorrect de-identification of images, incomplete study data received by the RCL, or perhaps the incorrect study was sent. This automated notification and tracking system should help to assure that nearly 100% of studies can be successfully transferred and archived for the LTRC in a timely fashion.

The MIDIA LTRC storage pool automatically transfers a copy of archived data to a CT Analysis Workstation for assessment by a CT 3D Analyst in the Mayo Advanced Image Processing/3D Laboratory. This analyst would determine whether the study sent from the clinical center qualifies for quantitative analysis by examination of the number or series, number of images and DICOM header elements relevant to CT technique. If the CT scan parameters include volumetric high-resolution imaging of the entire chest without intravenous contrast and are reconstructed in a non-edge-enhancing kernel or match those of the LTRC specific protocol, these images will be processed for quantitative information. The CT 3D Analyst would record the results from the quantitative analysis into the LTRC database. Upon completion of quantitative analysis and reporting, quantitative results, anatomic and parenchymal object map files and other study results are stored.

After processing by the 3D analyst, CT datasets will be reviewed by a LTRC radiologist for qualitative assessment and semi-quantitative reporting. The radiologist results (Form 51) would be recorded into the LTRC database through a secure web-based interface.

The clinical center DVD media will be stored for the duration of the LTRC protocol as an additional backup.

15.2 Storage

Validated studies received by the RCL will be archived in the MIDIA. This institutional archive resource will allow for storage of LTRC DICOM data in a high-availability dual-datacenter environment at reasonable cost. As part of the storage process to MIDIA, a final archive verification report will automatically be generated, which will be compared to the tracking information in the LTRC database for final data integrity assurance. The standard procedures utilized at the Mayo Clinic for storage verification of research related and clinical images to the MIDIA archive will be followed for LTRC data.

15.3 Distribution

Any data requested for distribution from the RCL to investigators will be done only at the direction of the DCC or NIH/NHLBI or their designee. Image data in DICOM format and object map files

will be copied from the RCL archives to appropriate portable media (DVD, Flash media, external hard drive, etc.). Receipt of the materials will be confirmed by the DCC and portable storage will be returned to the RCL by the requesting investigators for erasure and future use by the RCL.

15.4 Quality Assurance

The Mayo LTRC Radiology Core Laboratory (RCL) will design, implement, and maintain a Quality Assurance (QA) program for the clinical centers in order to ensure the consistency and comparability of the CT images data. The consistent high-quality acquisition of CT images is critical to the success of the qualitative and quantitative evaluation of the CT examination of participants in the LTRC. The assessment of pulmonary diseases studies within the LTRC may be compromised by inconsistent acquisition parameters or improper scanner calibration. In addition, the QA program will verify the qualifications of clinical center personnel and assist in their initial and ongoing training.

The RCL medical physicists and QA technologists will assure that all systems utilized for LTRC CT protocol image acquisition meet or exceed applicable state and federal regulations as well as professional recommendations. A complete quality assurance performance evaluation is performed on all of our CT systems at least once annually. In addition, the RCL medical physicists and QA technologists re-test systems after any major equipment (e.g., x-ray tube replacement) or software changes. The RCL Quality Assurance Group, under the direction of the lead LTRC CT physicist will be responsible for the ongoing process of monitoring and ensuring the quality of CT images and dosimetry data obtained at the clinical centers.

An experienced Mayo Clinic QA Technologist (Medical Imaging Technical Specialist) will serve as the LTRC QA and Training Technologist. This QA and Training Technologist reports directly to the RCL Quality Assurance Liaison, the lead LTRC CT physicist and the RCL core Administrative Group. This technologist will be responsible for supervision of the technical aspects of CT acquisition, maintenance of QC/QA procedures and initial on-site training at the clinical centers. A RCL Liaison (typically a Lead CT or QA technologist) will be appointed or designated at each clinical center (by that institution's radiology department and investigators at that institution), and will be directly supervised by the LTRC RCL QA and Training Technologist for training purposes. QA/QC certification and issue resolution and ongoing training of Clinical Center radiology personnel.

The QA program that RCL will require for all clinical centers is based upon the procedures that are recommended or required by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO), the National Council on Radiation Protection and Measurements (NCRP), the American Association of Physicists in Medicine (AAPM), the Council of Radiation Control Program Director (CRCPD), the Minnesota Department of Health (MDH), the International Electro-Technical Commission (IEC) and the ACR CT Accreditation Program.

Each clinical center will need to describe the CT equipment available at that institution for use in the LTRC study. Information regarding the equipment manufacturer, model and software version, optional features including dose reduction software, ad detector and tube design will be gathered. In collaboration with the investigators and radiology department at each clinical center, a sub-set of these CT systems will be selected for entry into the qualification process for the LTRC study. In addition, the clinical center investigators must supply to the RCL the credentials of the medical physicists and radiologic technologists who will be involved in the QA process and in the acquisition of images under the direction of the LTRC MOP.

The qualification process for a particular CT scanner for use in the LTRC protocols will involve the acquisition of clinical and phantom images, dose measurements, and the submission of

particular scanning protocols and image data to the RCL for analysis and verification. Phantom scanning and the evaluation of phantom images will be standardized and, where possible, automated. The process of quantifying the required performance will allow the LTRC to take advantage of changes in the CT technology or acquisition of new CT systems by the clinical centers during the duration of the LTRC study, as long as the new systems can perform in a manner that gives equivalent image quality and noise levels. Thus, as scanners are replaced by the clinical centers throughout the duration of the study, the LTRC project can utilize the new systems without concerns that the longitudinal validity of the data has been compromised.

The facility receiving initial LTRC qualification is qualified for the duration of the LTRC study. However, individual CT systems will be required to meet specific criteria on a regular basis for continued use in data acquisition. For sites that do not initially meet criteria for entrance into the LTRC for image acquisition, the RCL will provide specific recommendations regarding areas that could be improved for future qualification.

For new scanners added after qualification has been granted to a clinical center, or for systems having significant hardware or software upgrades to previously qualified equipment (including major assemblies such as the x-ray tube, detectors or reconstruction computers), the clinical center will be required to submit to the RCL the technical specifications for the scanner and a copy of the medical physicist's evaluation as specified in the LTRC MOP Appendix C "LTRC Radiology Manual." Thus each new system, or each system having major modifications, will require system qualification. The RCL will require the clinical center physicist to submit specific data including phantom images and dose measurements to the Radiology Center for approval.

The clinical centers will maintain a log regarding the results specific to the QA procedures for the duration of the LTRC study, and will send a copy of these results to the Radiology Center annually for review.

As part of the initial qualification process, a RCL investigator or collaborator will visit each clinical center to verify the qualifications of the clinical center staff, review their QA procedures, and assess the technical abilities and knowledge of the clinical center staff. Based on results, and at the RCL Administrative Group's discretion, additional site visits to clinical centers may be arranged.

15.4.1 LTRC Medical Physicist Qualifications

A qualified medical physicist at the clinical center (an individual certified by the American Board of Radiology in the subfield of Diagnostic Radiological Physics, or its equivalent) will be a key member of the LTRC clinical center CT QA process. This medical physicist must be familiar with the principles of CT imaging physics and of radiation protection; the guidelines of the National Council on Radiation Protection and Measurements; laws and regulations pertaining to the performance of the equipment being tested; the function, clinical uses and performance specifications of the imaging equipment; and calibration processes and limitations of the instruments used for performance testing. The clinical center physicist may be assisted by properly trained individuals in obtaining data; however, the medical physicist is responsible for and must be present during initial on-site evaluation of the clinical center by LTRC staff. Documentation to be sent to the RCL must be reviewed, approved and signed by the medical physicist. These requirements are equivalent to those required for ACR CT Accreditation.

15.4.2 Equipment Standards

CT equipment specifications and performance shall meet state and federal requirements and applicable ACR Standards.

15.4.3 CT Scanner Qualification for Use in the LTRC - Image Quality Phantom

The qualification of specific CT scanners for use in the LTRC study will require the use of a CT image quality phantom designated by the LTRC RCL. The ACR CT accreditation Phantom Gammex 464 will be purchased and sent to each clinical center, to ensure consistent QA measurements for all clinical centers. This phantom was designed by the ACR CT Accreditation Physics Committee for use on systems from any manufacturer. The phantom design is easy to use, measures all the relevant image quality parameters, and is made from solid materials that can be reproducibly manufactured. QA testing of the phantoms has shown less than 2 HU variance in the CT number of water, regardless of the CT system used.

The phantom is marked in such a manner as to make alignment very reproducible. Once set up, all image quality scans can be acquired using the clinical center's LTRC approved high-resolution chest scan protocol. Test objects at known locations will be used to provide the following quantitative measures of CT scanner performance:

- Scanner alignment light accuracy.
- Slice thickness.
- CT number accuracy of water, air, bone, acrylic, and polyethylene (mimic fat).
- Low contrast resolution.
- Image uniformity and noise.
- High-contrast spatial resolution.

The exact scan acquisition parameters to be used for the LTRC protocol will be utilized in the scanning of the Image Quality Phantom and the data obtained will be sent to the Radiology Center for further evaluation. If the image quality and dose data are acceptable, the particular scanner at the clinical center will be qualified for use in the LTRC. This will ensure that the images obtained with the clinical scan protocol produce consistent and comparable image quality at an appropriate dose. This image quality consistency is essential to the success of the quantitative analysis to be performed on the chest CT images in this study. Particular attention to the signal-to-noise ratios for the acquired scan, the high- and low-contrast resolutions, and the effect of the particular software reconstruction algorithm will be made. Data showing unusual results will be investigated using more in-depth measures of image quality, such as the measurement of the Modulation Transfer Function or the Section Sensitivity Profiles, in order to determine the adequacy of image data from a system for inclusion in the LTRC study.

15.4.4 Ongoing CT Scanner Quality Assurance Program

As outlined above, the performance of CT systems to be used at the clinical centers will be assessed with use of the ACR CT Image Quality Phantom prior to qualification for use in LTRC protocols. This will be done at the time of the visit from the QA and Training Technologist from the RCL. In addition, regular scanning of the same CT image LTRC Radiology quality phantom will be required. The frequency of such measurements shall be no less than monthly.

The phantom-based measurements will be performed, recorded and reported to the RCL. This on-going QA program will be monitored by the RCL Medical Physics and Medical Imaging Technology Specialists group, but will be administered by a qualified medical physicist or his/her qualified designee at each clinical center. In addition, a complete set of qualification images must be obtained and sent to the RCL after any major change in the scanner hardware or software.

If the results of a QA test fall outside the control limits, the RCL shall be notified and corrective action taken by the clinical center medical physicist and local service engineers. RCL physicists and other experts will be available to assist in prescribing corrective actions for unresolved problems. All deficiencies must be documented and service records maintained at the CT clinical center, and a copy of these should be included with the annual report to the RCL. It is expected that regular preventive maintenance be undertaken and documented by a qualified service engineer, according to the manufacturer's provided schedule and instructions. All test results, equipment deficiencies, corrective actions, and service records must be documented by the Clinical Center, with copies submitted annually for review by the LTRC Quality Assurance group and approval by the core RCL Administrative Group.

15.4.5 CT Scanner Dose Measurements and ALARA Program

Dose measurements for the scanners and scan acquisition protocol to be used in the LTRC study shall be conducted by a Qualified Medical Physicist according to the specific instructions contained in the LTRC MOP. Dose measurement shall be performed using the standard 32-cm diameter, acrylic CT Dose Index (CTDI) phantom prescribed by the FDA (Code of Federal Regulations 21). The medical physicist from the RCL will assist the medical physicist from the clinical centers in defining the exact acquisition parameters to be used for dose measurements, such that clinical dose estimates for the clinical scan protocol can be made from the dose measurements. The standard methods of obtaining CTDIs and Volume CTDI shall be employed, as described by the IEC, the ACR and the AAPM. This will include the use of the customary 100 mm long CTDI ionization chamber. Dose data shall be required for each CT system used in the LTRC, and any deviation of more than 10% from normative dose values of the given technique shall require investigation by the local medical physicist and service engineers. This on-going Dose QA program will be monitored by the RCL Medical Physics and Medical Imaging Technology Specialists group, but will be administered by a qualified physicist or his/her qualified designee at each clinical center. Dose measurements will be required annually, or after any major equipment component is replaced or modified. The dose data submitted by clinical centers will be analyzed in correlation with image quality measures from the Image Quality Phantoms and used to establish reference dose values for the LTRC clinical centers and, if necessary, to modify LTRC Protocols and sent dose limits.

The RCL medical physicists will assist the clinical centers with other questions regarding radiation safety or quality assurance. For each CT scanner qualified for use in the LTRC, the medical physicists will construct a detailed technique chart such that the dose is appropriate for participants of varying sizes. In this manner, comparable image quality (predominately image noise) can be achieved using doses that are As Low As Reasonably Achievable (ALARA).

At the RCL, two CT medical physicists will evaluate phantom images and accompanying dosimetry and protocol information.

15.4.6 Radiation Dose Optimization

The medical physicist at the RCL shall provide technique charts for all scanners included in the LTRC such that the appropriate radiation dose is delivered to study participants regardless of participant size. Such body size-specific acquisition parameters will allow comparable image noise levels for all participants. This consistency and comparability of the CT image data will reduce the likelihood that participant size will be a confounding fact in the quantitative analyses applied to the CT image data (such as histograms and texture analyses).

15.4.7 Reporting Duties

The RCL will verify the delivery of complete CT datasets and complete the CT Scan Inventory form. This report will include whether quantitative analysis was performed on the received datasets, the series and image counts of CT scans received, a qualitative analysis of the studies (with notation of specific quality issues if scan is suboptimal), a semi-quantitative analysis of the CT scan findings, a description of the distribution of emphysema and/or interstitial lung disease, notation and quantification of incidentally identified nodules and masses, the presumed radiologic diagnosis or diagnoses and any other general comments. These data will then be transcribed into the Advantage eClinical^{SM} data system within two weeks of the reception of CT scan data.

15.4.8 Radiology Center Image Quality and Protocol Compliance Assessment

As part of the semi-quantitative analysis by the radiologists at the LTRC RCL, subjective quantification of the quality of each study will be made. Specified CT artifacts such as participant motion, respiratory motion, abnormal image noise, beam hardening artifact, image in homogeneity, or image corruption will be noted. In addition, the ongoing data monitoring by the Image Transfer and Data Integrity Group will include regular review of DICOM header information in the image datasets from the Clinical Centers. Specific DICOM elements such as slice thickness, kVp settings, mAs settings (size-appropriate), slice thickness, slice interval, detector configuration, display field of view, and table speed will be compared to the specific protocols prescribed for each CT modality device. This will allow an assessment of LTRC Procedure compliance and clinical center technologist reliability.

Cases of improper protocol prescription or data entry will be documented and consistent errors or patterns of unreliable acquisition parameters from a clinical center may require retraining or intervention at the clinical center. If particular protocol issues cannot be resolved, these problems will be brought to the attention of the Steering Committee for further discussion and resolution.

15.4.9 Inadequate Study Procedures

Participants with inadequate completion of the LTRC-specific CT protocol exams by virtue of technical limitations - such as incorrect acquisition parameters, motion artifacts, and excessive image noise - should be immediately repeated if possible. For other studies deemed inadequate by the RCL, the participants should be rescheduled, if possible, for repeat CT as soon as possible by the clinical center investigators. In all cases, the need to obtain the LTRC-specific CT protocol or to obtain a repeat scan is at the discretion of the clinical center investigators.

15.4.10 Image QA/QC and Subjective Assessment

The RCL will maintain a QA validation procedure to validate the techniques used to acquire images at the clinical centers. The program will provide ongoing assurance of image quality and detail the specific procedures to assess image quality in the LTRC MOP. Each radiologist report will include an assessment of image quality and address any specific participant-related or unexpected image quality abnormalities, anomalies or artifacts.

These assessments will be logged and reviewed on a regular basis to assess for significant trends or correctable issues which would be addressed through contact with the clinical center CT Technologists Liaison. Studies deemed to have significant QA/QC issues or be non-diagnostic by the radiologist shall be reviewed at regular RCL Administrative Group meetings and, if deemed necessary, steps to correct or minimize systematic problems will be reported to the Steering

Committee for further analysis. The RCL will suggest to the Steering Committee any needed modifications in the methods of image acquisition and transmission.

The semi-quantitative and qualitative evaluation of pulmonary disease is inherently subjective, and depends greatly on the skill, training and experience of the radiologist. Because of this dependence, standardized terminology, accepted descriptive criteria, careful training and testing of reader performance is required. Our goal is to reproducibly assess the various pulmonary pathology within the LTRC studies and produce excellent inter and intra reader reproducibility.

Because analysis by experienced radiologists can be done quickly and expert interpretation allows differentiation of specific types of pulmonary pathology, semi-quantitative assessment is the preferred method for the evaluation of pulmonary disease. Inconsistently applied criteria or systematic bias can significantly reduce the power of the study.

15.4.11 LTRC Radiology Center Radiologist Viewing and Consistent Study Display

Images from the clinical centers will be transferred to a workstation for review and semi-quantitative analysis. This workstation will be similar in hardware and image viewing software features to a typical radiologist diagnostic workstation employed for the display of CT images. As such, this workstation will undergo the same regular display evaluation and calibration following the guidelines of the Medical Imaging Technology Services for the Department of Radiology at the Mayo Clinic. The combination of optimal acquisition parameters and thorough quality assurance programs at each clinical center, a careful integrity and tracking program, and a systematic training program for radiologists optimizes the quality and consistency of the quantitative and semi-quantitative data generated at the RCL.

16. CAPITATION PAYMENTS

16.1 Overview

Reimbursement for LTRC protocol activities will be accomplished through the use of a capitation reimbursement system. LTRC clinical centers and core laboratories will report to the NHLBI eligible capitation payments on their monthly invoice and/or quarterly financial report. Capitation payments will not be released until the LTRC DCC has provided verification to the Contracting Officer that the data is complete and accurate.

The DCC will generate lists monthly of the type of capitation that a LTRC clinical center and core laboratory can receive reimbursement for based on entry of designated LTRC forms. These lists will be provided to the NHLBI and the appropriate LTRC clinical center or core laboratory.

16.2 Clinical Center Capitation

DCC verification for clinical center capitation will be based on entry of study eCRFs in Advantage eClinical[SM] as identified in the table below:

Table 6: Capitation Criteria

Type	Description	DCC Verification
Enrollment	Enrollment and characterization of participant according to protocol.	• Six-Minute Walk • Medical History • Concomitant Therapy • Clinical Diagnosis • Pathological Diagnosis • Smoking History • Family History • Environmental/Occupational Histories • Spirometry • Blood draw • Laboratory testing of blood • Vital Status • Visit Documentation
Additional Characterization	Characterization in addition to what is required according to the protocol.	• Lung volume • Diffusing capacity (DLCO) • Arterial blood gas analysis
Tissue	Procurement of lung tissue, initial tissue processing, and delivery to Tissue Core Lab	• Site Specimen Inventory
Protocol CT Scan	Performance of LTRC protocol CT scan and delivery to Radiology Core Lab.	• CT Scan Inventory
Historical CT Scan	Acquisition of historical CT scan and delivery to Radiology Core Lab	• CT Scan Inventory

16.3 Radiology Core Laboratory Capitation

Capitation for the Radiology Core Laboratory (RCL) will be based on the number of LTRC participants from whom a CT image is received and analyzed according to the LTRC protocol manual. Either LTRC-specific imaging or historical exams that are received by the LTRC and reported by the RCL qualify for capitation. CT images that were obtained by protocol from the same participant on separate occasions are eligible for separate capitation. DCC verification for RCL capitation will be based on entry of the CT Scan Report eCRFs in Advantage eClinical[SM].

16.4 Tissue Core Laboratory Capitation

Capitation for the Tissue Core Laboratory (TCL) will be based on the number of LTRC participants from whom lung tissue is received, processed and analyzed according to the LTRC protocol. Lung tissues obtained from the same participant in separate surgical procedures on different days are eligible for separate capitation. DCC verification for TCL capitation will be based on entry of the Tissue Core Inventory eCRF in Advantage eClinical[SM].

APPENDIX A: OVERVIEW OF STUDY PROCEDURES

Table 7: Overview of Study Procedures

Procedure	Requirement
Demographics	R
Informed Consent and Eligibility	R
Visit Documentation	R
Adverse Events	AN
Clinical Dx Report	R
Concomitant Therapy	R
Central Pathology Report	R
CT Scan Report	R
Environmental Questionnaire	R
Family Hx Questionnaire	R
St. George's Respiratory Questionnaire	R
Laboratory Data	R
Local Pathology Report	R
Medical Hx Questionnaire	R
Occupational and Environmental Questionnaire	R
Pulmonary Function Tests*	R
SF-12 Health Survey	R
Smoking Hx	R
Symptom Questionnaire	R
Study Status	R
Six-Minute Walk Test	R
Vital Status	R

R: Form is required.

AN: Form to be completed only as needed.

* Spirometry is required but lung volume, diffusing capacity (DLCO) and arterial blood gas (ABG) components are optional.

APPENDIX B: PARTICIPATING INSTITUTIONS

The LTRC is being conducted by the NHLBI, National Institutes of Health (NIH), Bethesda, MD in collaboration with four clinical centers, a Data Coordinating Center (DCC) and two Core Laboratories. Each of the clinical centers, the DCC and the Core Laboratories has been contracted by NHLBI to perform certain duties for the study.

Detailed contact information on all study staff at all the participating institutions is provided on the LTRC study website (www.ltrcpublic.com). This directory is updated as needed.

I. NATIONAL HEART, LUNG, AND BLOOD INSTITUTE (NHLBI)

As the contracting institution, staff at the NHLBI in Bethesda, MD is responsible for the overall design and conduct of the LTRC. The Project Officer will oversee the study design, implementation, data quality and information dissemination. The Contract Officer will monitor the receipt of all deliverables required by the contracts and payments to the contracted institutions.

Contacts:

Thomas Croxton, MD, Project Officer

Sara Stoops, MBA, Contract Officer

II. CLINICAL CENTERS

Each of the four clinical centers has participated in the design of the LTRC and has agreed to have the necessary staff and resources to implement the Protocol. Each clinical center will be responsible for recruiting and retaining a minimum of 100 participants, collecting the required data, transmitting these data electronically to the DCC, and shipping specimens and scans to the Core Laboratories.

Contacts:

Andrew Limper, MD, Principal Investigator, Mayo Clinic Rochester, MN

Kevin Flaherty, MD, Principal Investigator, University of Michigan

Frank Sciurba, MD, Principal Investigator, University of Pittsburgh

Gerard Criner, MD, Principal Investigator, Temple University

III. DATA COORDINATING CENTER (DCC)

The DCC is located at The Emmes Corporation (Emmes), Rockville, MD and is responsible for study coordination, data management, study monitoring, quality control measures and data analysis. The DCC will maintain all study documents, the study website and the Internet data entry system. Additionally, the DCC will schedule conference calls and coordinate meetings.

Contacts:

Diane Brandt, Principal Investigator

Traci Clemons, PhD, Co-Principal Investigator

IV. RADIOGRAPHIC CORE LABORATORY (RCL)

The Radiographic Core Laboratory (RCL) is located at the Mayo Clinic – Rochester, MN and is responsible for developing the standardized procedures for obtaining the CT scans, interpreting, storing, providing a diagnosis, shipment of scans to investigators and providing data to the DCC for inclusion in the LTRC database. Additionally, the RCL is responsible for monitoring the calibration of the densitometers at each clinical center and the rotation of quality assurance phantoms between the clinical centers.

Contact:

Brian J. Bartholmai, MD, Principal Investigator

V. TISSUE CORE LABORATORY (TCL)

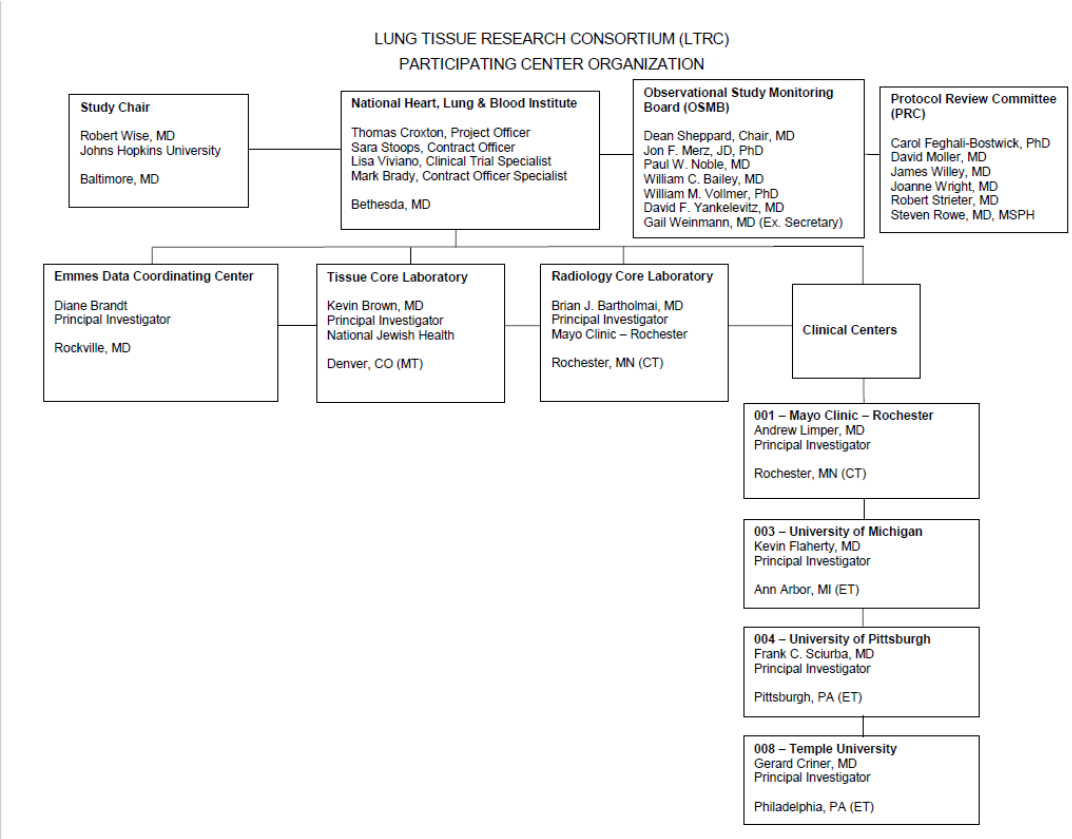
The Tissue Core Laboratory (TCL) is located at the National Jewish Health Center, Denver, CO and is responsible for developing the standardized procedures for obtaining the tissue specimens, storage of these specimens and shipment of specimens to investigators.

Contact:

Kevin Brown, MD, Principal Investigator

VI. ORGANIZATIONAL CHART

Figure 1: Organizational Chart



APPENDIX C: RADIOLOGY MANUAL OF PROCEDURES

The Radiology Manual of Procedures is documented as a separate document. It can be downloaded from the LTRC study website (www.ltrcpublic.com) by logging in and navigating to Resources>LTRC>Study Documents.

APPENDIX D: REFERENCES

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