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14. ABSTRACT The mission statement of the Lung Cancer Biospecimen Resource Network (LCBRN) states that the LCBRN "will collect, annotate, store, and distribute human lung cancer biospecimens in a manner that embraces the highest ethical standards for human subjects research, that conforms to the best practices of biorepository science, and that furthers basic, translational and clinical research in the understanding, diagnosis and treatment of this disease". Year 2 of the award has been spent increasing subject enrollment at all sites, building on the informatics infrastructure to support biorepository activity, creating the web site that serves as the interface between investigators, the public and the LCBRN, and establishing a biomolecular extraction technique that allows the LCBRN to provide investigators with nucleic acids and protein instead of the tissue samples themselves. Coordinators, procurement personnel and co-PIs continue to participate in monthly LCBRN conference calls, and all Resource Sites continue to ship biospecimens for permanent banking to the LCBRN Coordinating Center at regular intervals. Although the LCBRN was initially prepared to provide samples to investigators in September 2012, sample distribution has been delayed until the Coordinating Center completes molecular extraction of previously banked and tissue specimens as recommended by the EAB.					
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Section I: Introduction

The mission statement of the Lung Cancer Biospecimen Resource Network (LCBRN) states that the LCBRN “will collect, annotate, store, and distribute human lung cancer biospecimens in a manner that embraces the highest ethical standards for human subjects research, that conforms to the best practices of biorepository science, and that furthers basic, translational and clinical research in the understanding, diagnosis and treatment of this disease”. The specific aims to support this program include: 1) the creation of a Coordinating Center at The University of Virginia (UVA) that provides standard operating procedures (SOPs), standardized specimen collection kits, informatics infrastructure, quality control procedures and specimen storage as well as being the contact site for investigators using the resources of the LCBRN, 2) the creation of Biospecimen Resource Sites at UVA, the Medical University of South Carolina (MUSC) and Washington University in St. Louis (WUSTL) that will recruit subjects with lung cancer and procure the biospecimens, and 3) the establishment of a centralized biobank of high quality tissue, blood, urine, bronchoscopic washing and saliva samples from lung cancer subjects that are annotated to clinical, laboratory and radiographic data.

Section II: Progress

Subject Accrual

UVA, MUSC, and WUSTL received human subjects regulatory approval to begin recruiting patients on 03/17/2011, 04/19/2011, and 04/22/2011 respectively. Since final approval was not obtained until seven months from the start date of the grant period, the LCBRN as a consortium was behind in its recruitment goal of 50 subjects enrolled per site by the end of year 1. All Biospecimen Resource Sites strived to make up for this deployment lag time by recruiting a minimum of 100 subjects each with complete biospecimen collection by the end of Year 2. As of this reporting period, UVA has recruited a total of 131 subjects, MUSC 101 subjects, and WUSTL 126 subjects. Review of progress will continue to be monitored at the monthly Coordinating Conference calls during which each site will be asked to give an update on subject enrollment and follow-up collections.

Informatics

The LCBRN’s central database stores subject identification, clinical data and tracks biospecimen parameters. Although the patient modules of the database were in use at all Resource Sites during the first year of the award, the design and coding for the biospecimen modules were not complete until December 2011. The specimen modules track accessioning, biospecimen type, segmentation, preparation details, processing events, and sample location. After a brief testing period, the specimen modules were deployed in January 2012 while the backlog of specimen data, which was maintained by the LCBRN Network Manager using Excel spreadsheets, was successfully migrated into the database by March 2012.

Specimen data entry begins on the Specimen Data Entry tab where users may search on a subject ID or Aliquot ID. Once a subject or aliquot has been selected for the session, the previously entered patient demographics information will appear at the top of the screen along with a tabbed menu for either

tissue accessioning or fluid accessioning. The tabs show any specimen data entered for this subject previously and allows for additional entries as well as edits to any existing data. Figure 1 shows a screen shot of the Tissue tab which is used to accession tissue specimens, create aliquots and capture the events associated with procurement.

The screenshot displays the 'TISSUE' tab of a specimen management system. It is divided into several sections:

- Tissue Accessions (1)**: A table with columns for Specimen Number, Accession Date, Institution, Surg Path #, and Action. It shows one accession for specimen V2352A2352, dated 02/01/2012, from the University of Virginia, with Surg Path # 512-12345. There are 'add' and 'clear' buttons at the bottom.
- Tissue Specimens for Accession V2352A2352 (3)**: A table with columns for Site, Position, Provisional Dx Category, and Action. It lists three specimens: LUNG (RUL, MALIGNANT), LUNG (RUL, NORMAL), and BRONCHUS (RUL, NORMAL). Each row has an 'add' and 'clear' button.
- Notes for Aliquots of Selected Specimen**: A text area containing the message 'No notes for aliquots of the selected specimen.' with 'Cancel' and 'Save Content' buttons.
- Tissue Aliquots for Selected Specimen (2)**: A table with columns for Aliquot #, Status, Preparation Type, Preparation Details, Qty, Units, Location, and Action. It shows two aliquots: V00123T01 (Pending QC, Fixed, FFPE NBF 12-48 hr) and V00123T02 (Pending QC, Frozen, QF LN2). There is a 'save selected aliquots' button.
- Tissue Events for Aliquot V00123T02 (5)**: A table with columns for Event Type, Event Date, Event Time, and Action. It lists five events: Clamp time (12:30 PM), Excised from patient (1:00 PM), Received in surg pat (1:05 PM), Received by technici (1:10 PM), and Frozen (1:15 PM). Each row has an 'add' and 'clear' button.

Figure 1. Tissue tab of specimen module showing specimen accessioning, segmenting and procurement events.

Both the tissue and fluid tabs are currently operational and being used at all Resource Sites. Once specimens are shipped from the Resource Sites to the Coordinating Center, tissue specimens undergo histologic quality control (QC) and are further segmented into aliquots for distribution and molecular extraction. Both tissue and fluid aliquots are then given location codes and entered into the central database by the Network Manager.

The QC management screens are in their final testing phases. These modules will track both histologic and molecular QC data on tissue specimens. Figure 2 shows a screen shot of the QC module. QC information is currently being captured on paper by the reviewing pathologist at the time of histologic and molecular assessment and is then transferred to an Excel spreadsheet maintained by the LCBRN Network Manager. It is expected that this module will be tested, debugged and deployed by the end of

2012. QC data being captured on excel spreadsheets will be transferred into the central digital database soon thereafter.

The screenshot shows a web-based interface for specimen quality control (QC). The top navigation bar includes tabs for 'SUBJECT DATA ENTRY', 'SPECIMEN DATA ENTRY', 'SPECIMEN QC', and 'REPORTS'. The 'SPECIMEN QC' tab is active.

Below the navigation bar, there is a search section with 'Search Type' set to 'Subject ID', 'Search Text' set to 'V0200', and a 'Search Pending Only' checkbox. A 'Search' button and a 'Show All Pending' link are also present.

The main form area contains several input fields and dropdown menus for specimen details:

- QC Report Designation:** Tumor-FFPE
- QC Site:** (dropdown)
- Diagnosis:** (dropdown)
- Other Comments:** (text area)
- Submitting Diagnosis:** (text field)
- TTF-1:** (dropdown)
- Napsin A:** (dropdown)
- CK 5/6:** (dropdown)
- p63:** (dropdown)
- Chromogranin:** (dropdown)
- Synaptophysin:** (dropdown)
- Mucin:** (dropdown)
- Malignancy Type:** (dropdown)
- Tissue Type:** (dropdown)
- Diagnostic Comments:** (text area)

At the bottom of the form, there are fields for 'Assign to (Aliquot Numbers)' and 'Completion Date', along with 'Save', 'Edit', and 'Cancel' buttons.

On the right side of the form, there is a table titled '6 aliquots found' with columns 'Aliquot ID', 'Weight', and 'Status':

Aliquot ID	Weight	Status
V00123T01		Pending QC
V00123T02	.65 g	Pending QC
V00235N01		Pending QC
V00235N02	1.00 g	Pending QC
V00345B01		Pending QC
V00345B02	.32 g	Pending QC

Figure 2. Screenshot of the QC module – Depicts formalin fixed paraffin block (FFPE) block awaiting histologic review.

The latest IT infrastructure provided to the LCBRN by the Coordinating Center is the web site that serves as the interface between investigators and the public with the LCBRN. The site (<http://www.lcbnrn.org/>) contains information on LCBRN samples and collection protocols, the informed consent documents used at each Resource Site, as well as the application procedures to obtain specimens. Once received by the Coordinating Center, investigators applications will be reviewed by the Scientific Review Panel (SRP), which includes all LCBRN P.I.s and 3 academic faculty with funded lung cancer research programs. Although the LCBRN publicly announced an expected sample distribution date of September 2012, due to the recommendation by the External Advisory Board (EAB) to prioritize the distribution of DNA, RNA, and protein to investigators instead of whole tissue, sample distribution has been delayed while the backlog of tissue samples are undergoing molecular extraction.

Standard Operating Procedures (SOPs)

Additional SOPs dealing with biorepository activities have been added as needed since the end of year 1 and are available at the LCBRN Collab online resource (<https://collab.itc.virginia.edu/portal>) and on the LCBRN public website.

Table 1: LCBRN SOPs as of 10/19/2012

SOP#	Creation date	SOP Title
LCBRN SOP1	11/18/2010	Establishment, revision & documentation of LCBRN SOPs
LCBRN SOP2	12/17/2010	Tissue Procurement
LCBRN SOP3	11/23/2010	Procurement and aliquoting of serum
LCBRN SOP4	11/23/2010	Procurement and aliquoting of plasma and buffy coat
LCBRN SOP5	11/23/2010	Procurement and aliquoting of urine
LCBRN SOP6	1/23/2010	Procurement and aliquoting of saliva

LCBRN SOP7	11/23/2010	Procurement and aliquoting of bronchial lavage fluid
LCBRN SOP8	11/19/2010	Procurement and aliquoting of sputum
LCBRN SOP9	12/01/2010	Shipping of samples to the LCBRN Coordinating Center
LCBRN SOP10	11/18/2010	Venipuncture
LCBRN SOP11	03/24/2011	Creating LCBRN Specimen Labels
LCBRN SOP12	09/15/2011	Informed Consent
LCBRN SOP 13	06/15/2012	Archival Materials Request
LCBRN SOP 14	08/31/2012	RNA DNA Protein Isolation

Due to concerns raised by the EAB that DNA obtained from adjacent non-neoplastic tissue in lung resection specimens may not always serve as the optimal control for “normal” DNA, the LCBRN updated the plasma collection protocol (SOP4) to include the collection of the white blood cells present in the buffy coat after centrifugal blood plasma separation. All of the LCBRN Resource sites have successfully deployed this additional biosample collection.

In order to maximize the collection of frozen tissue samples, the LCBRN adopted the standard that any malignant or normal lung tissue received for procurement that is less than 200 mg should be frozen in its entirety. By following this practice, formalin fixed paraffin embedded (FFPE) materials is unavailable to cut the slides needed for the immunohistochemistry (IHC) necessary for assessing poorly differentiated tumors. For these cases, the Coordinating Center asks that biorepository personnel access the clinical archival materials at their respective sites to obtain FFPE blocks for unstained sectioning. These slides are then shipped to the Coordinating Center where IHC workup is performed. See LCBRN SOP 13.

As discussed earlier in this report, the EAB recommended the LCBRN develop a plan for DNA/RNA extraction including quality documentation outlining the specific molecular extraction protocol. At the time of this report, previously banked and prospectively procured tissue samples are undergoing extraction following the guidelines written in LCBRN SOP 14. This methodology utilizes organic extraction (Trizol) and alcohol precipitation, which obtains both high and low molecular weight RNA. Though more time consuming than silica matrix-based RNA isolation and not amenable to the robotic automaton available to the LCBRN Coordination Center, this protocol satisfies the EAB recommendation of ensuring investigators interested in microRNA analysis have RNA preparations suited for this purpose. Figure 4 demonstrates the presence of low molecular weight RNA species in test tumor extractions using the new SOP.

Coordination meetings and conference calls

LCBRN conference calls that include coordinators, procurement personnel and co-PIs have been scheduled on a monthly basis. Calls and meetings have occurred on the following dates:

10/27/2011

12/16/2011 - Conference call included webinar demoing specimen modules of the LCBRN database

01/20/2012
02/17/2012
03/23/2012
04/23/2012
05/21/2012
05/21/2012- Conference call between co-P.I.'s and Network Manager only
06/26/2012 - EAB meeting in Fort Detrick, MD
07/06/2012
08/17/2012
09/18/2012
10/22/2012 -2nd Annual LCB RN Coordinating Meeting

Topics for conference calls have dealt with subject enrollment, regulatory affairs, informatics and specimen quality control. Minutes are available at the LCB RN Collab online resource.

The 2nd annual LCB RN Coordinating will take place in Charleston, SC on 10/22/2012 and will be attended by the Co-P.I.'s, Clinical Research Coordinators, Network Manager, biorepository personnel and 2 representatives from the Congressionally Directed Medical Research Program (CDMRP). Items on the proposed agenda include subject enrollment, an update on follow-up specimen collections at each site, a demo of the QC module of the LCB RN database, and the EAB recommendations.

Tissue collection and quality control

Histologic and molecular quality control have been performed at regular intervals on tissue from all 3 Resource Sites. Figure 1 shows the cumulative RNA Quality Index (RQI) for tissue samples as of 05/14/12. RQI values of 7 or above indicate excellent quality RNA, values between 4 and 7 are considered moderate, and any values below 4 indicate poor quality RNA.

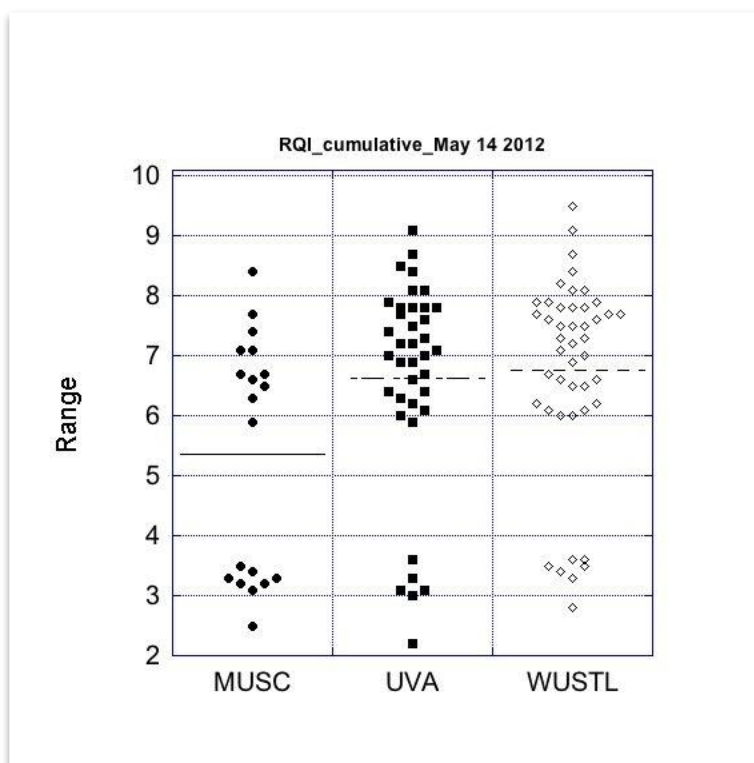


Figure 3: RNA RQI Scores of tumor samples from each Resource Site as of 05/14/2012

The majority of tissue samples from all sites fall into the moderate to excellent quality range. After examining the intrinsic tissue necrosis, warm ischemia time and cold ischemia time on a small subset of cases from all Resource Sites, there was no significant correlation found between the RQI scores and any of the aforementioned factors. Further analysis on a larger sample set will be performed prospectively. All Resource Sites will continue to follow the guidelines of known literature that states that collecting specimens quickly will result in higher RQI scores.

Key LCBRN personnel attended the annual EAB meeting in Fort Detrick, MD on 06/26/2012. One of the recommendations of the EAB was for the LCBRN to provide investigators with extracted nucleic acids or proteins from tissue rather than the actual tissue specimens themselves. In accordance with this recommendation, the LCBRN has adopted a protocol of biomolecule extraction (utilizing Trizol™ organic extraction rather than silica matrix adsorption) that results in the isolation of the full range of RNA molecular weights, and also isolates DNA and protein from the same tissue sample, further maximizing the availability of these biospecimen resources. (See SOP #14) The figure below shows microcapillary electrophoresis of RNA obtained from a collection of non-LCBRN tumor samples. Both high and low molecular weight RNAs are isolated using this technique.

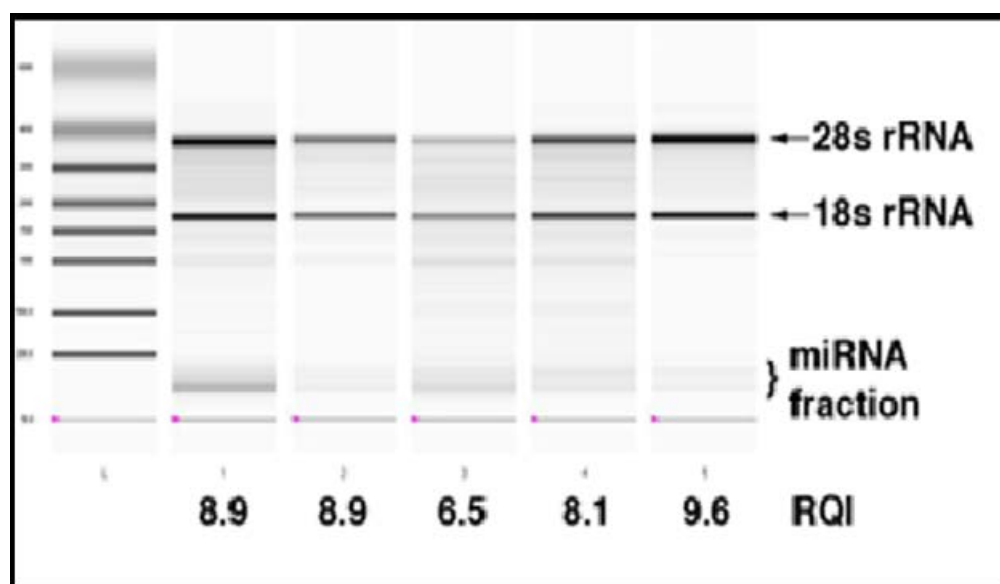


Figure 4. Microcapillary electrophoresis of RNA obtained from test tumor samples

The Coordinating Center is currently extracting the previously banked tumor and normal tissue samples in addition to any specimens procured prospectively.

Data Quality Assessment

The Data Quality Control Manager and Network Manager performed a data audit by sampling the Baseline form, Pathology and Staging form, Surgical Information form and Follow-up Forms from five randomly selected LCBRN enrolled patients at each Resource Site. Both tissue and biofluid collection forms from these same 5 patients were also assessed. By comparing paper and digital records, they were able to review the completeness of data annotation and follow-up conforming to specified time lines. The Data Quality Control Manager and the Network Manager have been working with the Caisis development team to ensure that data entry screens and procedures capture required data elements, follow a standardized workflow, and that valid data is being captured in the database. As a result of their review, updates to the database include the ability to track any newly developed primary tumor unrelated to lung cancer at follow-up, as well as the ability to track follow-up specimen collection more accurately for reporting purposes. The results of the data audit revealed no significant findings; however, the Network Manager will review best practices for data entry with all database users and will also determine a timeline for regular specimen shipments to the Coordinating Center at the annual Coordinating Meeting on 10/22/2012.

Section III: Key Research Accomplishments

- Creation and deployment of the specimen modules of the LCBRN database
- Migration of specimen data from excel spreadsheets into LCBRN database
- Creation and maintenance of the LCBRN public website
- Creation of the investigator application and investigator agreement forms, as well as the Scientific Research Panel (SRP) who will review these documents

- Successful implementation of monthly conference calls with minutes recorded and kept on LCBRN Collab online resource
- Successful deployment of the buffy coat isolation procedure at all Resource Sites
- Biospecimens shipped from all Resource Sites and stored at LCBRN Coordinating Center
- Histologic and molecular quality control performed at the LCBRN Coordinating Center with regular feedback provided to all Resource Sites
- Successful adoption of a new bimolecular extraction technique that will result in the distribution of RNA, DNA, and protein aliquots to investigators

Section IV: Reportable Outcomes as of 10/19/2012

UVA - Year 2 Metrics

Number of subjects currently enrolled in study: 131

Number of subjects who have baseline biofluid collection: 119

Percentage of subjects who have tissue collection: 86%

Number of subjects who have follow-up 1 biofluid collection: 37

Number of subjects who have follow-up 2 biofluid collection: 7

Number of subjects who have follow-up 3 biofluid collection: 2

Number of subjects withdrawn from study: 24

Reasons for withdrawal from study: Benign disease (15), Ineligible to continue (9)

Number of subjects withdrawn from follow-up: 6 (deceased)

WUSTL - Year 2 Metrics

Number of subjects currently enrolled in study: 126

Number of subjects who have baseline biofluid collection: 124

Percentage of subjects who have tissue collection: 77%

Number of subjects who have follow-up 1 biofluid collection: 38

Number of subjects who have follow-up 2 biofluid collection: 9

Number of subjects who have follow-up 3 biofluid collection: 4

Number of subjects withdrawn from study: 14

Reasons for withdrawal from study: Benign disease (10), Ineligible to continue (4)

Number of subjects withdrawn from follow-up: 4 (deceased)

MUSC - Year 2 Metrics

Number of subjects currently enrolled in study: 101

Number of subjects who have baseline biofluid collection: 90

Percentage of subjects who have tissue collection: 75%

Number of subjects who have follow-up 1 biofluid collection: 25

Number of subjects who have follow-up 2 biofluid collection: 3

Number of subjects who have follow-up 3 biofluid collection: 0

Number of subjects withdrawn from study: 10

Reasons for withdrawal from study: Benign disease (6), Ineligible to continue (3), Withdrew consent (1)

Number of subjects withdrawn from follow-up: 1 (deceased)

LCBRN Consortium –YEAR 2 Metrics

Number of subjects currently enrolled in study: 358

Percentage of subjects who have tissue collection: 81%

Percentage of subjects with a military history: 21%

Percentage of subjects who have never smoked: 6%

Percentage of subjects who are Caucasian: 89%

Mean age of subjects: 65 years

Section V: Conclusion

The LCBRN has met the majority of organizational metrics laid out in the Statement of Work for Year 2. Since the recruitment of subjects did not begin until the latter half of the first year of this award due to the requirement of obtaining regulatory approval from the DOD and local sites in a sequential manner, the goal of 150 subjects recruited per year was not met in Year 1. Since then, however, all Resource Sites have been able to overcome this deployment lag time by increasing recruitment efforts and striving for the goal of 100 subjects enrolled at each site by the end of Year 2. Two procurement sites were able to exceed this goal while the third site was only slightly under goal. At this rate of accrual, the LCBRN as a consortium should be able to easily surpass its goal of 150 subjects enrolled per year. In addition, the majority of tissue specimens collected thus far by the LCBRN have passed both histology and molecular quality control metrics, with feedback mechanisms in place to achieve improvements where needed.

Plans have been finalized to deploy a system for investigators to apply for specimens from the LCBRN and for a scientific panel to review the applications for suitability. The sample distribution date is still pending while the Coordinating Center completes biomolecular extraction on previously banked tissue specimens. It is anticipated that samples will be ready for distribution by the end of 2012.

The outcome of this project will be a bank of high quality and highly annotated tissue and biofluid samples from lung cancer patients that will support research into the molecular basis of this disease, the discovery of diagnostic and prognostic biomarkers and the validation of new biomarker assays. This resource will be available to the general research community, with a panel of experts ensuring the judicious use of this resource by projects with a significant likelihood of creating new knowledge of human lung cancer and new tools for the diagnosis and treatment of this disease.