

Lung Tissue Research Consortium User's Guide to the LTRC

What is the LTRC? The Lung Tissue Research Consortium (LTRC) provides tissues and data to investigators performing research on lung diseases, especially chronic obstructive pulmonary disease (COPD) and interstitial lung disease (ILD). The majority of ILD patients have Idiopathic Pulmonary Fibrosis (IPF). LTRC materials can be requested to facilitate investigations of clinical features, radiographic manifestations, and pathogenetic mechanisms of lung disease through histopathological studies of human lung tissues. Clinical and tissue resources of the LTRC come primarily from patients with COPD and ILD, though limited numbers of tissues may also be available from patients with other diseases. The LTRC serves as a repository for tissue specimens, blood samples, corresponding clinical data, physiological parameters, and CT scans from donor subjects with COPD and ILD.

How do I determine what tissues and data are available through the LTRC? The availability of subject materials in the LTRC data set can be reviewed on the LTRC public website at www.ltrcpublic.com. Subject materials may be located using a web-based search tool that permits query by diagnosis, lung function, age, demographic characteristics, and other features. After reviewing the website, if you are uncertain as to the availability of sufficient tissue samples that you need, you may email the LTRC Data Coordinator, at coordinator@c-tasc.com.

What types of clinical data, radiographic information, and tissue materials and formats are available through LTRC? The LTRC provides clinical data including but not limited to subject age, sex, smoking history and environmental exposures, as well as clinical data including pulmonary function tests, six-minute walk testing, and quality of life measures such as the St. George Respiratory Questionnaire. Complete cardiopulmonary exercise testing data is available on certain subjects. CT scans are available for most subjects, and results from quantitative analysis and anatomic image segmentation are available on selected subjects who have completed a volumetric high-resolution CT. Tissue materials obtained from subjects include formalin-fixed, paraffin-embedded tissues (FFPE), HOPE-fixed tissues, RNAlater-fixed lung tissues, flash frozen lung tissues, glutaraldehyde-fixed tissues, plasma and serum samples, and DNA obtained from peripheral blood. Potential uses of LTRC tissues include histopathology, immunohistochemistry, biochemical analyses, DNA and RNA analyses, and microarray and proteomic studies. Table 1 at the end of this User's Guide provides a convenient cross-reference of the various specimen types and potential research uses. Table 2 and Table 3 list the storage and shipping conditions for each of these specimen types. Table 4 lists the types of radiology-related data available from the LTRC.

What kinds of requests for tissue and data can be made? Requests for tissues and subject information available through the LTRC will generally fit into one of three categories, based upon the size of the study and the resources being requested. These are: pilot studies of tissues from small groups of subjects; comprehensive tissue studies involving many subjects and/or individual data; and studies not requiring tissue resources (clinical/CT data only).

Pilot studies of tissue groups will typically involve few subjects (up to two groups of eight), limited numbers of samples from each subject, and aggregated clinical data about the subjects (for example, group averages for age, FEV₁ % predicted, QOL scores, 6 min walk test distance, and CT emphysema score). Pilot tissue study requests will generally be used for proof of concept studies, investigations of new pathophysiological concepts, and studies designed to

generate preliminary data for larger studies or for grant applications. While sound scientific rationale will be required for such requests, extensive preliminary data will not be required.

Comprehensive tissue studies may involve up to 40 subjects. Greater numbers of biological samples and more detailed subject clinical information may also be requested than for pilot studies. Comprehensive study requests will generally represent definitive studies, investigations proving pathophysiological concepts, and studies designed to provide substantial insights into these lung diseases. In addition to sound scientific rationale, supportive preliminary data and proof of concept will also be needed for approval of these requests, commensurate with the amount and scarcity of the specimens being requested. A statistical plan including study design, analytical methods, and power analysis will be required of all such proposals. A realistic timeline for completion of the project should also be included.

Studies that do not require tissue resources may be performed with clinical information and radiographic data of the LTRC. For projects that do not involve banked tissue, nucleic acid, or blood sample resources, there is no limit imposed on the number of subjects that may be studied. A formal research plan, including design, methods, and power analysis, will be required of all such proposals. A timeline for completion of the analysis must also be provided.

How much does it cost to get tissues and data from the LTRC? At this time, the NHLBI is paying all costs for procuring, processing, preparing, and shipping LTRC specimens and data to qualified investigators. You must, of course, obtain funding from other sources for performing the research project itself.

How do I apply for LTRC materials? Clinical data, radiographic information, and biological samples can be obtained by completing all forms found in Appendix I through V of the LTRC Tissue Requisition and Publication Policy document which is downloadable from the website www.ltrcpublic.com. All requests require the investigator's biosketch, a detailed scientific proposal, and agreement to specific terms and conditions imposed by the LTRC. In particular, LTRC data and tissues may be used only for the specific study described in the application, and acknowledgement of LTRC support must be given in all publications and presentations of the study. If your institution determines that the proposed study involves human subjects, IRB approval or exemption must be provided at the time of application.

Can I obtain statistical consultation for designing my studies using LTRC resources? Statistical and study design assistance are available through the LTRC data coordinating center. As noted above, formal statistical methods, including power analysis, and planned analyses will be required of all formal studies. If local statistical support is not available, the investigator is strongly encouraged to contact the Data Coordinating Center (DCC) via e-mail to coordinator@c-tasc.com prior to application for LTRC resources.

How is the request reviewed and processed? The completed tissue request should be sent electronically to the DCC via e-mail to coordinator@c-tasc.com. A hard copy of Appendix IV with original signatures should be sent to the DCC. After an initial administrative and logistical review by DCC personnel, the proposal will be sent to a protocol review committee established by the NHLBI for formal scientific review of proposals. The recommendation from this review will be sent back to the DCC, who will contact you by E-mail. If your request is approved, the DCC will authorize and coordinate release of LTRC clinical information, radiographic data, and tissue and blood samples as requested. Tissue specimens will be shipped directly from the Tissue Core Center at the University of Colorado and radiographic data will be shipped from the

Radiology Core Laboratory (RCL) at the Mayo Clinic College of Medicine. All clinical information will be sent directly from the DCC.

Can I later request additional patient or radiographic information on the subjects in my study? The LTRC Data Coordinating Center will maintain the ability for individual investigators to obtain additional clinical or radiographic data, beyond that requested in the initial proposal, for a period up to two years or until after the manuscript related to the study has been published. After that time, the DCC will delete records of which tissues were provided to you. Requests for additional data should be directed to the LTRC DCC via e-mail to coordinator@c-tasc.com.

What if I wish to perform additional studies on residual tissue obtained from the LTRC Data Coordinating Center? To obtain LTRC tissue specimens, you must agree that any remaining portions of specimens and any new samples generated from the original LTRC specimens will remain the property of the LTRC and may not be used for any reason other than the originally proposed study without express written permission. In addition, you may not disseminate LTRC resources outside of your laboratory without written permission from the LTRC Protocol Review Committee or NIH. You must also agree that after completion of the project all remaining LTRC human specimen materials will be destroyed unless an application has been submitted to the LTRC requesting permission to perform an additional study with those materials. Additional studies that you wish to perform with LTRC tissue or data resources already on hand may require that you submit a new proposal for review and approval by the Protocol Review Committee.

How do I contact the LTRC, if I have questions? Most information concerning the LTRC clinical data and tissues are found on the LTRC website at www.ltrcpublic.com. Additional inquiries to the LTRC should be directed to the data coordinating center, which can be reached by phone (410-435-0663), FAX (410-435-0689), or e-mail to coordinator@c-tasc.com.

TABLE 1
Specimen Types Available through the LTRC

Specimen Types	Tissue samples available	Approximate number of samples per patient from tissue harvested	Standard aliquot per patient for distribution	Uses
Formalin Fixed, Paraffin-Embedded Tissue	blocks	1/biopsy 4-10/lobe 12-30/explant	1 block	Histopathology, DNA
	Slides 4-5 microns	100/block	≤20 slides	Histopathology, DNA
HOPE Fixed Tissue	blocks	1/biopsy 1-3/lobe 6-15/explant	1 block	Histopathology, DNA, RNA, protein
	slides	100/block	≤ 20 slides	Histopathology, DNA, RNA
	"slices" 20 microns	25/block	≤ 5 slices	DNA, RNA, protein, NOT laser micro-dissection or Histopathology
RNA Later RNA Preserved Tissues	aliquots	10/lobe 10-25/explant	30-100 mg	DNA, RNA, protein
Flash frozen	aliquots	5/lobe 10-25/explant	30-100 mg or 100-1000 mg	DNA, RNA, protein
Glutaraldehyde	aliquots	1-3/lobe	1 mm ³	electron microscopy
Plasma/Serum	aliquots	4-8/patient	0.5 ml	
DNA	aliquots	5-10/patient	20 mcg	

TABLE 2
SPECIMENS ARE STORED UNDER THE FOLLOWING CONDITIONS:

Paraffin blocks	Room temperature
Paraffin embedded slides	-20° C
HOPE blocks	-20° C
Frozen tissue	-80° C
RNAlater tissue	-20° C
Gluteraldehyde	4° C
Blood – DNA tubes	-20° C
Blood – plasma and serum	-80° C

TABLE 3
SPECIMENS WILL BE SENT TO REQUESTING INVESTIGATORS IN THE FOLLOWING CONDITIONS:

Paraffin blocks	Room temperature
Paraffin embedded slides	Room temperature
HOPE blocks and slides	Dry ice
Frozen tissue	Dry ice
RNAlater tissue	Dry ice
Gluteraldehyde	Ice packs
Blood – DNA tubes	Dry ice
Blood – plasma and serum	Dry ice

TABLE 4
THE FOLLOWING ARE THE TYPES OF DATA AVAILABLE FROM THE LTRC RADIOLOGY CORE LABORATORY (RCL)

<p>DICOM Datasets</p>	<p>'Full LTRC Three-Phase CT Scan' consists of volumetric non-contrast high-resolution CT of the chest with 1 to 1.25mm thickness images obtained as a contiguous volume on a multidetector scanner and reconstructed with 50% slice overlap utilizing a high spatial frequency algorithm appropriate for HRCT of the chest.</p> <p>'Basic LTRC High-Resolution CT' includes the three acquisitions of non-contiguous high-resolution images (1-1.25mm thickness) obtained with a gap of 9mm between the images.</p> <p>Other CT scans of the chest not obtained by a LTRC-specific protocol are available for most LTRC participants.</p>
<p>Quantitative Analysis Results</p>	<ul style="list-style-type: none"> A. <u>Lung volumes</u>. This includes total lung volumes as well as the subsets of Left and Right lungs, the lobes within each lung. Results for each lobe are subdivided into central and peripheral components. B. <u>Emphysema volume/score</u>. Specifically, this is the % pixels within the lung measuring less than -950 Hounsfield Units (HU). C. <u>Lung parenchyma histogram analysis</u>: Mean Density, Standard Deviation, Histogram Normality, Relative area below the 5th percentile of the histogram (Perc 5), and Relative area below the 15th percentile of the histogram (Perc 15). D. <u>Texture Analysis results</u>. Quantitative techniques utilized to estimate of the volume of specific parenchymal abnormalities including Honeycombing, Ground Glass Density and Reticular Infiltrates. E. <u>Tracheal branching analysis</u> include cross sectional area, length and estimated wall thickness at the midpoint of the trachea, main bronchi and lobar bronchi.
<p>Segmentation Results /Anatomic Object Maps</p>	<p>These digital files represent the anatomic structures supine inspiration dataset for all Full LTRC Three Phase CT Scans have associated 'object map' files created of the chest that are determined automatically by the image processor as well as some structures manually identified by a radiologist or technologist during the analysis. These files identify each pixel within the CT volume as belonging to a particular body part or type of pathology.</p>