Department of Health and Human Services
Centers for Disease Control and Prevention
Agency for Toxic Substances and Disease Registry

National ALS Biorepository Pilot Study Final Meeting

March 2-3, 2015
Summary Report

Table of Contents

TABLE OF CONTENTS **2**
ACRONYMS USED IN THIS DOCUMENT **3**
PURPOSE **4**
CALL TO ORDER **4**
OVERVIEW OF THE NATIONAL ALS BIOREPOSITORY PILOT STUDY **4**
POST-MORTEM COLLECTIONS **11**
IN-HOME BIOSPECIMEN COLLECTIONS **32**
RECOMMENDATIONS FOR LONG-TERM IMPLEMENTATION **44**
NATIONAL ALS REGISTRY PRIORITIES AND FUTURE DIRECTION **48**
FACILITATED DISCUSSION TO DEVELOP EXPERT PANEL RECOMMENDATIONS **52**
CLOSING REMARKS **65**
PARTICIPANT ROSTER **66**

Acronyms Used in This Document

Acronym	Expansion
ALS	Amyotrophic Lateral Sclerosis
ALSA	Amyotrophic Lateral Sclerosis Association
ALSFRS	Amyotrophic Lateral Sclerosis Functional Rating Scale
ATSDR	Agency for Toxic Substances and Disease Registry
BUSM	Boston University School of Medicine
CASPIR	Specimen Packaging, Inventory, and Repository
CDC	Centers for Disease Control and Prevention
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FTD	Frontotemporal Dementia
FTLD	Frontotemporal Lobar Degeneration
GLP	Good Laboratory Practices
GUID	Globally Unique Identifier
iPS	Induced Pluripotent Stem
IRB	Institutional Review Board
MDA	Muscular Dystrophy Association
MTBI	Mild Traumatic Brain Injury
NCI	National Cancer Institute (NIH)
NDI	National Death Index
NDRI	National Disease Research Interchange
NEALS	Northeast Amyotrophic Lateral Sclerosis Consortium
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NOK	Next of Kin
NYGC	New York Genome Center
OMB	Office of Management and Budget
PALS	People With ALS
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PLP	Periodate-Lysine-Paraformaldehyde
PTSD	Post-Traumatic Stress Disorder
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
RFA	Request for Applications
RNA	Ribonucleic Acid
SOP	Standard Operating Procedure
SSN	Social Security Number
TBI	Traumatic Brain Injury
TREAT ALS	Translational Research Advancing Therapy for ALS
UK	United Kingdom
US	United States
VA	(United States Department of) Veterans Affairs

Centers for Disease Control and Prevention (CDC)
Agency for Toxic Substances and Disease Registry (ATSDR)
National ALS Biorepository Pilot Study Final Meeting

Minutes of the Meeting
March 2-3, 2015

Purpose

The purpose of this meeting was to provide stakeholders an update on the National ALS Biorepository Pilot Project activities.

Call to Order

Bob Kingon, MPA
Facilitator
McKing Consulting Corporation

Mr. Kingon called the meeting to order and welcomed those present. He went over the meeting ground rules, reviewed the agenda, and had everyone introduce themselves. A participant roster is included at the end of this document.

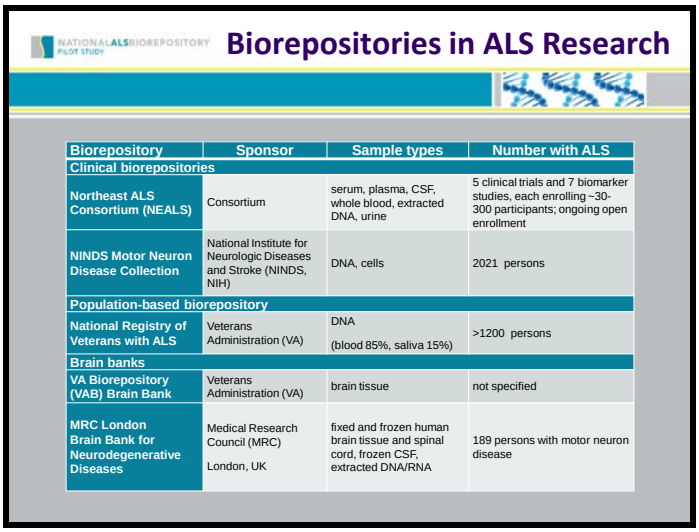
Overview of the National ALS Biorepository Pilot Study

Wendy E. Kaye, PhD
Study Director
National ALS Biorepository Pilot Study
McKing Consulting Corporation

Dr. Kaye thanked everyone for attending the meeting, especially those who were impacted by the inclement weather.

As a reminder, she explained that a biorepository is a collection of biological specimens (e.g., blood, urine, and tissues) stored for future use by researchers. No analyses are being performed on the specimens at this time. Biorepositories have been used in Amyotrophic Lateral Sclerosis (ALS) research to identify genes associated with ALS (family studies), monitor response to treatment (clinical trials), and search for evidence of environmental causes (registries). ALS biorepositories could be used in the future to validate biomarkers (exposures, diagnosis), classify ALS subtypes (prognosis, treatment), and discover underlying pathobiology.

About four years ago, at the time the study was begun, an extensive review was performed of various existing biorepositories. There are several existing biorepositories related to ALS, some of which are clinical and others of which are population-based. The following table outlines the existing biorepositories:



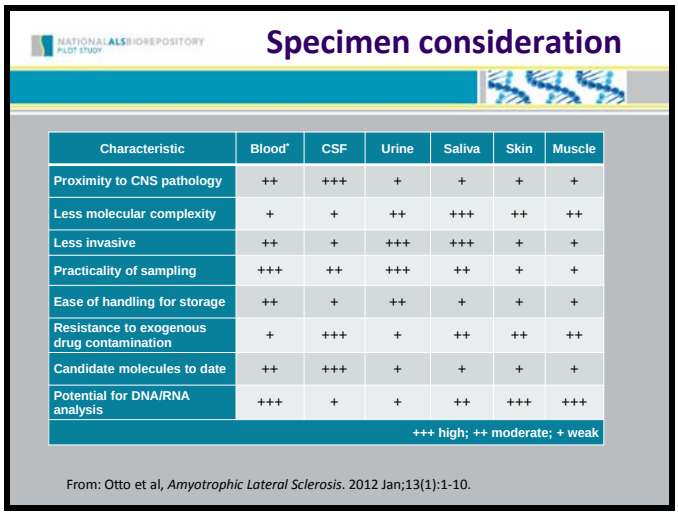
Biorepository	Sponsor	Sample types	Number with ALS
Clinical biorepositories			
Northeast ALS Consortium (NEALS)	Consortium	serum, plasma, CSF, whole blood, extracted DNA, urine	5 clinical trials and 7 biomarker studies, each enrolling ~30-300 participants; ongoing open enrollment
NINDS Motor Neuron Disease Collection	National Institute for Neurologic Diseases and Stroke (NINDS, NIH)	DNA, cells	2021 persons
Population-based biorepository			
National Registry of Veterans with ALS	Veterans Administration (VA)	DNA (blood 85%, saliva 15%)	>1200 persons
Brain banks			
VA Biorepository (VAB) Brain Bank	Veterans Administration (VA)	brain tissue	not specified
MRC London Brain Bank for Neurodegenerative Diseases	Medical Research Council (MRC) London, UK	fixed and frozen human brain tissue and spinal cord, frozen CSF, extracted DNA/RNA	189 persons with motor neuron disease

The rationale for establishing a biorepository for the National ALS Registry, which was mandated by Congress, was to correlate biomarkers with extensive epidemiologic data collected by the National ALS Registry; enroll a nationally representative, population-based sample of participants (not selected by geographic area, exposure, or clinical characteristics); and increase the number of biological specimens available for research on ALS.

The goal of the pilot study was to pilot methods for collecting and banking biological specimens from participants in the National ALS Registry in order to assess the potential for developing a comprehensive, national research resource associated with the National ALS Registry. The objectives of the pilot study were to maximize scientific potential, given the National ALS Registry parameters; maximize cost-efficiency; make recommendations for long-term sustainability; and recommend a process for providing access to researchers.

In March 2012, ATSDR convened a large meeting of experts in ALS, biorepositories, and biomarkers. A draft protocol was discussed, and participants provided input into the draft ALS biorepository pilot study protocol regarding sample size and follow-up, specimens to be collected, and potential research uses. Some of the research considerations were for the biospecimens collected from participants to complement registry epidemiologic data; allow comparisons with other studies; maximize scientific utility within National ALS Registry constraints; and be “future-proof” (e.g., amenable to emerging technologies and research priorities).

As part of the March 2012 meeting, a list of specimen types that would be desirable was shared from a paper by Otto [Otto et al, *Amyotrophic Lateral Sclerosis*. 2012 Jan;13(1):1-10]. The following table illustrates the specimen collections considered and their potential for being useful in ALS:



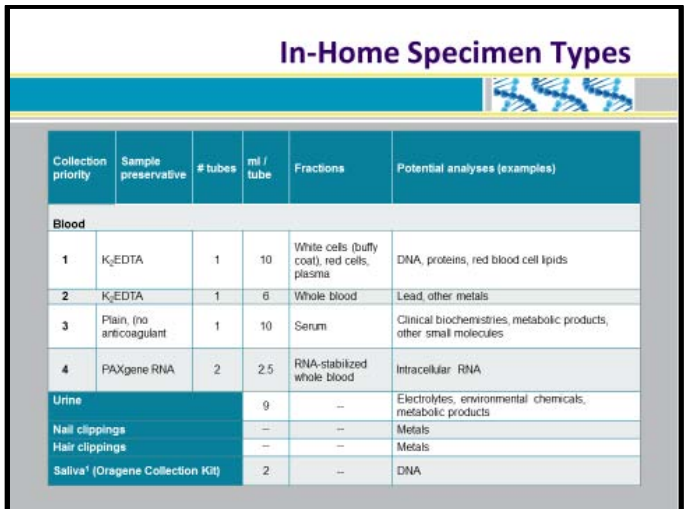
Specimen consideration

Characteristic	Blood*	CSF	Urine	Saliva	Skin	Muscle
Proximity to CNS pathology	++	+++	+	+	+	+
Less molecular complexity	+	+	++	+++	++	++
Less invasive	++	+	+++	+++	+	+
Practicality of sampling	+++	++	+++	++	+	+
Ease of handling for storage	++	+	++	+	+	+
Resistance to exogenous drug contamination	+	+++	+	++	++	++
Candidate molecules to date	++	+++	+	+	+	+
Potential for DNA/RNA analysis	+++	+	+	++	+++	+++

+++ high; ++ moderate; + weak

From: Otto et al, *Amyotrophic Lateral Sclerosis*. 2012 Jan;13(1):1-10.

The final recommendations were to collect specimens from 300 participants on two occasions, approximately 6 months apart; collect some blood metals free and urine specimens; and add a specimen processing form to collect information necessary to interpret the specimen analyses. The following table delineates the in-home collection specimen types:



In-Home Specimen Types

Collection priority	Sample preservative	# tubes	ml / tube	Fractions	Potential analyses (examples)
Blood					
1	K_2EDTA	1	10	White cells (buffy coat), red cells, plasma	DNA, proteins, red blood cell lipids
2	K_2EDTA	1	6	Whole blood	Lead, other metals
3	Plain, (no anticoagulant	1	10	Serum	Clinical biochemistries, metabolic products, other small molecules
4	PAXgene RNA	2	2.5	RNA-stabilized whole blood	Intracellular RNA
Urine			9	--	Electrolytes, environmental chemicals, metabolic products
Nail clippings			--	--	Metals
Hair clippings			--	--	Metals
Saliva[1] (Oragene Collection Kit)			2	--	DNA

The specimens were designed to be drawn in the home, which is why skin biopsies were not included. The specimens are shipped at ambient temperature to the laboratory where aliquots are prepared as follows:

Whole blood (10 ml EDTA)

- ❑ Plasma 0.5 ml aliquots (8)
- ❑ Buffy coat 1.0 ml
- ❑ RBC 1.0 ml (2)

Metals Free blood (6 ml EDTA)
- ❑ Whole blood 1.8 ml (3)

Blood (8 ml red top)
- ❑ Serum 0.5 ml (8)

PAXgene tube (2)

Urine
- ❑ 1.8 ml (10)
- ❑ 4.5 ml with Hg preservative
- ❑ 10 ml (3)

Hair
- ❑ ½ oz. glass jar

Nails
- ❑ 2 ml cryovial

Postmortem tissue specimens include brain, spinal cord, cerebrospinal fluid (CSF), bone, muscle, and skin.

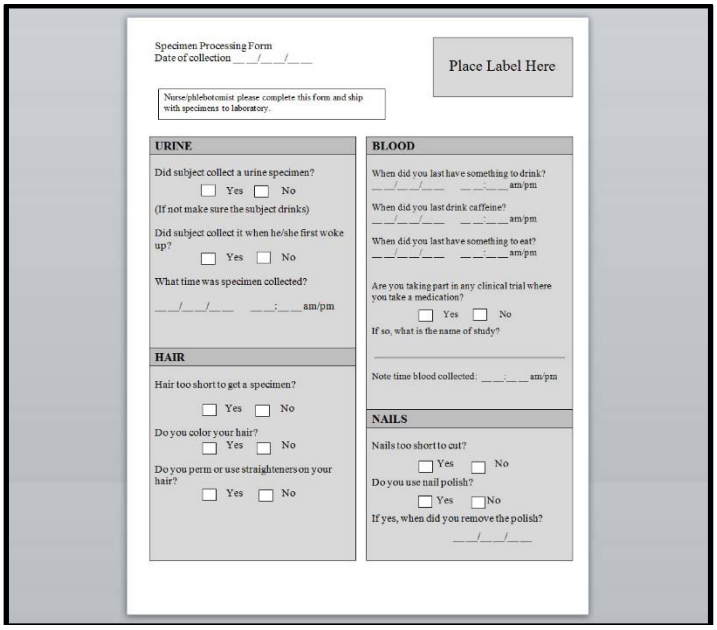
In terms of eligibility for the study, participants must be enrolled in the National ALS Registry. They must have agreed to be contacted about ALS studies through the National ALS Registry, and they must be willing to have someone come to their houses for specimen collection. Any stage of disease progression was permitted.

Recruitment was proportional to the state population. Individuals were selected from the National ALS Registry based on state. An email announcing the project was sent by the National ALS Registry to those selected. A follow-up email was sent from McKing with more information about the project. Additional follow-up emails were sent, up to a total of four. Outreach was done by the Muscular Dystrophy Association (MDA) and the Amyotrophic Lateral Sclerosis Association (ALSA). An alert was also placed on the ATSDR website. In addition to those who were actively recruited, volunteers were accepted after they were verified to be in the National ALS Registry and to live in a state from which participants were still being actively recruited.

A package of information was sent to anyone expressing interest in the project, with follow-up with potential participants approximately 1 week later to answer questions and go over the consent form. Participants who signed consent forms returned them to McKing, and McKing scheduled collection appointments.

The specimen collection kits were created and distributed by Fisher. McKing ordered the kits to be shipped to the participants' houses. The types of kits include: Total Kit, Blood Only, Urine Only, and Saliva Only. Some adjustments had to be made to the types of kits based on various situations, such as being able to collect everything but blood during a visit. The kits were also adjusted to include a sharps container. Here is a picture of a kit:

The phlebotomists who visited the homes for sample collections were also supposed to fill in the following form describing information such as the time the urine specimen was collected, when the individual last had something to eat or drink or last had caffeine.:



Specimen Processing Form
Date of collection __ __/__ __/__ __

Place Label Here

Nurse/phlebotomist please complete this form and ship with specimens to laboratory.

URINE

Did subject collect a urine specimen?
☐ Yes ☐ No
(If not make sure the subject drinks)

Did subject collect it when he/she first woke up?
☐ Yes ☐ No

What time was specimen collected?
__ __/__ __/__ __ __ __:__ __ am/pm

HAIR

Hair too short to get a specimen?
☐ Yes ☐ No

Do you color your hair?
☐ Yes ☐ No

Do you perm or use straighteners on your hair?
☐ Yes ☐ No

BLOOD

When did you last have something to drink?
__ __/__ __/__ __ __ __:__ __ am/pm

When did you last drink caffeine?
__ __/__ __/__ __ __ __:__ __ am/pm

When did you last have something to eat?
__ __/__ __/__ __ __ __:__ __ am/pm

Are you taking part in any clinical trial where you take a medication?
☐ Yes ☐ No

If so, what is the name of study?

Note time blood collected: __ __:__ __ am/pm

NAILS

Nails too short to cut?
☐ Yes ☐ No

Do you use nail polish?
☐ Yes ☐ No

If yes, when did you remove the polish?
__ __/__ __/__ __

Because of issues with phlebotomists completing the form, there are some suggested revisions if the project goes forward.

Collections were only supposed to be done Monday through Thursday, because they needed to be done in time to be shipped by FedEx the same day for next day delivery. McKing sent letters to confirm appointments, and calls were made to phlebotomists to answer questions. Phlebotomists were also supposed to call participants the evening before the appointment to reconfirm that they were going to be there, get directions, et cetera.

McKing worked with a variety of phlebotomy services to find phlebotomists who would go to people's homes, using multiple providers to ensure coverage in rural areas; provided minimal standards for phlebotomists; and provided training materials for collecting and shipping specimens. As noted, collections were supposed to occur on Monday through Thursday only, and specimens were supposed to be shipped overnight to the laboratory. Once in the lab, blood and urine aliquots were created, PAXgene ribonucleic acid (RNA) vials were frozen as received, and hair and nails were stored as received.

Some protocol changes were necessary. Permission was sought from and granted by the Institutional Review Board (IRB) to increase frequency of recruitment and follow-up emails, given that many potential participants did not check their emails regularly. The sample size was increased from 300 to 330 to increase the number of paired samples, because approximately 15% to 20% of participants were unable to complete their second draw due to death or illness.

There were a number of challenges. Responses to recruitment emails were slow, and the National ALS Registry does not collect any other contact information (e.g., telephone numbers, mailing addresses). In terms of in-home collections, some potential participants did not want people coming to their homes. Finding reliable phlebotomists across the country was challenging. In 2013, there were issues with very high summer temperatures that required changes in packing and shipping procedures. There was also the addition of specimen quality assurance (QA) three years into the project.

Other related project activities include specimen governance and assessment of long-term storage options. The goals of governance are to manage the use of the biorepository for relevant research; assure and facilitate researcher access to samples and data; assure that public presentations of findings are accurate and objective; establish guidelines for authorship and acknowledgements; and maintain a record of proposed and published research so that it can be reported back to people with ALS (PALS). A decision was made to have a review panel at ATSDR to assess requests to use specimens. The membership and procedures of the panel would be based on guidance for the National ALS Research Committee established by ATSDR, which is already reviewing requests for researchers to recruit participants from the registry. Investigator obligations would include Confidentiality and Data Use Agreements, Material Transfer Agreements, and project updates.

The specimens are currently being housed at Fisher, but long-term storage options must be considered for where the samples will be stored once the project ends in September 2015. There have been discussions with other biorepository programs at CDC and the National Institutes of Health (NIH). Several organizations were contacted to learn more about biorepository capabilities, including the CDC / ATSDR Specimen Packaging, Inventory and Repository (CASPIR), commercial organizations, academic institutions, and other organizations. The goal is to recommend what type of organization is best suited to store National ALS Biorepository samples after September.

In terms of next steps, a few people still need to have their second collections completed. Quarterly contact will continue with post-mortem participants, and post-mortem donation collection will continue as well. The QA analysis of specimens will also continue. A final report and recommendations will be developed for the long-term implementation of a biorepository.

In conclusion, Dr. Kaye recognized the National ALS Biorepository Pilot Study team members:

- Wendy E. Kaye, PhD, Director
- Laurie Wagner, MPH, Study Coordinator
- Leandrea McGill, MPH, Participant Coordinator
- Kelly Hallock, BA, Participant Coordinator
- Ebony McGriff, PhD, MSW, Participant Coordinator
- Ariel Davis, BA, Research Assistant
- Maggie Ritsick, MPH, Project Manager

Discussion Points

Dr. McQuillan pointed out that the number of specimens that have been collected for the 330 participants will suggest what type of repository is needed, and whether CASPIR will be suitable.

Dr. Kaye replied that there are less than the million range, so CASPIR confirmed that they could house the samples.

Dr. Corriveau asked whether deoxyribonucleic acid (DNA) is being extracted.

Dr. Kaye indicated that it was not part of the protocol to extract anything. That was part of QA analysis, and DNA is now being extracted.

Dr. Horton asked what the patient feedback has been on the overall process. Many of these patients go to clinics to have blood drawn, et cetera. It seems that because phlebotomists are being sent to people's homes, it has to be more convenient for patients.

Dr. Kaye responded that many people live pretty far from a referral center and a clinic. Even people who were early on in their disease and did not have as much disability from it liked someone coming to their home when it was convenient for them. The flipside is that some of the phlebotomists were not as good as they would have preferred (e.g., were late, could not draw the blood, et cetera).

Dr. Weisskopf requested further information about where the recruitment lists comes from, and whether as part of that process they would be able to obtain information on people who were invited but did not participate.

Dr. Kaye replied that the lists came from the National ALS Registry and included identification numbers and the states in which they were located. She then went through the lists and selected people proportionally based on what she needed in what state. She gave that list to ATSDR and they emailed those people to let them know that there was a study that might interest them. Simultaneously, they provided McKing with some identifiers so that they would know if somebody called them they were on the list. They know who was invited but did not participate. The caveat is that a fairly significant portion of those persons were deceased. Though some searches were done, contact was never made with some people and it was

unclear whether they were alive. It would probably be interesting to compare those who said they were not interested. An email address is required to register with the National ALS Registry, but some people are not active email users and may have only gotten an email address so they could register. Some of them are not checking this email address, which is why having only the email address makes recruitment somewhat more difficult.

Dr. Van Den Eeden asked whether they would be presenting more details on the responses.

Dr. Kaye affirmed that these data would be presented during this meeting.

Dr. Corriveau asked whether more specifics would be discussed regarding the intended use of the samples (e.g., biomarker discover, genetics discovery, and so forth). Specific things about the sample collection seem important in that context such as whether shipping is at room temperature and method of subsequent plasma preparation.

Dr. Kaye replied that this could probably be further discussed as part of the Facilitated Discussion session.

Dr. McQuillan asked whether consideration had been given to assessing the National Death Index (NDI), which is a CDC registry, to cross-check for the people who did not respond.

Dr. Kaye indicated that while they do this for other projects, it is complicated and was not part of the protocol for the pilot.

Post-Mortem Collections

Pilot Study Overview, Findings, and Outcomes

Wendy E. Kaye, PhD, Project Director
Laurie Wagner, MPH, Study Coordinator
National ALS Biorepository Study
McKing Consulting Corporation

Dr. Kaye presented an overview of the post-mortem recruitment and collection processes. Eligibility for the post-mortem study was similar to that for biospecimen collections. Participants had to be enrolled in the National ALS Registry, agree to be contacted by the National ALS Registry about research studies, sign Health Insurance Portability and Accountability (HIPAA) Authorization to contact his/her neurologist, have eligibility confirmed by the treating neurologist, and have a signed family authorization form.

There was an effort to recruit people whose disease was more progressed, but who were cognitively able to sign a consent form. Given that only 30 participants were being recruited for post-mortem collections, no effort was made to achieve geographic distribution. The recruitment process was similar, with an email sent by the National ALS Registry announcing the project to those selected. A follow-up email was sent from McKing with more information about the project, and up to four additional follow-up emails were sent as needed. There was outreach by MDA and ALSA, as well as an alert on the ATSDR website. Volunteers who were enrolled in the National ALS Registry were accepted as well.

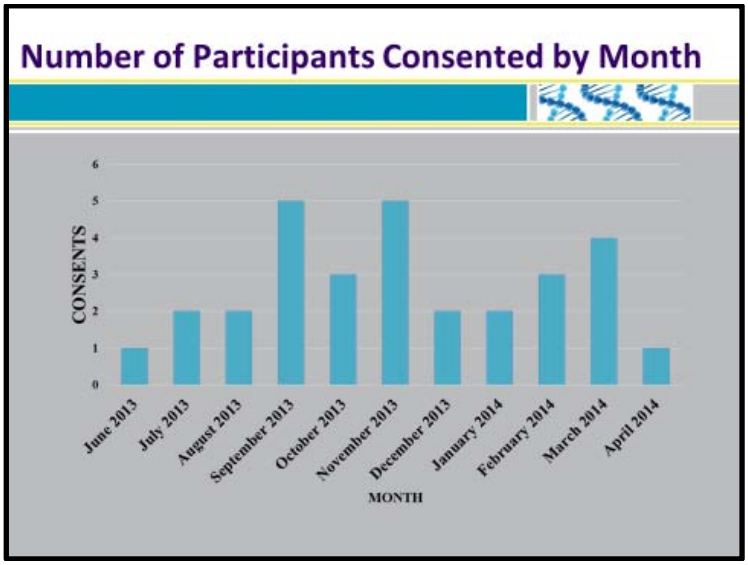
A package of information was sent to anyone expressing interest in the project, and McKing followed up with potential participants about a week later to answer questions. Treating neurologists were contacted to confirm eligibility. Some assessment had to be done to determine cognitive ability to consent. An effort was also made to determine whether there were any family dynamics that might hinder the donation process. For example, Texas has a strange law on a family member being able to consent for someone who is incapacitated. If there are four siblings, all four siblings have to agree and all four have to be consented. Consenting of participants and their family member(s) was done in participants' homes. A call is made quarterly to assess disease progression and update plans as needed.

The only protocol changes made to this portion of the project was the addition of skin as a sample type. Some participants were required to be re-consented if they had already been consented for the full postmortem collection. They were contacted about the addition and was sent a packet of information. Consent was done over phone, and participants mailed the signed consent form to McKing. The participants that had not been previously consented, signed consents for both parts of the postmortem study (i.e., brain/spinal cord and skin) at the same time.

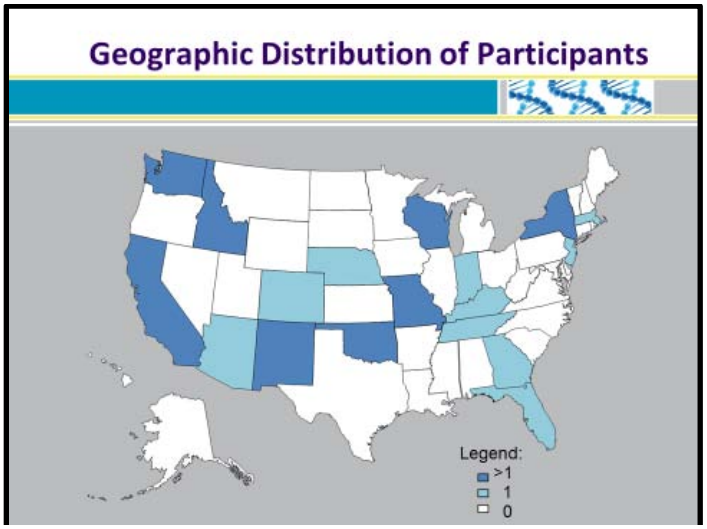
The following is a picture of the postmortem collection kit used to ship the brain, spinal cord, and CSF to Boston:

Ms. Wagner presented some of the preliminary results for the post-mortem component of the study. As a reminder, recruitment for this portion of the project began in April 2013. ATSDR sent 144 initial emails. McKing followed up with 441 emails over the four rounds of emails that were sent. As a result of those emails, information was received by calls or emails from family members that 26 were deceased and 5 were not interested. Of the potential participants, 97 received information packets. After contacting neurologists, 33 were interested and deemed to be eligible for the study. Of those, 30 were consented for the study as that was the maximum sample size. Of the participants, 27 were later consented for the addition of skin to the sample types collected. Of the 3 who were not consented, 1 died before skin was added to protocol, 1 refused, and 1 died before consent was completed. There were 9 individuals who declined participation in the post-mortem component, but participated in biospecimen collection. All participants in the post-mortem portion also participated in the biospecimen collection.

This table offers an overview of the number of participants consented by month for the post-mortem study:

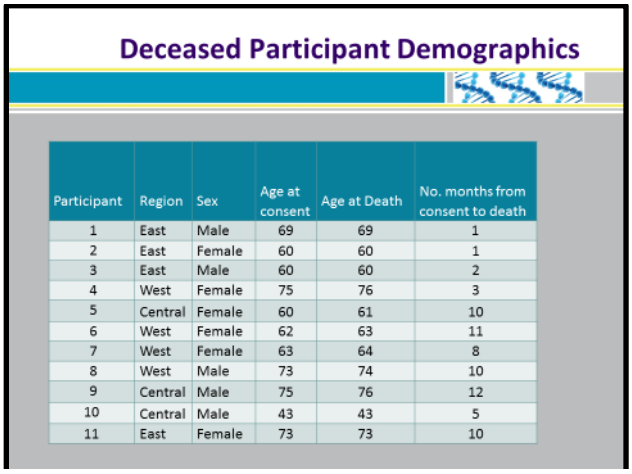


Participation increased during September, October, and November of 2013. This may be attributable to some of the outreach emails and postings on the websites of ATSDR, ALSA, and/or MDA. Sometimes it had to do with the timing during which visits could be made to participants' homes to complete the consent process. The number of males tended to vary across age groups, while the predominance of females were in the 50 to 59 and 60 to 69 age groups. There were 15 male and 15 female participants, which was not intentional. As shown by the following map, post-mortem participants are located in different regions:



The distance participants live from an MDA or ALSA referral center was assessed, and 50% were determined to live 50 or more miles from a referral center.

A variety of kits were built, including 25 full kits, 31 bone and muscle kits, 29 CSF kits, and 31 skin kits. All of the kits are pre-positioned with dieners at locations that are in close proximity to the potential donations. Thus far, 11 participants have died. All of them donated brain, spinal cord, CSF, bone, and muscle. As mentioned earlier, 9 of the 11 participants also donated skin. This table provides some general information about the 11 participants:



Deceased Participant Demographics

Participant	Region	Sex	Age at consent	Age at Death	No. months from consent to death
1	East	Male	69	69	1
2	East	Female	60	60	1
3	East	Male	60	60	2
4	West	Female	75	76	3
5	Central	Female	60	61	10
6	West	Female	62	63	11
7	West	Female	63	64	8
8	West	Male	73	74	10
9	Central	Male	75	76	12
10	Central	Male	43	43	5
11	East	Female	73	73	10

The 11 deceased participants were relatively well-distributed in the regions. There are 6 females and 5 males so far. Their ages range at consent was 43 to 75, and age at death was 43 to 76. The amount of time that each was in the study ranged from 1 to 12 months from the date they were consented until the date they died.

There were some challenges for the post-mortem part of the study. Being able to assess eligibility and consenting quickly was difficult. As mentioned earlier, these potential participants were the sicker of the population and time was of the essence to get them consented quickly. It took a few months to complete the re-consenting for the addition of the skin specimen. McKing's Participant Coordinator was able to make calls to everyone and 27 signed skin specimen collection consents forms were received in the mail. The McKing had to follow up closely to make sure everything was signed and in the office. One issue that remains a challenge is working with participants to make final arrangements. McKing has donation plans that they review with participants. Often, they are not ready to make these final decisions because they need to choose a funeral home and make arrangements for their final days. Some participants to date have not completed their donation plans. Finding dieners in remote locations and assuring diener coverage at all times has been very challenging. At this point, there is coverage and McKing has worked closely with NDRI to ensure this. Designing the kits for additional specimen types was also challenging. Making sure that specimens are shipped quickly can be difficult due to issues such as weather, diener availability, and the timing of the call from the participant's family. Making sure specimens are received and processed quickly poses additional challenges. Initially, some skin specimens were contaminated with mold. NDRI and Zen-Bio worked together to eliminate this issue, which has not occurred since the changes were made.

Next steps are to continue to make quarterly calls to the participants in the post-mortem portion of the study. Donation retrievals will continue as they occur, and a plan will be developed to transition all of the participants to another study when this pilot ends in September 2015.

Discussion Points

Dr. Corriveau asked whether the consent is narrow or broad with respect to disease and with respect to the science that they are told and the sharing that will be done with the samples.

Dr. Kaye replied that the consent is broad in terms of sharing the specimens, and both the in-home and post-mortem consent forms say that the specimens have to be used for ALS-related research. "Related" is a broad term.

Ms. Wagner added that participants usually want the research to be ALS-related, and that gives them comfort.

Though more males typically develop ALS than females, Dr. Horton was curious about the fact that more females than males consented.

Dr. Kaye clarified that in the post-mortem component, an equal number of males and females consented at 15 each. The ratio of males to females is currently 62% to 38% in the biospecimen component.

Ms. Wagner added that they had to be pretty open with selection, because everyone is not interested in that portion of the study.

Dr. Sorenson noted that the map of the United States (US) showed a few higher population states that have no representation (e.g., Ohio, Michigan, Texas, and Pennsylvania). He asked for more details about how these were targeted, and whether it was a random sampling.

Dr. Kaye replied that based on the date ATSDR provided, she selected people who had a diagnosis at least a year in the past. She weighted selection more toward people who had been diagnosed a year and a half to two years before, knowing that the pilot project had a defined timeframe and they are trying to complete as many donations within that timeframe as possible. One of her parameters worked too well, and nearly everyone she selected had already passed away. It was just based on diagnosis, and there was nothing related to geography. In addition, she received calls from people who were interested because they heard about it from other people who agreed to participate. Many people who called in voluntarily to express an interested were not enrolled because their state already had participants.

Dr. Sorenson asked whether the post-mortem participants are aware of the fact that the plan is to roll them into another program after September 2015.

Dr. Kaye replied that if ATSDR goes forward with the study, it will be fairly easy to roll them from the pilot into the full-scale study. If ATSDR chooses not to go forward, a list has been compiled of other groups and there will be a conversation with each participant to let them know that the pilot is over, that there are other projects, and let them know that they would have to consent into the new project. However, if ATSDR moves forward, they will simply be moved into the ATSDR project. The Veterans Administration (VA) has already agreed to take any Veterans. NDRI has several other projects for which they make post-mortem collections in their network. The plan for the donation would not have to be changed if they rolled into one of those.

Dr. Goutman thought the consent rate for the post-mortem specimens seemed somewhat low at about 25%, and he asked whether there had been any effort to engage the patients' neurologists to assist with consent. The University of Michigan has a robust brain bank, and their participation numbers increased significantly once he began consenting all of the patients. He thinks that if patients are approached by someone they do not know or who is not an MD, their willingness to participate is much lower.

Dr. Kaye clarified that 97 potential participants received a packet of information. Of those, only 33 signed the HIPAA authorization. Of the third who signed the HIPAA authorization, 30 consented. She suggested that he raise the issue regarding MDs consenting patients the next day during the "Facilitated Discussion to Develop Expert Panel Recommendations."

Dr. Horton added that this is all the more reason that ATSDR must reach out not only to patients, but also neurologists and others who see ALS patients on a daily basis to let them know that this is part of a larger effort.

Dr. Kaye pointed out that 27% of the participants in the post-mortem study live more than 100 miles from a referral center, which suggests that they may not be presenting to the referral center on a regular basis. That is not to say that having physicians involved in the consenting process would not be beneficial, but if it is not done both ways, the population is likely to be biased.

Dr. Weisskopf noted that the 33 were the ones deemed eligible. It was not clear to him how they went from 97 to 33.

Ms. Wagner clarified that not everyone who received a packet expressed an interest in this component of the study.

Dr. Kaye added that only 33 people returned their signed HIPAA authorization, and all 33 were deemed eligible.

While Dr. Weisskopf understood the eligibility criterion to be competent to sign the consent creates problems for patients with frontotemporal dementia (FTD), and will likely rule out anyone with signs of FTD.

Dr. Kaye responded that this would depend upon where they are in their disease. From an IRB perspective, being competent to sign a consent is a lower threshold than being competent from a legal perspective. It is true that at a certain point someone with ALS-FTD would be deemed not able to sign the consent form, but someone early on in disease would be allowed to sign a consent form.

If the study moves forward, Dr. Weisskopf wondered whether the goal would be to try to target people at an earlier stage of ALS. Dr. Kaye replied that this would be the goal moving forward.

Dr. Van Den Eeden asked what the rough distribution was from the time of diagnosis.

Ms. Wagner replied that we did not have that data at this time but once participants were in the project, they were treated on the same level. Some participants who had ALS longer were still living, and some who had not been diagnosed long have passed away. So, it is unclear whether this is a good correlation.

Dr. Kaye added that the first 25 or so participants recruited were selected from the registry, and did not capture the right population. They were getting people who were just diagnosed, for example. They took some of those, but then subsequently tried to weight it more toward those at a later stage. People who called to volunteer could have been anywhere in terms of their stage. At least of third of these people were volunteers and were not actively recruited. One person started out in the biorepository portion and had completed one collection, then thought about it some more and decided they wanted to enroll in the post-mortem component as well. So, it is somewhat of a mismatch.

Ms. Wagner can look at the date of diagnosis and the length of time until they died just to have a window of how long people are surviving with the disease. Perhaps this information could be added to the final report.

Postmortem Tissue Recovery and Shipping

John Lonsdale, PhD
Vice President, Partnership Development
National Disease Research Interchange (NDRI)

Dr. Lonsdale indicated that NDRI's role in the pilot project has been the coordination of the post-mortem collections to date. NDRI was founded in 1980 by Lee Ducat who founded the Juvenile Diabetes Research Foundation (JDRF). NDRI is based in Philadelphia and is a 501c3 non-profit corporation that has been funded by NIH for over 30 years. There are over 200 peer-reviewed publications a year by NDRI-supplied investigators annually. NDRI's scope is wide, as is the range of tissue samples the organization provides.

NDRI has four primary types of donor profiles:

- Deceased Transplant Donors (organ, tissue, eye)
- Living Donors
- Patients Participating in Clinical Studies
- Pre-registered Research Donors

NDRI matches donor referrals that come in through a massive procurement network of partners comprised of 240 sites and more than 200 recovery specialist with the tissue needs of approved researchers in NDRI's database. The breakdown of the 240 recovery sites is as follows:

- 56 Organ Procurement Organizations
- 23 Tissue Banks
- 46 Hospital Donor Sites
- 18 Satellite Tissue Source Sites
- 37 Eye Banks

NDRI is a 24/7 call center, and has a logistics facility. NDRI's Rare Disease Private Donor Program is comprised of 446 current living registrants with 54 rare diseases represented, 534 registrants with tissue recovered. Overall, 4344 tissues procured with 71 rare diseases represented.

NDRI began working on the ALS Biorepository project in April 2013, and has engaged in a weekly project teleconference with the McKing team. In terms of the donation plan, McKing tells NDRI about prospects prior to consenting and NDRI conducts a preliminary search for a recovery specialist. McKing obtains consent, securely sends NDRI a copy of the consent form, family authorization, and preliminary donation plan that includes the funeral home, donor's address, and next of kin (NOK) contact information. NDRI uses the donor location and funeral home to match with a contracted recovery specialist. Donor distribution by state is shown in the table.

State	Number of Donors	Disposition
MO	**4**	3 Pending, **1 Recovered**
WA	**3**	1 Pending, **2 Recovered**
CA	**3**	Pending
NM	**2**	1 Pending, **1 Recovered**
WI	**2**	1 Pending, **1 Recovered**
NY	**2**	1 Pending, **1 Recovered**
ID	**2**	1 Pending, **1 Recovered**
OK	**2**	1 Pending, **1 Recovered**
NJ	**1**	**Recovered**
CO	**1**	Pending
NE	**1**	Pending
KY	**1**	Pending
MA	**1**	Pending
TN	**1**	Pending
GA	**1**	**Recovered**
AZ	**1**	**Recovered**
FL	**1**	Pending
IN	**1**	Pending
18 STATES	**30 Donors**	**19 Pending, 11 Recovered**

The more than can be put into place prior to a participant's death, the greater the chances of successful tissue recovery. NDRI contacts the recovery specialist and funeral home to discuss and arrange recovery logistics, including equipment availability, and NDRI sends them kits and standard operating procedure (SOP). McKing and NDRI developed a long and complex SOP, which drives the entire process from the dissection through to the packaging, preservation, and shipping. NDRI periodically re-contacts the recovery specialist and funeral home every couple of months, given that months or years could pass between the initial contact and the actual recovery. NDRI securely sends McKing a completed donation plan that includes contact details, body transportation details for the family, et cetera.

The following are photographs of the kit:

The large box is lined with polystyrene and there are nine freezer bricks, which are layered on the bottom pre-frozen and a cardboard piece is placed on top of those to keep them from moving around. The four large freezer bricks are placed around the sides, because those will not fit once the bucket is in place. The large freezer brick goes on the bottom of the bucket, and on top of that goes the foam with the notch cut out that allows the brain stem to not be disturbed when the brain is put in. The brain goes into the bucket and the circular foam is wrapped around the brain to keep it from moving. Finally, the last piece of circular foam goes on top. So, the brain is kept cool and cushioned in the bucket. The spinal cord in a bag goes on top of the round foam and the last two pieces of cushioning go on top to make sure that the spinal cord does not get crushed. The CSF goes on top of that, the bucket is sealed, and the last piece of cardboard goes on top to keep the whole thing from moving around. The last piece of polystyrene is placed on top, and the box is closed. There is a separate kit for bone and muscle that includes biospecimen containers with formalin and biohazard bags. There is also a separate kit for skin that includes biospecimen containers with saline and biohazard bags.

In terms of tissue recovery, NDRI is notified of the death by McKing. At that time, NDRI contacts the recovery specialist to confirm the time to arrival at recovery site and estimated time to complete the recovery, and confirm the availability of kit/equipment and that freezer bricks are frozen correctly. NDRI re-sends the NDRI Tissue Procurement Form and reviews the SOP with the recovery specialist. NDRI contacts the funeral home, updates the funeral director, and confirms that the patient can be picked up. NDRI also contacts the family to let them know that NDRI has set up the transportation of the body with the funeral home and confirm details. NDRI then updates McKing. The recovery specialist calls NDRI upon arrival at the funeral home and NDRI sets up a shipping “will-call” with the courier. The recovery specialist calls NDRI when close to completion, and NDRI activates the shipping process. NDRI updates McKing again and sends all tracking information to McKing and Fisher BioServices in Boston. NDRI confirms with the funeral home that the body is being returned to the family if transport is necessary, and updates the family upon completion of the recovery.

Regarding the current status, 30 donors have been consented. Of these, there are 19 pending recoveries, 16 of which have been assigned recovery technicians with kits (3 technicians have multiple cases). There are 15 confirmed funeral homes and 4 not yet provided by the family. The completed recoveries include 1 in 2013, 9 in 2014, and 1 in 2015. The samples consist of 11 whole brain, CSF, spinal cord, muscle, and bone and 9 skin samples. As noted, the skin sample was a later addition to the recovery protocol and required re-consenting.

The total recovery time was between 1 to 3 hours, with an average of 1 hour 45 minutes. The time from NDRI notification to recovery start was 4 to 26 hours, with an average of 13 hours. The total time from tissue recovery to shipment for the brain, CSF, and spinal cord (fresh) was 6 hours 47 minutes to 22 hours 35 minutes, with an average of 12 hours. The total time for skin (fresh) was 7 hours to 49 hours 30 minutes, with an average 27 hours 30 minutes. The total recovery time for bone and muscle (fixed) was 6 hours 47 minutes to 22 hours 35 minutes, with an average of 12 hours.

There have been some challenges. There are a lot of moving parts, there is weather, and there are many other things that can go awry. Finding a recovery specialist in the first place who is available 24/7 is difficult. There are donors in the backwoods of Idaho and it may be hard to find a recovery specialist who is closer than 200 to 300 miles away and who cannot get there for 3 to 4 hours. Therefore, NDRI spends a lot of time talking to people trying to find the recovery specialists who it is believed will do the best job. Back-up recovery specialists are also identified, given that the first choice may not always be available. An up-to-date schedule is maintained to speak with the recovery specialists to address issues like coverage for vacations, et cetera because if they have not heard from NDRI for a few months and are planning a vacation, they many not think to notify NDRI. Keeping kits accessible can also be challenging. There was a recent case in which the recovery tech went on vacation and locked the kit in his office, so it was not available to the back-up recovery specialist. In that case NDRI had to courier a new kit out. Finding a facility for the recovery can be problematic if the funeral home cannot be used and the recovery specialist does not have a facility. This is where the network comes in, as NDRI can work with hospitals and others in the network to find a recovery suite.

There can also be challenges at the time of recovery. For example, the recovery specialist or the funeral home may not be available. There are many timing issues in terms of communicating with the family, funeral home, and recovery specialist and arranging transportation if the recovery is not being done at the funeral home. All of that is dependent upon pronouncement of death. Even if NDRI receives a call from McKing at midnight, there have been cases in which it took another 2 or 3 hours for death to be pronounced. Nothing can be done before then. Sometimes a recovery kit is not pre-positioned and must be delivered, but NDRI does keep spare kits on hand and components can be obtained from network partners.

Consistency of supplies can be problematic. There have been changes in the brain kit specifications. In early 2014, the height of the brain/spinal cord pail was increased by 1 ½" by the supplier (Exakt Technologies), resulting in an inability to close the box with freezer bricks under the pail. Temperature validation was not available for the shipping boxes without the bottom freezer bricks. The solution was to order 2" taller removable box side insulation panels, and grow the outer box height. The deployed kits were recalled from recovery personnel and replacement parts were included in the new kits and the kits were redistributed.

Inadequate preparation of freezer bricks can be a challenge. Freezer bricks should be frozen and stored at -20°C. Storage at -80°C can damage specimens and bricks. There can be a potential issue of insufficient freezing time if the kit is not pre-positioned and is sent out from NDRI at the last minute. Another issue was that early skin samples were growing out contaminated cultures, but the skin kits did not include antibacterial/antifungal agents. NDRI proposed this could be remedied by including pre-measured vials of 100 X antibiotic concentrate of 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL in each skin kit with a shelf-life of 24 months. The agents would be mixed with PBS shipping/preservation medium at the time of recovery.

Discussion Points

Dr. Sorenson asked whether Dr. Lonsdale had data on the time of death to the time of notification. Brain tissue degrades very quickly after death. For biorepository purposes, the sooner it is collected the better.

Dr. Kaye indicated that McKing has this information. They carry the notification phone 24 hours a day, and notification is done within an hour. There was one call that was made to the office phone instead of the 24 hour cell, causing a delay.

Dr. Horton asked whether there is a breakout of rural versus non-rural. He would think the rural recoveries would take longer.

Dr. Kaye replied that because the kits are prepositioned and arrangements are made in advance, rural does not necessarily take longer. Part of it has to do with getting the samples on a plane. All of the samples from Idaho and Washington go out of the same airport, so the limitation there is being able to get to the last flight out. They try to put all of the samples on direct flights.

Skin Processing and Fibroblast Development

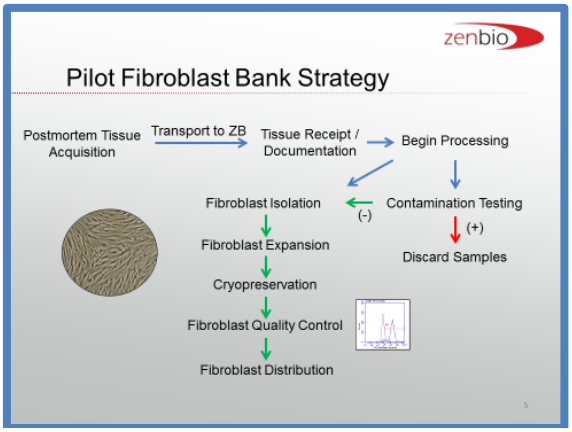
Ben Buehrer, PhD
Vice President and CSO
ZenBio, Inc.

Dr. Buehrer indicated that ZenBio, Inc. is involved in establishing the fibroblast bank from the post-mortem skin tissue. Zen Bio, Inc. was established in 1995 and is a very small company in Research Triangle Park, North Carolina. The company developed the first human primary adipocyte system, and continues to provide human primary cell-based systems to researchers worldwide. They began in the metabolic disease area, providing the first human primary adipocyte system. The company has branched out since then into several other areas, including cancer. In the late 1990s, ZenBio identified the adipose derived stem cell technology. That technology was spun out into a different company, while ZenBio remained a provider of research reagents outside of that technology. Contract research was included in 1996. Approximately one-third of the company's current business is performing contract research using primary cell systems created in-house. That also includes performance of custom cell isolations, as in the National ALS Biorepository Pilot Study. The company has had Good Laboratory Practices (GLP) certification since 2008.

The Fibroblast Bank Project was set up to determine the feasibility of establishing a post-mortem dermal fibroblast bank from donors in the ALS registry. The initial conception was to be able to establish cells from 15 out of 30 donors. The basic idea is to isolate fibroblasts from the skin tissue and characterize, expand, cryopreserve, and quality control (QC) them and then make them available / distribute them to approved ALS researchers. The Pilot Fibroblast Bank project was initiated in September 2013.

Once everything was in place, there were a few activities required before starting. The first task was to amend the existing IRB to include acquisition and use of human skin tissue. Dr. Kaye and her group took care of this very quickly. In addition, it was necessary to re-consent existing registrants and change the existing consent form to allow for skin collection. Before receiving the samples, it was necessary to prepare and distribute the 30 collection kits, which had to fit within the existing collection package. It was also necessary to establish and disseminate the tissue collection protocol, and work out the logistics for acquisition and shipment of tissue. That was handled primarily by McKing and NDRI.

When written out in a schematic as shown here, it seems like a very easy and straightforward process; however, there are always challenges:



The general process is that the tissue is procured, packaged, and transported to ZenBio. Upon receipt, ZenBio documents arrival of the tissue and assigns it an internal identification number with which to refer to it internally. No personal identification information is provided to ZenBio. McKing is contacted to confirm receipt of the tissue, and provide them with the identification number so that it can be linked with the actual number for the sample. At that point, the process begins to isolate the cells. That process includes a contamination test. Samples that test positive have to be thrown away, given that there is no good way to clean up cells from bacterial or mold contamination. Negative samples are then put through the process enzymatic digestion. Several processes were tested to determine the best approach. Nonetheless, the initial isolation is done followed by expansion to have a feasible number of cells with which to work. Passaging is kept to a minimum in order to retain as close to the donor specimen as possible. The cells are then cryopreserved. A few cells are then thawed to ensure that they maintained their morphology. Everything is then in place for distribution as necessary to ALS researchers, which will depend upon moving forward. ZenBio ships worldwide daily.

Some challenges were encountered. Given that live cells are being isolated, time is critical for viable fibroblast isolation. Fortunately, skin and fibroblasts are pretty hardy so they do not degrade as rapidly as other tissues may. Nevertheless, the shorter the timeframe from collection to receipt to isolation, the better the yields. The number of passages can also be kept to a minimum, making them more useful. There is limited notice to coordinate post-mortem tissue collection. The tissue samples also must be collected in an aseptic, preferably sterile manner. Skin is a difficult tissue in that regard because it comes into contact with bacteria, molds, et cetera. This was an issue early on. It is also important to minimize transport time to improve viable fibroblast isolation, but there are limitations to airline schedules. Courier or shipping company coordination for delivery on holidays and weekends is challenging. In addition to coordinating collection and transportation, technical staff must also be coordinated to receive tissue samples on short notice including nights, weekends and holidays. Sterility must be maintained throughout packaging, transport, and delivery for this component of the project.

The first skin tissue was received in March 2014, with 9 skin tissues received to date. These are the results from the 9 samples:

ZB Internal ID	Outcome	Cell number
DF031314C	Contamination	NA
DF061814A	No Growth	NA
DF062314B	No Growth	NA
DF082914	Contamination	NA
DF090314B	Fibroblasts Isolated	26 x 10^6 cells
DFM091714A	Fibroblasts Isolated	52 x 10^6 cells
DFM100514A	Fibroblasts Isolated	34 x 10^6 cells
DFM100914C	Fibroblasts Isolated	82 x 10^6 cells
DFM011915A	Fibroblasts Isolated	3.4 x 10^6 expanding

It is not uncommon when tissues are first received from new groups that it takes a while to get everything in place. ZenBio was in contact with McKing all along, and they were coordinating with NDRI. Most of the issues were worked through. The internal identification numbers refer to the date the samples are received, which is easy to do and is a means to coordinate with the sample collection. The first sample was contaminated, and two samples received in June 2014 had no growth. Due to the concern that these samples might arrive contaminated as well, they were treated more rigorously to decontaminate them. However, most of the living cells were killed. Subsequently, some of the protocol techniques were changed to ensure that collection was much more sterile and aseptic. Since then, fibroblasts have been isolated from 5 donors in a row ranging from 26 to 82 million cells, with one currently in expansion that was recently received.

Once the cells are received and expanded out, they are cryopreserved. The QC phase has also begun on those samples to ensure that they are at least 80% to 90% viable when thawed, maintain a reasonable growth rate so that they proliferate, and that they can expand through several passages so that they will be useful in a laboratory setting. Testing is done to ensure that they are actually fibroblasts, which can be determined by the morphology and assessment of typical cell surface markers. This also offers an idea of the purity of the culture and whether there are any contaminating endothelial or epithelial cells, which is unlikely but possible. Lot verification is done through short tandem repeat (STR) analysis to verify that there is a single donor in the collection. This is used for paternity and other testing. It does not indicate anything identifiable about the donor except their sex. However, it does offer a fingerprint if something short tandem repeats within certain genetic loci that can be matched up later in the process. For example, a researcher receives a specimen, passages it out, and can do this analysis to ensure that it correlates with the original sample they received. This ensures that there is consistency of the donor information throughout the process. Purity analysis is performed to ensure that there is greater than 95% fibroblasts in the product, and that the cultures are mycoplasma free.

In summary, procedures have been established for tissue acquisition and shipment to reduce the chances for contamination. An optimal procedure has been established for fibroblast isolation from post-mortem skin sample. Three common isolation procedures were compared, one of which seems to work every time. That also helps to reduce some of the contamination carry-over. QC control procedures are in place to establish culture purity and characteristics, cell validation, and sterility. Based on the initial outcome study, fibroblast isolation from post-mortem tissue appears to be feasible and is anticipated to provide viable cell culture reagents for continued ALS research.

Discussion Points

Dr. Corriveau said his experience with fibroblasts over a number of years has been moving to a place such that every fibroblast line must be karyotyped because of the frequency with which gross chromosomal changes are introduced during the fibroblast culture process. If this is not done, it results in investigators receiving fibroblasts among which a certain number will fail for karyotype if checked. This results in a lot of wasted resources and time.

Dr. Buehrer indicated that ZenBio, Inc. has not typically incorporated this. However, it would be very good to include specifically for this resource because it must be exactly identical to the donor and the disease state of the donor as opposed to anything that might have occurred during the process.

Dr. Corriveau clarified that he was not even talking about that level of sophistication. He just meant fails for karyotype triplication or other obvious karyotype that confounds the use of the fibroblasts. While this is not a best practice yet for repositories, in his opinion it absolutely has to be.

Dr. Buehrer agreed that the more QC, the better the utility of the biobank.

Brain, Spinal Cord, and CSF Processing and Storage

Thor D. Stein, MD, PhD
Department of Veterans Affairs
Boston University School of Medicine

Dr. Stein explained that Boston University School of Medicine (BUSM) is involved in multiple biorepository brain banks through a number of affiliations, including the following:

- ❑ National ALS Biorepository*
- ❑ VAB Brain Banks*
  - ALS
  - Gulf War
  - Post-Traumatic Stress Disorder (PTSD) for veterans and non-veterans
- ❑ Boston University Alzheimer's Disease Program*
- ❑ Chronic Traumatic Encephalopathy Center*
- ❑ Bedford and Boston VAMC
- ❑ Framingham Study*
- ❑ Centenarian Study*

For the VA ALS brain bank, BUSM recruits veterans across the country; follows subjects for life; collects clinical data, including ALS Functional Rating Scales (ALSFRS); and collects post-mortem tissue (brain, spinal cord, and CFS). The VA ALS bank is following subjects in 48 states, and has recovered specimens from 37. Also within the VAB Brain Banks is the Gulf War Bank; the Post-Traumatic Stress Disorder (PTSD) bank, which was recently begun; and the Traumatic Brain Injury (TBI) Brain Bank. These are national banks that will enroll from throughout the country. BUSM performs the neuropathology for Boston University Alzheimer's Disease Center. An offshoot will become integrated as the Chronic Traumatic Encephalopathy Program, which will assess athletes, veterans, and other individuals who have had a long history of repetitive mild TBI (MTBI). Interestingly, they find that about 12% to 14% of these individuals also have ALS. This is turning out to be a fairly significant co-morbidity in this group who develop traumatic neurodegenerative disease, which is a very interesting potential link to study. BUSM collects cases from local VAs in Bedford and Boston, which is important because this is a good source of controls. Controls are very difficult to enroll. The Boston VA has an autopsy service for people who pass away from other medical issues, who can serve as neurodegenerative controls. They process the post-mortem samples from them (brain, spinal cord) in an identical manner as the ALS brains. BUSM performs neuropathology for the Framingham Heart Study, which is another source of controls, as well as for the Centenarian Study.

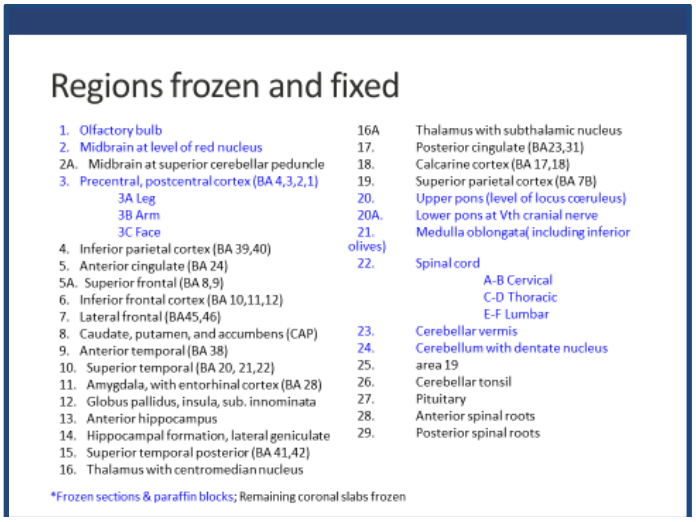
All of the studies with asterisks are nationwide, referral-based. Thus, BUSM has experience with the trials and travails of collecting throughout the country, which provides some unique difficulties. BSUM receives a lot of support from Boston University and the VA. All of the brain banks are housed at the VA, even the Boston University-centered studies. The neuropathology laboratory at the Bedford VAMC has three wet laboratories (1000, 600 and 400 square feet), each with two hoods, two tissue processors, an automated staining system, microtomes, cryostat, cassette and slide labeler, and a photomicroscope / image analysis room, eight ultralow freezers, and three deli-style refrigerators. VA Boston in currently under renovation, which includes an additional 800 square foot laboratory equipped for immunohistochemistry and molecular biology; additional office space; and new equipment that is already in use including

Agilent Bioanalyzer, Aperio slide scanner to scan in the slides for digital representation and opportunity to perform some routine immunostains, Zeiss fluorescent stereology system with Apotome for more advanced serology, and additional ultralow freezers and refrigerators.

In terms of the functions of the ALS biorepository in Boston, specimens are received in a fresh state, chilled and are assigned a code number and basic information is collected (e.g., age, gender, postmortem interval). The specimens are immediately processed, which involves the following:

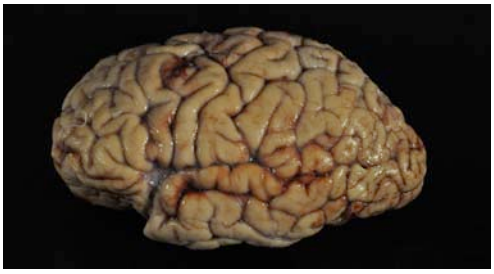
- ❑ Grossing: digital imaging, one hemisphere frozen, one hemisphere in fixative
- ❑ CSF processing: spun down in metal-free tubes, supernatant and pellet frozen
- ❑ Tissue quality control: RIN, pH
- ❑ Storage: frozen, paraffin blocks, slides, database input
- ❑ Neuropathology report generation for research investigators and families, if they desire
- ❑ Distribution of samples, eventually

For the neuropathology workup, the brain is hemisected with one half for frozen tissue and one half for histology. Half of the brainstem and cerebellum will be frozen for future molecular work. The other half will be fixed for at least two weeks in a special fixative, periodate-lysine-paraformaldehyde (PLP). This is a lighter fix than formalin and it preserves the antigenicity, which makes it easier to detect different protein accumulations. The disadvantage is that it requires refrigeration, so it takes up a lot of space. Once that hemisphere is properly fixed, it is then cut, photographed, and blocked. Blocks are processed and sections are cut and stained with LHE, Bielschowsky silver, and immunohistochemistry. The following is a list of all of the regions that are blocked:

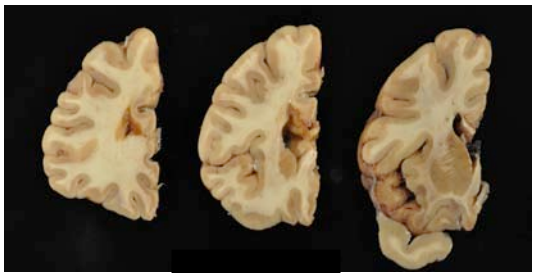


These are all made into paraffin blocks from about 40 different regions. For the ones in blue, the frozen half is also sub-dissected out. These include high demand regions, such as the cortex and spinal cord, for which there will be a lot of interest. A subset of this is processed into histological slides that are then used to make the diagnosis and generate a report.

Dr. Stein reviewed their first case to illustrate what they do and the pathology that they observe and the importance of having a thorough neuropathological work-up. This case was a 69 year-old man who presented with motor symptoms in 2008 and was diagnosed with ALS in 2011. He had a stroke in January 2013 and died 6 months later. At autopsy, his brain weighed 1150 grams (which is on the low side as the average should be about 1300 to 1400 grams); and showed mild frontal, temporal, and parietal lobe atrophy with a cavitated infarct involving the anterior corpus callosum as well as anterior spinal nerve root degeneration. The following is a photograph of the left hemisphere after fixation:

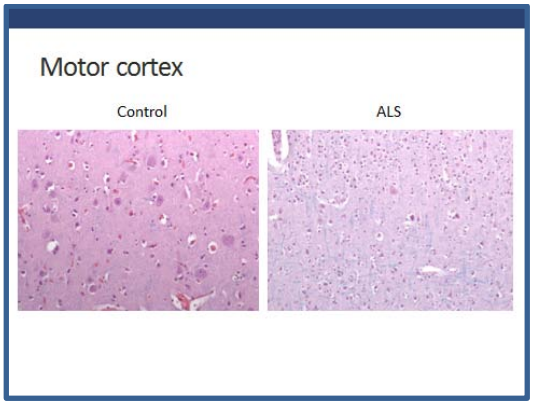


The coronal sections show an area consistent with a hemorrhagic stroke:

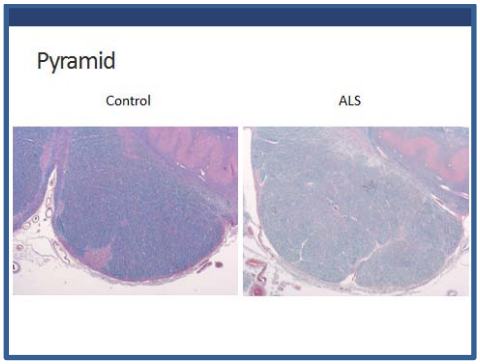


In the brainstem, there was a little bit of pallor in the nigra that is seen in Lewy body disease. The spinal cord showed the classic picture of ALS, with marked degeneration of the anterior spinal nerve roots.

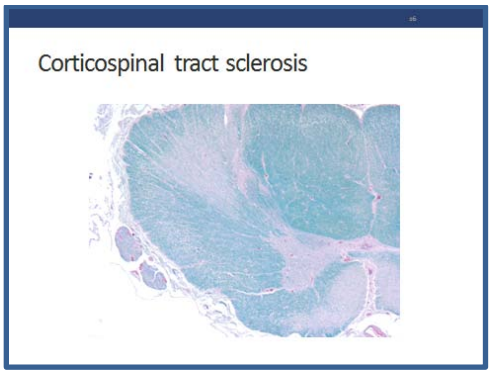
Microscopically, the following shows the difference in the motor cortex in a control and a case of ALS:



The following shows that the tracts coming down from the motor cortex are degenerated. This is the pyramid. Again, it is pale and degenerated on the right side:



In the spinal cord, the corticospinal tracts are also degenerated, which is where the disease gets the later part of its name lateral sclerosis:



Almost all sporadic forms of ALS will have inclusions of TDP-43, and these are typically linear inclusions in the cytoplasm illustrated in the following:

A number of additional stains are also done to cover a variety of neurodegenerative diseases, including the following:

- Beta-amyloid (AD)
- Tau (AD, FTLD, and CTE)
- Alpha-synuclein (LBD)
- TDP-43 and p62 in cerebellum (C9ORF72)

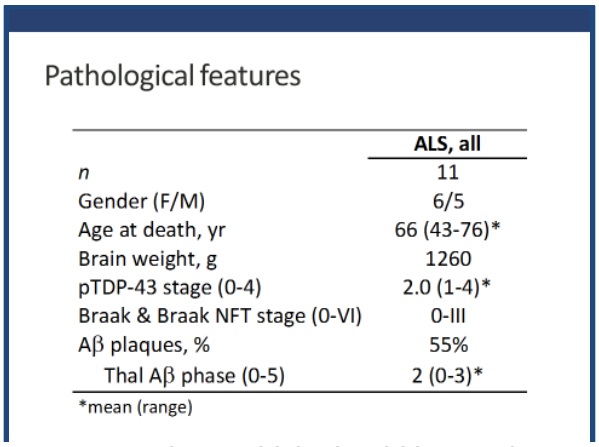
A number of neuropathology analyses are run, including the following:

- TDP-43: motor cortex, hippocampus, striatum, midbrain, medulla, and spinal cord
- Lewy bodies: olfactory bulb, medulla, pons, midbrain, hippocampus, amygdala, entorhinal cortex
- Tau: cortex, limbic, brainstem, spinal cord
- Other proteins: p62, ubiquilin, FUS

All of these are reported out on the pathology report, with stages and semi-quantitative measures generated on various parameters (beta-amyloid burden, NFTs, vascular disease, Lewy bodies, TDP-43) using standard published neuropathological criteria. The same is done for all of the Boston University Brains Banks, which allows for comparison across cases. Returning to the case, the diagnosis was clearly ALS. He also had a number of other pathologies, which is not uncommon. He had severe arteriolosclerosis, classically thought to be due to hypertension. He had one cavitated infarct involving the anterior corpus callosum, one subacute infarct within the right putamen, multiple remote microinfarcts in the caudate and putamen, and one remote microinfarct within the lateral midbrain. He also had the neuropathological changes of Alzheimer disease, but not sufficient for a diagnosis. He also had brain stem predominant Lewy body disease.

The ALS Brain Bank has had 11 subjects thus far, including the following diagnoses and pathological features:

- ALS (all, one case with primarily LMN involvement)
- Frontotemporal lobar degeneration (FTLD; 0)
- Alzheimer disease (2 with neuropathological changes)
- Lewy body disease (1)
- Vascular disease (1 with infarcts)
- Chronic traumatic encephalopathy (0); there are a few cases in the VA brain bank who have a significant TBI history and CTE



Pathological features

	ALS, all
n	11
Gender (F/M)	6/5
Age at death, yr	66 (43-76)*
Brain weight, g	1260
pTDP-43 stage (0-4)	2.0 (1-4)*
Braak & Braak NFT stage (0-VI)	0-III
Aβ plaques, %	55%
Thal Aβ phase (0-5)	2 (0-3)*

*mean (range)

Two quality control measures are used; pH which should be as close to physiologic as possible and the RNA integrity number (RIN). The average pH is pretty good at 6.2 and the average RIN is about 5.5. Different researchers interpret this differently depending upon what kind of RIN they want and the type of study they would like to conduct. All of these except one have been over 4, and that one had some other issues. Overall, they have done pretty well.

Once more cases are accumulated, data storage will have to be addressed. For the VA ALS study, a TissueMetrix database is used to track all of the specimens, their locations, and the various endpoints associated with them. It also allows for mapping of freezer space and barcode labeling of specimens. This is a cloud-based service that is scalable, allows for a storefront option for researchers, and allows them to drill down to determine how many cases of pure ALS there are, how many have other co-morbidities, what types of clinical information is involved, et cetera. Neuropathological data are recorded in a Microsoft Access database where the extent and degree of pathology can be recorded using semi-quantitative neuropathological measures. That will allow query of multiple variables, and once a larger number of cases has been accumulated, to pull out different values that may be of interest.

Some decisions had to be made regarding specimen distribution in terms of whether to put a lot of work in at the outset once the tissue is received, or more work later when it is sent out. This depends upon which regions are involved and the ease of dissecting out those regions. Areas anticipated to be in high demand and standard regions are processed at the time of dissection so that they are ready for immediate distribution. They do have the capability for generating frozen cut sections, which is particularly important for some of the high demand regions for which there is not a lot of tissue. They can also provide paraffin slides and paraffin blocks, and can perform DNA and RNA isolation. Frozen tissue blocks are available for distribution from the 11 cases, including 66 frozen motor cortex blocks and 165 spinal cord tissue blocks. Fixed tissue blocks are available for 451 total tissue blocks covering all of the regions shown earlier, of which 66 are motor cortex blocks and 77 are spinal cord tissue blocks. Multiple sections of spinal cord are put in one block so that it can be better screened. There are also 66 aliquots of CSF.

There have been a number of challenges, including the following:

- ❑ Getting tissue from around the country in a timely and appropriately packaged manner
- ❑ Balancing up-front tissue dissection with later distribution
- ❑ Storage issues, given that the samples add up quickly
- ❑ Data storage
- ❑ Tissue tracking

- ❑ The review process for tissue requests
- ❑ Tissue distribution and services offered

Discussion Points

Dr. McQuillan asked whether they charge a fee for the specimens.

Dr. Stein replied that they received NIH funding for some of the other banks and they do not currently charge. However, there has been discussion of generating a model of cost recovery at some point.

Dr. Sorenson asked what the criteria were for the 11 cases for establishing whether there was FTLD degeneration or not.

Dr. Stein indicated that basically it is the pattern of TDP-43 pathology and the extent of involvement. Typically, the type of inclusion will vary. There are four types of TDP-43 inclusion patterns. One case had some hippocampus involvement that might make one think about FTLD, but it did not meet other criterial for a diagnosis

Ms. Bledsoe asked whether any thought had been given to querying the researcher community to determine what types of services they might be interested in once the samples are made available.

Dr. Stein responded that they have not done so yet in terms of these samples, although they have done that for the VA bank to assess people's interest, particularly with the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS) there was consideration about tissue types. Most people are interested in motor cortex and spinal cord.

In response to a question from Dr. Antao regarding sample distribution, Dr. Stein said their biggest stumbling block on the VA side was putting a lot of work into collecting tissue, categorizing it, and getting it into the bank. However, they have had a difficult time getting it out. They are trying to make a push for that, but there have been a number of different stumbling blocks. The application and review process is very difficult and onerous, which has deterred some investigators. They have distributed five or six over the past year or two.

Dr. McQuillan asked how/whether they advertise or have a catalog.

Dr. Stein replied that another issue on the VA side is that they have been limited as to what they can do and where they can go. They have not been able to get the word out as much as they would like to, but are starting to do that more. They have been going to meetings to try to let people know about this resource and that it is available to everyone, VA or not, and they want people to request tissue. They have a very limited storefront, web-based catalog where investigators can see what tissue is available.

Dr. McQuillan said she has the same problem, so she was hoping Dr. Stein had the answer.

Ms. Bledsoe emphasized that marketing is critical and that it is not easy to do. Going to scientific meetings, having a booth, publishing in the literature, and making sure that the community is aware of the samples sounds pretty easy. However, this is not always so easy to do. There is another strategy that is up to the funding agencies, which is to tie a program announcement or some sort of funding initiative to using samples from the bank, and to put

money behind it in order for it to be effective. The National Cancer Institute (NCI) did this, and it was pretty effective for using some of the sample collections.

Dr. Corriveau stressed the importance of having a catalog that people recognize as such when they Google. He also liked the idea of linking the samples to funding announcements. In terms of quality, this makes the filter all the more important. Of course, if there is an announcement people will apply and they will use the samples to do whatever the funder says.

Dr. Mehta indicated that ATSDR currently has a Request for Applications (RFA) for the Registry. It is not based upon the biorepository samples, but it encourages individuals to use the registry as a recruitment tool. However, if they start requiring use of the biorepository or the registry for recruitment, that becomes contractual language rather than an investigator-initiated R01 RFA.

Dr. Corriveau said he was surprised to hear a RIN of 4 as being acceptable, though he has not used the RIN number much himself. It seemed to him that a RIN of 4 imposes certain limitations and he wondered what those might be in terms of limitations of the frozen tissue for something like biomarker discovery, for example.

Dr. Stein replied that it would depend upon the goal. It is probably less important in terms of protein biomarker studies. The main issue is messenger RNA studies. In terms of the histology and immunohistochemistry, tissue with very low RIN will still respond robustly to various immune-stains. A lot of protein work can be done with that. Ultimately, it will be up to each investigator to decide what is acceptable.

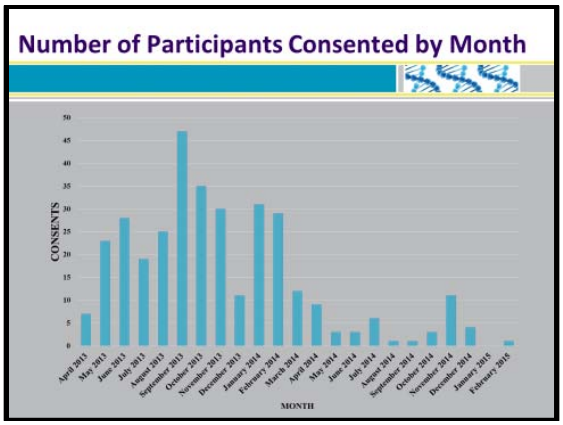
Dr. Corriveau said this is why he could not agree with the RFA to drive use of the resource; i.e. because if money is set aside without sufficient assurance about Q/C of the samples, investigators may be driven by the opportunity to apply for support instead of by the value and quality of the resources (e.g. tissue) available..

In-Home Biospecimen Collections

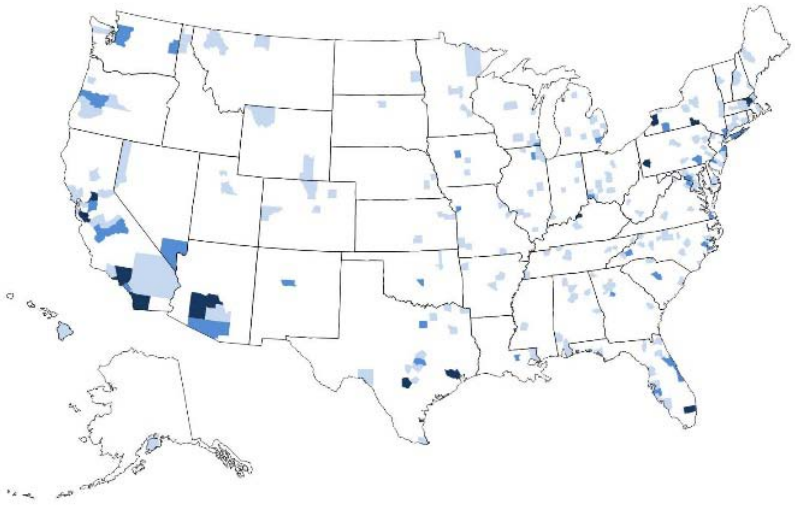
Pilot Study Findings and Outcomes

Laurie Wagner, MPH
McKing Consulting Corporation

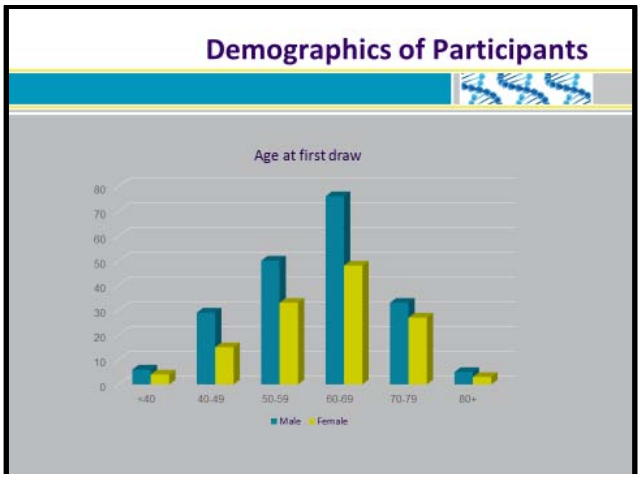
Ms. Wagner reminded everyone that recruitment for the in-home biospecimen collection portion of the study began in April 2013. ATSDR sent 1078 emails, and McKing followed up with 2972 emails to assist with recruiting. Of the potential participants, 71 were deceased, 80 were not interested, and 464 received information packets. Of those who received the information packets, 338 consented and 9 withdrew after consent (3 died before collection, 2 due to illness, and 4 for other reasons). Of the remaining consented participants, 329 have completed first draws. The following graphic shows the number of participants consented by month:



The number of participants consenting was slow in the beginning, but the number gradually grew as the months passed. The greatest number of participants were consented in September 2013. While Ms. Wagner has not cross-checked the dates of outreach and when ATSDR published email blasts through ALSA and MDA; however, there was an increase in calls when those blasts went out. Some of the enrollment peaks may be due to outreach. The number of participants consenting was lower beginning in August 2015, and no new participants were consented in January 2015. The geographic distribution of the participants is depicted in this map:



Participants are located throughout the US. There is at least one participant in each state, including Alaska and Hawaii. In terms of distance participants live from an MDA or ALSA referral center, 90 (27%) are less than 25 miles; 62 (19%) are 25 to less than 50 miles; 89 (27%) are 50 to less than 100 miles; and 88 (27%) are 100 or more miles. The figure shows the demographics of first draw by age range and sex:



The age ranges are fairly evenly distributed between males and females participating in the biospecimen component, with 199 males and 130 females. As expected, the highest number of first draws occurred among the higher age ranges, 50 through 69 years of age.

Second draws began in 2013. As of the end of January 2015, 251 participants have completed the second draw and 49 withdrew after first draw. Of the 49 who withdrew after the first draw, 32 died before the second collection, 2 were too sick to complete it, 3 could not be contacted to schedule an appointment, and 12 were not interested.

Regarding kit distribution, 588 full kits with all of the items shown earlier were created. As the study progressed, it became clear that blood only, urine only, and saliva only kits were needed. McKing worked with Fisher to create these additional kits and 24 blood only, 14 urine only, and 21 saliva only kits were distributed. Of the full kits, 580 (98.6%) have been returned. The remaining kits were not returned because the participant cancelled or died before his draw. Of the additional kits created, 19 (79.2%) blood only, 14 (100%) urine only, and 15 (71.5%) saliva only have been returned.

In terms of the specimens collected among the 329 first draws, 309 participants have had at least 1 vial of blood drawn, 296 have had all 5 vials of blood drawn, 319 have provided urine, 310 have provided hair, 308 have provided nails, and 15 have provided saliva. Among the whole blood specimens collected from 307 participants, there are 2214 plasmas (0.5 ml) from 305 participants, 307 buffy coats (1.0 ml) from 307 participants, and 585 RBCs (1.0 ml) from 298 participants. From the 307 participants who contributed metals free blood, there are 887 aliquots. From the 301 participants who gave specimens of plain blood, there are 2117 aliquots of plasma. PAXgene vials were collected from 307 participants, from which there are 607 vials of RNA. From the 319 participants who provided urine, there are 3142 1.8 ml vials, 297 4.5 ml vials with hg preservative, and 813 10 ml tubes. There are 310 hair samples, 308 nail samples, and 15 saliva samples. Hair, nail, and saliva samples are stored on shelves.

The ALS Registry includes surveys that participants can complete, so Dr. Kaye matched the data to see how many of the in-home collection participants completed at least one survey. Of the 329 in-home collection participants, 280 (85%) completed at least 1 survey and 255 completed surveys 1 through 7. This is good information because it means that background data, demographics, and disease progression can be matched with the in-home collection participants. This will be especially beneficial for researchers when the specimens become

available to them. The Disease Progression Survey can be completed multiple times and was counted as completed if done at least once.

There have been many challenges. Specimen collection forms are included in the kits, and the phlebotomists are supposed to complete them when collecting the specimens. However, many forms have been submitted in which all of the dates were not filled in. Some forms were cut into pieces and attached to the tubes. Sometimes the answers on the form do not agree with specimens received. For example, the form may indicate that there are nails but says there are not. Questions have been misinterpreted. For example, the question "When did you last have something to drink?" was answered "2009." Sometimes the phlebotomists have skipped the questions regarding hair and nails, which affects the specimens received. These issues have occurred frequently and have necessitated making calls to the phlebotomy companies and / or labs to acquire further information. If the biorepository moves forward, a recommendation will be made to revise the form to make it clearer.

What can go wrong does go wrong. A number of issues have arisen that were never anticipated, which highlights the reasons for conducting a feasibility study. There have been considerable issues related to phlebotomists, including the following:

- ❑ Phlebotomist confirms but does not go to appointment
- ❑ Phlebotomist reschedules appointment without consulting McKing or the phlebotomy company
- ❑ Phlebotomist reschedules appointment to a day or time when next-day FedEx delivery is not possible
- ❑ Phlebotomist takes specimens home and places them in the refrigerator instead of shipping
- ❑ Phlebotomist leaves specimens at the facility and does not call for pick up
- ❑ Phlebotomist acts unprofessionally, resulting in upset participants
- ❑ Phlebotomist is not well-trained, though more training is now required prior to working on this project
- ❑ Phlebotomist does not include or does not accurately complete the specimen collection form, and the Coordinators have to call them to complete it over the phone to the best of their ability

There are other general issues that relate to phlebotomy as well. There is no direct supervision/ observation at the site of collection. The tubes have to be drawn in a specific order, and the phlebotomists are supposed to use only the materials provided in the study kits (e.g., needles and gloves) to assure metals free collection. Some phlebotomists have eventually admitted that they did not use the materials provided. In addition, it has been difficult to schedule draws in some of the extremely remote locations. However, with creativity and diligence, they have been able to work this out.

Regarding next steps, approximately 20 second collections are left to complete, data will continue to be cleaned, and QA will be conducted on the specimens collected.

Discussion Points

Dr. Van Den Eeden requested clarification on the number of emails handled.

Ms. Wagner clarified that McKing could send up to four emails to each person. ATSDR sent the initial email, and then McKing followed up with frequent emails.

Mr. Kingon observed that they appeared to have started out with a number of phlebotomy companies, and then continued with two or three that worked.

Ms. Wagner replied that they initially started with one company. At first, she just presumed that it was simply more challenging and problematic than anticipated. They later decided to add additional phlebotomy companies. As they began to work with other companies, it became obvious that it did not have to be that difficult. There are two companies that work well and should get them through the end of the project. A lot of the phlebotomists only did a single visit; however, some did several draws. When the same phlebotomist does several draws, they tend to do a better job. McKing does no guarantee that a participant will get the same phlebotomist for the first and second draws, but the same phlebotomist can be requested and will be sent if available.

Dr. Goutman inquired as to whether there are any reliability data for phlebotomists in urban versus rural areas. He wondered whether some of the issues could be resolved if participants in more heavily populated areas could get to pre-specified ALS clinics or NEALS sites for sample collection, and continue to use the phlebotomy services in the rural areas. That may at least help to improve the reliability of the samples from some of the more urban areas.

Dr. Kaye said there were just as many problems in Los Angeles as in rural areas. For example, some phlebotomists in Los Angeles did not want to drive move than 10 miles. While she agreed that engaging ALS clinics would be beneficial, the problem is that it is an IRB nightmare. They would have to be IRB-approved in every center where they wanted to draw specimens. For example, Emory will not permit the drawing of specimens in their clinics to go to other studies. They will allow draws for their studies, but not for outside studies. It would be very complicated. Also, collecting only from those who go to referral centers will result in biased samples or have two tiers of specimens. These are all issues that can be taken into consideration if the project goes forward.

Ms. Ritsick added that consideration was given to the option of using Quest Diagnostic centers to have their blood drawn, but they could not guarantee that their phlebotomists would use only the materials in the collection kits.

It seemed to Dr. Corriveau that because one really valuable sample from this is the DNA, using Quest Diagnostics would be fine. Samples are being shipped overnight before plasma is even being made. How much difference would it make to use Quest Diagnostics?

Dr. Kaye reiterated that Quest Diagnostics did not want to guarantee the metals-free collection, so that would have to be given up.

Ms. Wagner stressed that the issue was using McKing's supplies. They could not conduct draws otherwise.

Dr. Kaye added that Question Diagnostics was very interested and said they could do it, but they did not want to be liable for guaranteeing that the phlebotomists would follow the directions and use only McKing's supplies. Without that requirement, they would have been an option.

In response from Dr. Traynor regarding use of the samples, Dr. Kaye replied that ATSDR is assessing this now. They have to determine a mechanism for applying for the specimens, reviewing applications, and distribution of the specimens. That will probably occur sometime next fiscal year. She stressed that they do not even have the full collection yet.

Dr. Traynor emphasized that they have a really good dataset and collection, but that it is only as good as the use that it is put to, and that they need to focus on getting samples to the researchers. The community has something to gain from this if the samples are made more generally available.

Collection Kits, Processing Samples, and Storage

Scott Hixon
Fisher BioServices

Mr. Hixon explained that Fisher BioServices is part of Thermo Fisher, the world leader in serving the science community. They have a global scale with over 50,000 employees worldwide. The company has four premier brands: 1) Thermo Fisher Scientific, which is the laboratory equipment division; 2) Life Technologies, which was just incorporated into the portfolio in January 2014 and is the life science research division; 3) Fisher Scientific, which provides supplies, containers, and lab equipment (Fisher BioServices is part of this brand); and 4) Unity Lab Services.

Fisher BioServices offers biobanking and biorepository services, cell therapy solutions, clinical trial sample management, and biologic-API management. Within those groups, there is clinical trial kit production such as those for the ALS Bioregistry, laboratory processing, qualification / validation, and cold-chain logistics. Fisher BioServices's information management system allows them to manage all of the specimens from collection through receipt of the samples, processing, and analyses. While Fisher BioServices does not perform any in-house analyses, they can distribute samples for analyses and store the resulting data. Samples are stored at various temperatures, and samples and kits are distributed through a large distribution channel. Fisher BioServices' US repositories are located in:

- Vacaville, California
- Detroit, Michigan (managed for someone else)
- Franklin, Massachusetts
- Rockville, Maryland (multiple locations)
- Frederick, Maryland
- Germantown, Maryland

One of Fisher BioServices' roles in the ALS Biorepository study is to create collection kits at the direction of McKing. Fisher is responsible for the acquisition of supplies: design, assembly, and distribution of the sample collection / shipping kits. As mentioned earlier, there are various types of kits for this study:

- Blood/Urine Collection kits
- Urine Only kits
- Blood Only kits
- Oragene Kits (for saliva samples)
- Post-Mortem Bone and Muscle Collection
- Post-Mortem CSF Collection

The kits contain pre-paid air bills, collection forms, phlebotomist guidelines, and shipping instructions.

Another role that Fisher BioServices plays in the ALS Biorepository is as a central processing lab. All laboratory procedures are developed and approved before samples can be processed. This includes client work instructions, SOPs, and forms that were developed specifically for this project. The procedures and forms are distributed internally for approval, and then to the client for their approval as well. All documents are in a controlled format, and include a change control process if a procedure needs to be modified. Additionally, any deviations from the controlled procedures require formal documentation. All laboratory technicians are trained on these procedures, and must demonstrate that they can perform the processes before they are permitted to work on the project. All training is formally documented. Fisher BioServices maintains full chain of custody for all incoming samples. This includes notification that samples are incoming and FedEx receipts. There is a control process for transferring samples within the facility as well. Samples that are destined for immediate storage are transferred to the repository, while samples to be aliquoted are transferred directly to the laboratory. After processing, aliquots are transferred to the repository for permanent storage. The following laboratory services are provided once the collected kits arrive at Fisher BioServices.

Whole blood is processed into:

- 6 ml EDTA: 3 whole blood aliquots into prescreened cryovials
- 10 ml EDTA: 8 plasma, 1 buffy coat, and 2 red blood cell aliquots generated in standard cryovials (not enough CDC prescreened cryovials were set aside for this part of the study)
- 10 ml Red Top: 8 serum aliquots generated into standard cryovials

Urine cup (approximately 60 mL to 100 mL) processing includes:

- 10 aliquots in CDC prescreened cryovials
- 1 aliquot containing a mercury additive in prescreened CDC vials
- 3 aliquots in prescreened CDC vials

Repository services for the ALS Biorepository study include secure storage and management of the samples collected at -80 degrees (e.g., blood, urine, saliva, bone and muscle, nail, hair). A very robust Inventory Management System is used that captures, stores, and manages all data associated with the samples related to collection and processing. Collection data includes; collection date, sample type, volume, barcode template, temperature monitor status, comments, and barcode (Patient ID). Aliquots are created for blood, serum, plasma, and DNA.

Some of the challenges faced associated with the kits were; the first set of kits produced were ID-specific, i.e., they had a bar code associated with them but did not get assigned to the patient until they were used. Just-in-time kit production was needed for the second round of kits, and the same patient ID had to be matched that was used on the first set. This resulted in turnaround time issues with patient-specific kits for the second visit kits. The short-term solution was to provide bulk unlabeled kits and barcode sets to McKing for distribution, but this bypassed Fisher BioServices' quality group. Kits are made in batches, so a "B" prefix was added to the original visit ID to maintain the relationship between the two draws. Summer temperatures required a change in the instructions to inform the sites to refrigerate the gel packs to accommodate the raise in temperature. Another challenge was the need to develop blood only kits, which were designed to offer a second chance at blood collection. Urine only kits were also developed, which were designed to provide a replacement kit if the phlebotomist missed the visit since the patient would have already collected the urine. Kits are created through a controlled batch record process, so new batch records and work instructions had to be generated to accommodate changes to existing kits.

For sample receipt and inventory there were some challenges faced as well including inconsistent information provided by phlebotomists as Ms. Wagner mentioned earlier. For example, a form indicated that no hair was collected but a hair sample was included. Another issue was missing collection times and/or dates. Fisher is tracking this information in its system, so if there are no dates included on the forms, they cannot be entered into the system. In the beginning, the phlebotomists did not fully understand what paperwork needed to be returned, and returned all paperwork contained within the kits, causing confusion for Fisher. Changes have been made to the Information Management system to help with upfront data validation. Changes were made to several fields so that if a field was not filled out or the date was incorrect, this would be flagged before going into the system. The upfront data validation helped to clean up some of the issues.

Regarding laboratory services challenges, two different vial types (pre-screened and standard) were used for processing samples, which increased the processing time. The two different vial types made the process multi-step. If all tubes were pre-screened, Fisher would be able to process in one continuous step and would be more cost-effective. Currently, this requires splitting the processing into separate functions. Additionally, the tubes came with the caps separated. If they were together, it would save time in the lab when processing samples.

The client requested that CDC pre-screened transfer pipettes be used to generate aliquots. This is fine for small numbers of samples, but is sub-optimal because there are variations in aliquot volume due to transfer pipet variations and operator consistency with volume estimation. This can lead to inconsistent sample volumes across the range of aliquots. If the intent of the pilot is to create data for the main study, as long as sample quantities are similar there is minimal issue.

All samples are currently being processed manually, even samples that could go on an automated platform. However, automation or semi-automation would be required if the incoming sample demand is significantly larger. There are some automated throughput processing considerations if the main study moves forward. Pre-screening would be required for pipette tips used with automation. Throughput will be significantly higher with pipetting automation. Using all prescreened vials would be recommended for use with automation.

If the study does not move forward, several issues will have to be addressed. Fisher will have to determine how to close out the project. If a termination letter is received, Fisher will supply final reports regarding shipping, receiving, processing, and inventory. A determination must be made about what to do in terms of material review and disposition. The CDC supplied kit components and the samples that are stored with Fisher will need to be dispositioned. The options are for Fisher to continue to store them, ship them to another facility, or destroy them. Final invoices will be issued. Any additional activities will need to be discussed with the Project Manager and may incur additional costs.

Discussion Points

Dr. McQuillan noted that whole blood and RBCs do lyse at ˉ80 degrees, and that she was thinking about this in terms of the recommendations and what to do with specimens. Mr. Hixon replied that ˉ80 degrees was the requested temperature.

Ms. Gunter asked in which facility the ALS Biorepository samples are stored, and whether they are stored at temperatures other than ˉ80 degrees.

Mr. Hixon responded that the ALS Biorepository samples are stored in their main Rockville, Maryland facility. The samples are stored at multiple temperatures.

Dr. Berry commented that typically, the buffy coat and red cells can be stored at ˉ80 degrees without lysing. The issue pertains to the process used to get them to ˉ80 degrees. The process for cryopreserving the cells will impact whether they lyse. They can be stored at ˉ80 degrees if they have not lysed during the freezing process.

Mr. Ally added that no cryopreservation process is currently being used, so they will lyse.

Dr. Corriveau asked why they were preparing buffy coats for DNA versus extracting it from the whole blood.

Mr. Hixon replied that as far as he knew, there was no plan to extract the DNA right away and they have not extracted any of the samples.

Dr. Berry thought there was a more efficient way to use the same volume of blood. If they wanted to use the plasma out of the sample, they could certainly save the buffy coat.

Dr. Corriveau said there was nothing technical about why they could not have buffy coats, but it was unclear why they wanted them.

Dr. Kaye responded that collecting the buffy coat was one of the recommendations in 2012, so they did.

Dr. Corriveau said his perspective was that if there is not a rationale for collecting buffy coats, it should be stopped.

Dr. Berry said he thought the rationale was that this is the source of DNA for the study, so it was collected to have a source for the DNA. The reason to save it as buffy coat rather than extracting the DNA at the outset was a current cost issue.

Dr. Corriveau agreed that extracting DNA was a good reason to have the buffy coat, but it is much cheaper and more straightforward to simply extract the DNA from whole blood without having to first prepare the buffy coat.

Dr. Traynor thought some background regarding the technology might be useful. With next generation sequence, the DNA requirement is dramatically dropping. Only a small amount is needed for whole genome sequencing, so put into perspective probably all that is needed is the blood.

Ms. Gunter clarified that the specimen collection was designed before a lot of the work on DNA extraction, whole blood, or even the red cell pellet was getting published as much. Using the buffy coat seemed like the appropriate method, because they would have that product from the separation of the whole blood. It was the easiest thing to get and save as a rich source. When they did the extraction would be highly dependent upon when funding would be available.

Dr. Corriveau said from his experience, more DNA can be obtained more easily from whole blood than from buffy coats.

Dr. Kaye said the thought going forward is that DNA would be extracted as part of the processing, which would address the issue.

Quality Assurance of Samples

Wendy E. Kaye, PhD
Senior Epidemiologist
McKing Consulting Corporation

Dr. Kaye noted that she meant to mention earlier that in addition to all of the other services NDRI provides, they also take responsibility for keeping track of all stating laws and ensuring that all state laws are adhered to in the collection of postmortem specimens. For example, New Jersey passed a law indicating that organ donation cannot take place in funeral homes. She thought this was to stop illicit organ procurement. This meant that even though they are not procuring organs for live donation, it was felt that that law applied. Therefore, none of the post-mortem collections can be done in funeral homes in New Jersey. They have to be done in a medical facility, or the decedent has to be transported to the NDRI facility in Philadelphia. That is just an example of one of the many things NDRI is keeping track of to ensure that everything is done appropriately.

Regarding QA, originally when the study was designed, there were plans for temperature monitors within the boxes. Therefore, temperature was the assessment of specimen quality to a certain extent. During the 2014 annual meeting, concerns were raised about the quality of specimens and what other quality controls there were besides temperature. In consultation with some laboratory experts, they tried to identify some assays that would measure specimen quality. Sometimes it is difficult to find a quality assessment for a specimen when what the

specimen will be used for is unknown. The decision was made to make sure that DNA could be extracted from the specimens and phenotype them. They had to assure specimens were labeled to the correct people, and assess the quality and quantity of the DNA extracted.

There were concerns about specimens not being spun down immediately and the plasma removed. One thought with regard to the issues of cell lysis was that the hemoglobin in the serum and plasma could be measured. If it was below what would be expected to be circulating as free heme, then cell lysis was probably not occurring and the samples were in good shape. They agreed to conduct analysis on approximately 30% of the specimens collected to date. Specimens were selected from both the first and second collections. Dr. Kaye systematically selected a person to contribute a sample, and then alternated between the first and second collections to ensure that they were evenly distributed in first and second collections and from early in the study to late in the study. Then they had to retrieve the appropriate aliquot for the specific analysis. Fisher BioServices pulled the specimens for them that were selected.

In terms of the demographics of the people selected, most of the people were between 50 and 69 years of age, but there were some people in all age groups. Of those selected, 117 (63.6%) were males and 64 (36.4%) were females. The country was divided into three large regions: West, Central, and East. Of the samples selected, 74 (42%) were in the East, 63 (35%) were Central, and 39 (23%) were in the West.

Each collection kit included a TrekView® temperature data logger, which can be used about 26 times and the data can be download via a USB port:

The temperature range is set, and an alarm goes off if the temperature goes outside the range. Because some temperatures were found to be too warm early on, an additional cool pack was added to the collection kit. Participants were directed to place cool packs in the refrigerator before their collection appointment. That seemed to address the issue, because they stopped getting alarms on the TrekViews®.

DNA has been extracted from the first 90 specimens selected. The method was changes to increase the DNA yield. She considered yields of ≥ 100 µg to be “good.” Overall, 58% had a “good” yield and 21% had an “adequate” yield. The first 13 looked pretty bad, but that was before the DNA extraction method was changed. After the method change, 67% had “good” yield and 22% had an “adequate” yield. A 260/280 analysis method was used to measure the quality of the analysis. This is a standard to using DNA for downstream measure, and 92% met the accepted industry standard for DNA that can be used in downstream applications such as polymerase chain reaction (PCR), DNA sequencing.

In order to try to evaluate the quality of not spinning the specimens and freezing them before they were shipped, the hemoglobin concentration in serum and plasma was assessed. Approximately 62 µg/ml would be free heme that was circulating, and it appears that there was a lot of cell lysis in the tube that was used for serum. This tube was collected third in the list, and there is not always a very good correlation. The plasma may be great in the first tube. Because it is not bad in both of them, it does not seem to be a shipping issue. Instead, it appears to be a collection issue. The heme levels in the plasma is quite good, so it looks like the samples are coming in good shape. The thought is that more analyses need to be performed. Part of it may be the gauge needle being used, and by the third tube they are causing some cell lysis. A fairly small gauge needle has to be used, because the patients may be really sick. It may be that by the third tube, it is just becoming more difficult to get the blood. They can tell this to a certain extent by whether they got tubes 4 and 5, which would give some indication that they were beginning to have difficulty collecting the blood.

Looking at the heme levels in the serum and plasma simultaneously per person, almost always the serum heme is higher than the plasma. Some are very good on both, but for some of them the plasma is fine and the serum is off the chart. The plan is to go back through all of the notes to assess what may have caused some of these.

In terms of next steps, the QA analysis will be continued on the rest of the specimens. The QA analysis will be incorporated into the specimen processing instead of having to pull the aliquots out and test them later. The magnetic bead based method will continue to be used for DNA extraction, because it is getting so much better yield. Options will be evaluated for increasing viability of specimens. It was suggested that to get a cleaner and more pure specimen, small amounts of serum plasma could be manually taken off after it is spun and freeze that down before the machine is used to pull out the serum and aliquot it out. Therefore, the top purest specimen could be set aside and the rest could be done by machine.

Discussion Points

Mr. Ally explained that the first step in harvesting the aliquots is to centrifuge the blood tubes. The hypothesis is that if a small aliquot of the serum and plasma are removed first, this would essentially result in a cell-free aliquot that subsequent freezing should not affect.

Ms. Gunter said she knew the literature on plasma and serum hemoglobin goes back decades, but she wondered whether the 62 µg/ml threshold was the same for both. The serum hemoglobin levels would be expected to be somewhat higher simply because of the process of clotting causes shearing of some RBCs, and therefore would elevate the level of free Hb level in the serum. .

Mr. Ally agreed that practically, this could be part of the explanation. The literature does not differentiate between the roles of the two. He agreed that it would make more sense because of the lack of anticoagulant, there would be a lot more squeezing of the cells.

Dr. Kaye asked everyone to spend the evening thinking about whether the ALS Biorepository should move forward and, if so, what should be included / not included, how the process might be changed to make it better, et cetera.

Recommendations for Long-Term Implementation

Wendy E. Kaye, PhD
Senior Epidemiologist
McKing Consulting Corporation

Dr. Kaye pointed out that based on the results thus far, they have shown that the ALS Biorepository is feasible, with some caveats pertaining to potential improvements if the project goes forward. With that in mind, McKing developed some recommendations for in-home and post-mortem collection.

Regarding in-home collection, donating specimens to the biorepository should be a choice within the National ALS Registry in order to enhance enrollment. Upon enrollment, registrants would be able to click a box indicating interest in hearing more about the biorepository. Additional contact information should be collected, such as mailing address and phone number, to facilitate contact. Selection of participants from those who express interest should continue, maintaining geographic representativeness and distribution of people from rural and urban areas and clinics and non-clinics in order to have good representation of all patients who have ALS. From a logistics and sample size standpoint, consenting/collecting only once from 400 to 500 participants per year seems more feasible than collecting from 300 people twice. Many people do not make it to their second collection, and there are a lot of costs associated with tracking, scheduling, et cetera to complete a second draw. Those funds could be utilized to increase the number of people from whom specimens are collected versus trying to get two samples from the same person, especially if one of the primary goals is to have DNA.

For specimen types and processing, it is feasible to continue collecting blood and urine. As part of the process, rather than putting the blood directly into storage, DNA should be extracted from one blood tube during the processing. The DNA should be aliquoted, frozen, and made another type within the repository. RNA should be extracted from the PAXgene tube, and also should be aliquoted and frozen. Given that there are many issues associated with collecting the metals free tube, McKing would recommend that metals free blood tube collection be stopped until demand for the specimens is assessed. Consent forms could be written in a more open ended manner that would permit up to a certain amount of blood and certain number of tubes. The types of tubes might vary from year to year depending upon need. For example, if the metals free tubes samples become depleted, they could be added back in. There are currently about 1500 metals free aliquots, which may be sufficient for a while.

While it is cheap and not difficult to collect hair and nails, these samples still have to be stored, inventoried, and tracked. As with the metals free tube, collection of hair and nails should be stopped until demand for the specimens is assessed. Collection of hair and nails could be done for a limited time when current specimens are depleted. Saliva specimens should continue to be collected from those who cannot give blood, and the saliva kits should be processed to extract and freeze DNA aliquots. Consideration should be given to collecting saliva on interested people with ALS (PALS) not selected to donate blood.

Quality assurance and processing of existing samples should continue. DNA should be extracted from the remaining blood and saliva specimens, and RNA should be extracted from the PAXgene tubes. Although the manufacturer of the PAXgene tubes indicates that they have a 4 to 5 year shelf-life frozen, the lab has reported that they are observing degradation in about a year. Therefore, it is important to extract all of those specimens immediately before further degradation occurs.

Regarding post-mortem collection recruiting and consenting, donating specimens to the biorepository should also be a choice that can be selected after registration. Again, additional information, such as mailing address and phone number, should be collected to facilitate contact. Approximately 20 to 30 participants should be consented/collected from per year. Quarterly contact should be continued. The estimate is that if 20 to 30 people per year are consented, eventually a steady state would be achieved in which there would be approximately 20 to 30 collections per year.

In terms of specimen types and processing, collection of the brain, spinal cord, and CSF should continue. As with some of the biological collections, perhaps collection of bone and muscle specimens should be discontinued until its usefulness is assessed. Consideration could be given to adding this back for a limited time when current specimens are depleted. Though it has been shown that fibroblasts can be done fairly successfully from the post-mortem skin tissue, consideration must be given to how many are needed and whether it is important to do this for everyone.

Creation of donation plans with pilot study participants should continue. It helps with the planning, and has resulted in successful collection. IRB approval should be obtained to move the pilot study participants into the larger study if it goes forward or to re-consent them to other studies if necessary. Not everyone wants to make a post-mortem donation, so it is important to honor the wishes of those who have already consented to participate by moving them into the larger study if it goes forward or into another study.

Regarding the overall operation, ATSDR would need to integrate the biorepository into the protocol for the National ALS Registry protocol. They are currently being operated independently for a number of reasons. However, it would be advantageous to integrate the protocols, amend the IRB protocol to include the donation of specimens, amend the Office of Management and Budget (OMB) package to include specimen donation, and update the National ALS Registry website with application materials for use of specimens, catalogs, procedures, et cetera.

With respect to maintenance, Ms. Ritsick has been investigating the type of private laboratory the might be used to maintain the specimens long-term. McKing has talked to a variety of people at various repositories (academic, commercial, CDC, NIH. et cetera), and would recommend that the specimens be maintained at a private laboratory. If the project moves forward and extracting DNA continues to be part of the process, CDC's Specimen Packaging, Inventory, and Repository (CASPIR) does no processing. The distribution of specimens should be integrated into the biorepository operation, meaning that whomever is housing the specimens would assist in the facilitation of the review process of applications for specimens, maintain an inventory of available specimens, and retrieve and ship specimens to approved researchers. Consideration should be given to obtaining approval to charge a minimal fee for retrieval, shipping, and custom dissection of brain or spinal cord.

Discussion Points

Dr. Van Der Eeden asked whether there were any plans to provide assay results back to the program for broader sharing.

Dr. Kaye indicated that the second meeting in 2013 dealt with governance of the specimens. At that time, there was discussion regarding a requirement to provide some of the data back to the biorepository so that people would not have to conduct the same testing repeatedly. While the details have not been finalized, this has been discussed.

Regarding skin sampling, Dr. Bruijn asked whether there had been any dialog about the movement of science toward making induced pluripotent stem (iPS) lines from the blood. There is a different collection process, so it is important to be mindful of how to deal with this.

Dr. Kaye replied that there had not been any discussion regarding this issue, but that it could be included in the recommendations discussion for moving forward.

Regarding the maintenance of the specimens in the private laboratory, Ms. Gunter requested clarification about whether this meant combining all of the samples, including what Dr. Stein is storing.

Dr. Kaye replied that they consider Dr. Stein's laboratory to be private versus a government laboratory. The post-mortem specimens would be maintained by BUSM. The blood, urine, hair, and nails would be housed in a private laboratory. If some wanted specimens from both, that would be coordinated.

Dr. Sorenson wondered about what is going to be done with the in-home blood collection. It seemed like what was proposed for the future was somewhat of a departure from what has been done in the pilot study. Recalling the discussion from the previous day regarding the merits of cryopreservation and extracting the DNA, there did not seem to be a strong consensus regarding what should be done. That may warrant further thought and discussion. It seemed that what Dr. Kaye just proposed would not allow for serum to be preserved.

Dr. Kaye clarified that the recommendations she presented during this session pertained to the changes that they would suggest. Consideration needs to be given to how serum and plasma are being collected. Though Dr. Corriveau was unable to stay for the second day, she was able to speak with him before he left. He suggested that there might be a two-tiered approach and that everything does not have to be collected from everybody. Perhaps everyone would provide a blood sample to get DNA, while others under a different circumstance in a controlled setting may give the blood sample, serum, and plasma so that these could be spun down and frozen immediately. These are all considerations for the recommendation discussion period regarding how ATSDR might structure the biorepository in the future. These were just McKing's initial thoughts prior to having heard the previous day's presentations.

Dr. Horton asked whether there was any idea what the demand might be for these samples. Obviously, ATSDR is strongly considering moving forward. If there is not going to be a significant demand, perhaps they should start small and ramp up to collect more samples in subsequent years. Knowing how many samples are requested in a given year would help to inform how ATSDR structures its biorepository if they move forward.

Dr. Berry reported that he was recently assessing this. The Northeast ALS Consortium (NEALS) has received an increasing number of requests each year since about 2009 when they began formalizing the process of sending out samples. There is still an opportunity for many more requests. This is partly a matter of letting scientists know what is available, and what the benefits are of using these samples. Over the last year, NEALS has had about 10 requests. The requests often are for aliquots from 50 plus patients, and are typically for matched samples such as serum and RNA or serum and DNA. NEALS has CSF, so they are often matching CSF and plasma. One challenge is that there may be 10 requests, and there may be projects that would not be conducted otherwise. However, if the request is for samples from 150 people, a large biorepository is needed. If ATSDR starts small, the benefit of each of those requests is smaller. It may be that there are 100 people requesting 10 samples, which may not be the best use of a biorepository like this anyway. The best use may be to have 10 people requesting 200 samples, but it is necessary to be prepared for that.

Dr. Horton pointed out that because they do not know what the demand will be for the 11 existing post-mortem samples, perhaps in the first year ATSDR could collect in-home samples only while the demand for the existing post-mortem samples is assessed. If there is demand in subsequent years, they could then move to a post-mortem model.

Dr. Mehta asked what the annual budget is for the NEALS repository.

Dr. Berry responded that they have always operated on a “shoestring,” but recently received some funding from ALSA and another foundation. They now have a full-time coordinator who can focus on this, as well as some time from investigators and a project manager and have been able to build a team around this. They have found that during the years they have been barely getting by, they have created a lot of problems that the team is now having to back-track. This highlights the advantage of building a team that can pay attention to this.

Ms. Bledsoe emphasized that one of the major issues in the field is sustainability and concerns about underutilization of samples. Especially given the suggestion of the need to assess demand for certain types of specimens, she wondered whether it would be worthwhile to conduct some market research to query the community about what they are looking for. This should not be difficult, and it probably would be possible to acquire a list of ALS researchers from NIH. This would raise issues regarding conducting a survey and OMB clearances, but it might be worthwhile to avoid ending up with an expensive collection that will not be used.

Dr. Lonsdale reported that NDRI serves a significant number of researcher requests for post-mortem ALS samples, which they recover from donors outside of this program. He believes there is a demand, though it is probably fragmented. An expansion of this project would be a great way to consolidate those requests. Demand has been consistent over the years, and they have definitely observed an uptick over the last couple of years.

Dr. Bruijn agreed that one of the issues about demand is certainly related to marketing and people knowing about it. She is working with the VA, which has a fabulous collection that is not being used as much as it should be. Part of it is motivated around funding for projects to use the samples. ALSA is very engaged in building up and helping with the NEALS repository, and has opened a second site for the repository. They are also very engaged with Translational Research Advancing Therapy for ALS (TREAT ALS) which will also be doing this. ALSA’s incentive through TREAT ALS is going to be a program to fund biomarkers studies. ALSA’s intention is, with NIH, to perhaps publish a call for funding. ALSA would not limit the request for funding and use of samples to what they are investing in, but would open it out to the VA bank

and others to indicate what is available. The message and funding behind it are important. Although there are a lot of activities connected to the collection of CSF and blood, the greatest challenge is the post-mortem tissue. There is always an interest from the patient community to participate, and they receive many calls. Getting that organized and getting a commitment into that area would be very helpful.

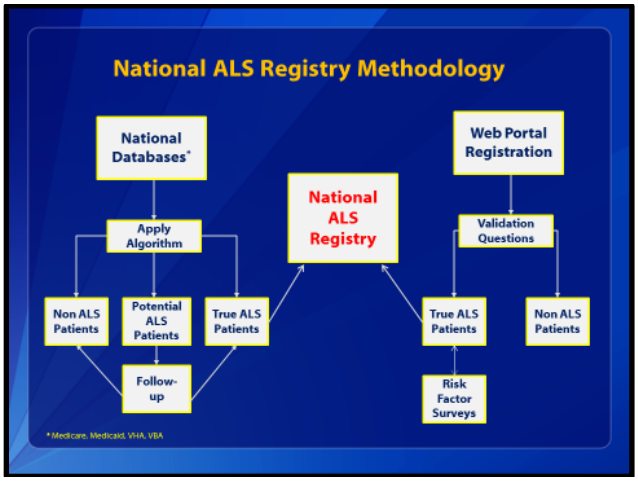
Dr. Berry recently spoke with Lyle Ostrow who fills frequent requests for his tissue repository, though they tend to be smaller numbers. The requests for post-mortem tissue are usually smaller, but there are more of these requests.

Dr. Kaye added that Dr. Corriveau also suggested that as part of the cataloguing of specimens, each repository should also promote/advertise all of the other repositories that are available. Then if someone needed 600 specimens, they could obtain 300 from ATSDR, 200 from NEALS, and 100 from NIH. This would permit investigators to increase their samples sizes significantly. She also mentioned that another reason for using Boston University for this project is because they are involved in the VA project, and all of the specimens have been processed in the same way. Thus, specimens that have been processed exactly the same way could be obtained from both banks through them and the samples.

National ALS Registry Priorities and Future Direction

D. Kevin Horton, DrPH, MSPH
Chief, Surveillance and Registries Branch
Division of Health Studies
Agency for Toxic Substances & Disease Registry

Dr. Horton discussed what he considers to be some of the priorities for the National ALS Registry, including the potential biorepository component. He acknowledged that there were some new participants in attendance and that while there had been significant discussion regarding the repository, he wanted to ensure that everyone understood how the National ALS Registry functions. The National ALS Registry takes a two-pronged approach as depicted in the following graphic:



Like many non-infectious diseases, ALS is not a notifiable disease except in Massachusetts which started a registry a couple of years ago. Given that, ATSDR had a challenge from the outset in terms of how to identify new and existing cases of ALS in a country of 315 million people. With the two-pronged approach, the first approach uses large national databases such as Medicare and Medicaid. An algorithm is devised and applied to these large databases to characterize people into several categories. Those who are classified as not being cases will not go into the ALS Registry, those who are classified as true cases will be moved into the Registry, and those who are classified as potential ALS cases because there is insufficient information are set aside for future assessment when subsequent years of data are collected. The algorithm is comprised of a number of components, including the ICD code for ALS. Though a number of cases are identified this way, the ICD code for ALS alone cannot be relied upon because ICD coding is notorious for errors. Prescription drug use for Rilutek® (riluzole) is also part of the algorithm, because it is the only Food and Drug Administration (FDA)-approved drug for ALS. If a person is taking Rilutek®, this is a pretty good indication that he/she has ALS. The algorithm also assesses frequency of visits, given that many ALS patients go to a clinic quarterly.

The second prong of the two-tiered approach to the National ALS Registry is web portal registration, which is the way most PALS know of the registry. Upon enrollment, PALS complete a series of validation questions. Depending upon the responses, a registrant is also classified a Non ALS Case, True ALS Case, or Potential ALS case. Critical about the online portal is that it is more real-time. Some of the administrative databases have a considerable lag-time, so it can be two to three years before ATSDR receives data from them. One of the goals of the registry is to assess the risk factors for ALS and a number of risk factor modules have been incorporated into the registry. Dr. Horton believes the modules are critical for the biorepository as well. A logical question regards whether a person will be counted twice, but they are not because the subjects are matched on Social Security Number (SSN) for both tiers. This is a list of the current risk factor modules and the number completed of each by patients who enroll through the web portal:

Survey (n=17)	Release Date	No. Completed
Demographics	October, 2010	5295
Occupational history	October, 2010	4852
Military history	October, 2010	4775
Smoking and alcohol history	October, 2010	4704
Physical activity	October, 2010	4549
Disease progression (ALSFRS)	October, 2010	4473
Family history of neuro. diseases	October, 2010	4458
Clinical data (e.g., devices used, body onset)	November, 2013	1250
Open-ended etiological questions	November, 2013	1153

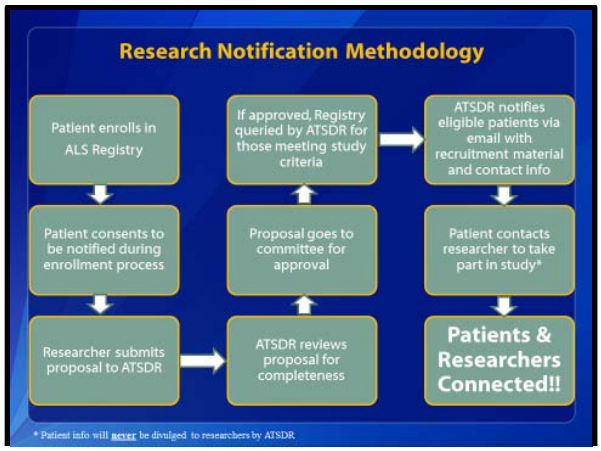
Lifetime residential history	May, 2014	982
Lifetime occupational history	May, 2014	958
Residential pesticide use	May, 2014	900
Hobbies with toxicant exposures	August, 2014	671
Caffeine consumption	August, 2014	644
Reproductive history (women)	August, 2014	493
Trauma history	December, 2014	310
Health insurance status	December, 2014	310
Total (as of 2/24/2015)	---	**40,777**

Many of the survey topics are described in the literature as being associated or potentially associated with the development of ALS. The modules were rolled out using a staggered approach. The first seven were launched on the same day the online portal was launched. The additional modules were rolled out once they were developed, vetted, and tested. All modules are designed to be taken one time, with the exception of the Disease Progression Module, which is a longitudinal module that individuals take multiple times each year to assess their disease progression.

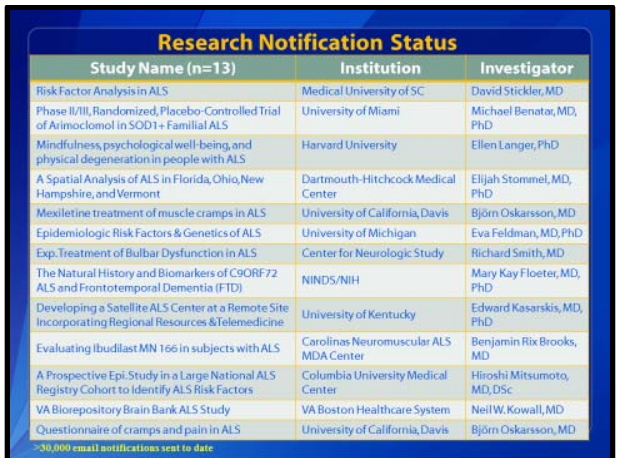
ATSDR was directed by Congress to build this registry, and now steps are being taken to enhance the Registry to make it stronger and more useful to PALS and researchers. The current priorities of the registry are to collect and analyze data to quantify the US ALS burden and examine risk factors, as mandated by Congress; determine whether to launch a biorepository component to facilitate research or rely on other repositories; utilize the registry to help researchers recruit for clinical trials and epidemiological studies; and fund external research to better understand the risk factors.

Regarding the major findings, the first report covered the time period from October 19, 2010 through December 31, 2011. During that timeframe 12,187 persons were identified through the portal and national data bases as definite ALS cases and were included in the registry. This results in an estimated prevalence rate of 3.9 cases/100,000 persons. This is approximately what is reported in the literature, though it is somewhat low. This is a new registry, so it is understood that not every case has been captured. Some people use private insurance rather than Medicare or Medicaid, so they have not been captured. The findings are largely consistent with what has been observed in European ALS registries, and some of the small-scale epidemiological studies that have been conducted in the US. For example, ALS is more common in whites, males, non-Hispanics, and persons aged from 60 through 69 years. Males had a higher prevalence than females. The lowest number of ALS cases are in persons aged 18 through 39 and > 80 years [Mehta, P et al; *MMWR*, Surveillance Summaries / Vol. 63 / No. 7 July 25, 2014].

In terms of considerations for a National ALS Biorepository, biosamples (e.g., blood, tissue) are important in ALS research to better understand the genetics of ALS. In Dr. Horton's opinion, what would set this apart from other biorepositories are the risk factor surveys. The ATSDR Biorepository would link the epidemiologic and risk factor survey data with biosamples. No other such ALS biorepository exists domestically or abroad. The ATSDR Biorepository would be nationally representative, and the data collected would be available to ALS scientists for their own research. As Dr. Kaye's group demonstrated, it is feasible to collect samples from people in rural areas who may not go to referral centers. Dr. Horton emphasized that he was not trying to take anything away from other biorepositories. Instead, he views the ATSDR Biorepository as being able to fill a gap if ATSDR decides to launch it. The National ALS Registry has already been opened up to researchers for recruitment purposes. Patient recruitment, in general, can be difficult. The National ALS Registry links PALS with scientists who are recruiting for research (e.g., clinical trials, studies), and a high percentage of PALS are signing up to be linked with researchers. Researchers across the US are using the tool for recruitment purposes. Dr. Horton briefly described the National ALS Registry research notification process, which is depicted in the following graphic:



ATSDR is not involved in any of these studies other than to facilitate the research notification process. Thus far, the agency has helped to link PALS and researchers for the following 13 studies:

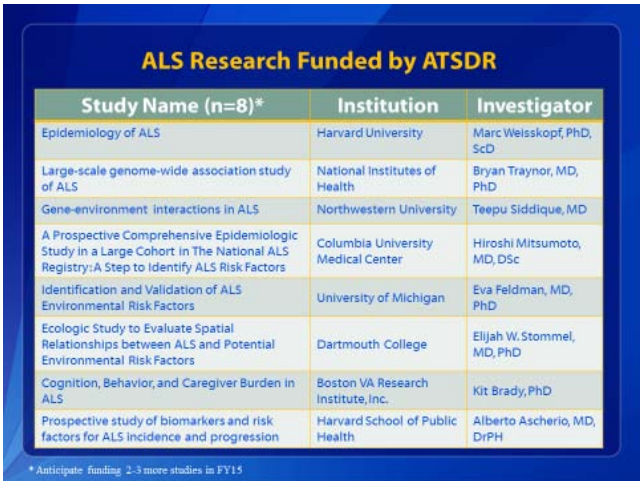


Research Notification Status

Study Name (n=13)	Institution	Investigator
Risk Factor Analysis in ALS	Medical University of SC	David Stickler, MD
Phase II/III, Randomized, Placebo-Controlled Trial of Arimoclomol in SOD1+ Familial ALS	University of Miami	Michael Benatar, MD, PhD
Mindfulness, psychological well-being, and physical degeneration in people with ALS	Harvard University	Ellen Langer, PhD
A Spatial Analysis of ALS in Florida, Ohio, New Hampshire, and Vermont	Dartmouth-Hitchcock Medical Center	Elijah Stommel, MD, PhD
Mexiletine treatment of muscle cramps in ALS	University of California, Davis	Björn Oskarsson, MD
Epidemiologic Risk Factors & Genetics of ALS	University of Michigan	Eva Feldman, MD, PhD
Exp. Treatment of Bulbar Dysfunction in ALS	Center for Neurologic Study	Richard Smith, MD
The Natural History and Biomarkers of C9ORF72 ALS and Frontotemporal Dementia (FTD)	NINDS/NIH	Mary Kay Floeter, MD, PhD
Developing a Satellite ALS Center at a Remote Site Incorporating Regional Resources & Telemedicine	University of Kentucky	Edward Kasarskis, MD, PhD
Evaluating Ibudilast MN 166 in subjects with ALS	Carolinas Neuromuscular ALS MDA Center	Benjamin Rix Brooks, MD
A Prospective Epi. Study in a Large National ALS Registry Cohort to Identify ALS Risk Factors	Columbia University Medical Center	Hiroshi Mitsumoto, MD, DSc
VA Biorepository Brain Bank ALS Study	VA Boston Healthcare System	Neil W. Kowall, MD
Questionnaire of cramps and pain in ALS	University of California, Davis	Björn Oskarsson, MD

>30,000 email notifications sent to date

ATSDR strives to fund as much external research as possible to learn more about ALS etiology and risk factors. Information gleaned from that research will help ATSDR prioritize topics for future risk factor surveys, and add to the literature. The following ALS research has been funded by ATSDR:



ALS Research Funded by ATSDR

Study Name (n=8)*	Institution	Investigator
Epidemiology of ALS	Harvard University	Marc Weisskopf, PhD, ScD
Large-scale genome-wide association study of ALS	National Institutes of Health	Bryan Traynor, MD, PhD
Gene-environment interactions in ALS	Northwestern University	Teepu Siddique, MD
A Prospective Comprehensive Epidemiologic Study in a Large Cohort in The National ALS Registry: A Step to Identify ALS Risk Factors	Columbia University Medical Center	Hiroshi Mitsumoto, MD, DSc
Identification and Validation of ALS Environmental Risk Factors	University of Michigan	Eva Feldman, MD, PhD
Ecologic Study to Evaluate Spatial Relationships between ALS and Potential Environmental Risk Factors	Dartmouth College	Elijah W. Stommel, MD, PhD
Cognition, Behavior, and Caregiver Burden in ALS	Boston VA Research Institute, Inc.	Kit Brady, PhD
Prospective study of biomarkers and risk factors for ALS incidence and progression	Harvard School of Public Health	Alberto Ascherio, MD, DrPH

* Anticipate funding 2-3 more studies in FY15

A new funding announcement was posted on January 14, 2015. ATSDR hopes to fund two to three more research projects this year, and anticipates that the funding will be in place by late summer to early October.

Dr. Horton concluded that, in his personal opinion, an ATSDR Biorepository makes sense and that ATSDR can fill some critical gaps by launching the biorepository. It is not the agency's intent to crowd out others who already have biorepositories in place. If ATSDR can bring more samples on line, pair them with risk factor data, and make them available to researchers, this should be beneficial.

Facilitated Discussion to Develop Expert Panel Recommendations

Bob Kingon, MPA
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During this session, Mr. Kingon invited participants to engage in a discussion regarding the development of Expert Panel recommendations. He indicated that participants would be voting on the recommendations, with the exception of ATSDR and McKing staff who would not be permitted to vote. The vote/responses for questions posed for discussion are grouped following the question under which they were raised:

General Discussion Points

Given that it is difficult to make decisions without understanding the approximate costs involved and finances, Mr. Gibson requested that they talk about those issues before beginning the recommendation discussion.

Mr. Kingon replied that is approach to this session was not to be concerned with the overall cost of moving forward with the registry, given that it will have to fit within the priorities and context of what ATSDR has in terms of resources. He requested additional input from Drs. Horton and Kaye.

Dr. Kaye replied that this is a difficult question because it depends upon the specimens collected, the processing, the desired number to collect, et cetera. As a guess for in-home collection only, 500 specimens a year would cost approximately $1.5 to $2 million. Post-mortem is more of a per collection cost, with an estimated $30,000 per collection including identification, ensuring eligibility, consenting, collection, processing, and storage.

Dr. Horton emphasized that everything depends upon demand. At this point, the demand is unknown. If ATSDR decides to move forward with this process, they would begin with in-home pre-mortem sample collection. If demand for the existing post-mortem specimens is determined to be significant, more post-mortem collections could be added in subsequent years. They have to start reasonably, and then ramp up.

Mr. Gibson stressed the importance of marketing to the research community to articulate what this additional information regarding risk factors could drive.

Ms. Bledsoe requested clarification of “long-term.”

Dr. Kaye replied that “long-term” means that the registry will not stop with the pilot study and there will be additional years of collection, but not necessarily that the registry will continue forever. Moving forward, the program would have to be evaluated annually to determine whether the samples are being utilized, if there are enough samples being collected, if the right types of samples are being collected, et cetera. Funding is also important. Even if funding runs out and more samples cannot be collected, the existing samples have to be maintained.

Dr. Horton replied that agency funding cycles are generally three to five years.

Mr. Gibson inquired as to how the results of the pilot study would be announced to the ALS community and Congress, and when the meeting report would be completed.

Dr. Horton replied that the pilot project does not end until September 2015. At that time, McKing will develop a report. There will be some sort of public version of the recommendations, but how ATSDR will publicize that has not yet been determined.

Ms. Ritsick pointed out that collections are scheduled all the way through August 2015, so final data will not be in until the end of August or early September. McKing can certainly create a draft for ATSDR to review, but the numbers will not be final until September.

Dr. Kaye added that the meeting report with the recommendations would be completed within a couple of months of the meeting. This meeting was pushed up considerably so that if the recommendations were favorable, ATSDR would have the opportunity to move forward in the next fiscal year without any downtime.

Dr. Horton emphasized that it is important to understand that this is not strictly an ATSDR decision. The agency is looking to the experts in the scientific community to help inform the decision regarding whether moving forward with the biorepository is feasible and worth ATSDR's time.

Ms. Bledsoe stressed the importance of having all of the processes and procedures in place before announcing the biorepository in order to avoid frustrating the scientific community.

Dr. Bruijn agreed, but thought they could announce that the concept is in development and indicate that there would be no access to samples at that point.

Dr. McQuillan emphasized the importance of ensuring the safety of the samples, regardless of where they are stored, to avoid an incident such as the disaster at the McClain Brain Bank.

Dr. Kaye said they have had an alarm go off, which was dealt with quickly. It did turn out to be a false alarm. Fisher BioServices has repositories in multiple locations, and one thought McKing had regarded whether to divide the collection in half and place each collection in a different location.

The denominator for the vote was determined to be 12.

Should ATSDR implement a long-term biorepository activity?

VOTE: Continue the Biorepository Activity	
Support With No Major Concerns or Reservations	7
Support With Major Concerns or Reservations	5
Do Not Support	0

- Sustainability must be thoughtfully addressed. Consideration must be given to when collection should be stopped, which is not a trivial question. Develop a business plan, do some market research to assess need, and ask critical questions about the current science, and where the science might move over the next three to five years. Flexibility to modify with changes in the science and demands of the researchers is essential.

- It is vital to maintain a good sense of how/if the samples are being well-used, so that it does not just become a collection that is not being used.

- Conduct some case studies to determine what type of research the samples can be used for, as well as who might fund those studies. For example, if studies are going to be conducted with serum to assess progression, it is important to determine the progression markers and how often they have been collected.

- One strength of this resource is the ability to enroll patients who live a distance away from NEALS sites or other tertiary or referral centers. There is a potential for duplication of efforts, given that there will be patients in other repositories who also are in the National ATSDR Biorepository. Establish a way to ensure that samples are not sent for the same study twice.

- The focus on post-mortem tissue collection is very important. Asking a patient for their post-mortem tissue is a very personal question. It touches on the features of one's mortality, and is a very difficult conversation to have. The need to have post-mortem tissue is extremely important, but the way in which that is implemented is equally important. It would be very valuable to engage the patient's physician, who is most likely to be discussing end-of-life issues with him/her. Sometimes patients will not sign a consent form, because they cannot sign their life away even if they want to share their tissue. Carefully addressing this issue could help to increase the yield.

- Develop a strong marketing plan in coordination with other repositories such as NEALS, VA, and other resources that are not necessarily ALS-specific. There are a number of similar resources, but it is unclear how/if they are being utilized.

- Controls are also very important, but are much more difficult to get into a very specific ALS biorepository. There are many synergistic efforts that can be marketed in a much more effective way.

- It is important to evaluate the value added of the risk assessment surveys to determine whether it actually is an "add" and, if so, what the "add" is, and whether these data will actually be used and promoted by epidemiologists. The National Institute of Environmental Health Sciences (NIEHS) might fund this type of study. There has been some very good work in the collection of environmental exposures in the Netherlands and as part of the European efforts.

- The fundamental problem for etiological studies is controls. Risk factor data is tremendous, but the issue depends upon who is being compared. The place of best footing in terms of bias concerns would be to study issues of progression. Then it will be a matter of how well FRS is being collected repeatedly from people. From the incident standpoint, it is somewhat trickier because of identifying appropriate controls. Another way risk assessment will be beneficial is with gene-environment interaction. There are some approaches for that for which controls are not needed.

- This is not the best of circumstances because controls are not being collected prospective at the same time in the same way, so it is going to be an issue.

- For the biorepository studies, NEALS tends to collect controls as part of the study. They have a longitudinal study that is focused on obtaining spinal fluid every four months. That study is very intensive for the people who are in it, and the decision was made that including controls in it was probably not the right use of research. However, that study is focused on assessing progression of the disease. Biobanking studies that are assessing who gets the disease and who does not assemble a cohort of patients and controls contemporaneously.

- NDRI is setting up an autism study with the National Institute of Mental Health (NIMH) to collect autistic brains post-mortem. For that study, prospective age/sex matched control collection is being done in parallel.

- Post-mortem tissue is more challenging in many of the NEALS studies. There is a growing effort to do multi-center post-mortem collection, but much of this has been done at individual institutions, and how people deal with controls has varied from place to place. This is a major issue for researchers using the tissue, given that they need to have comparable controls.

- In terms of how to prevent overlap of biofluids and tissues from one group to another, NEALS, NIH, and NIMH are moving to the use of the Globally Unique Identifier (GUID). Though previously there were some concerns, it has become more commonplace to use it. NIH is beginning to require it for certain diseases. The NEALS IRB now understands it and has put some fairly standard language in place. This is going to be a very important piece of information to link people.

- ATSDR is fully aware of and very interested in the GUID and has had a number of conversations with NIH about this. The immediate problem is that nine pieces of information have to be collected to generate a GUID. The National ALS Registry collects only two of them. For the other seven variables, ATSDR will have to go to its IRB and OMB. OMB is not a quick process. Dr. Kaye has already developed a one-pager for ATSDR's IRB. Once that approval is received, negotiations will begin with OMB. If OMB views this as a non-substantive change, a full OMB package would not be required.

- There are some major efforts in the ALS field to do genome sequencing. Project MinE is going to start in Europe and is being support by ALSA here in the US. There is also the New York Genome Center (NYGC) and some projects that are feeding into a large consortium of samples that are going to NYGC. This is for sequencing, the data from which would then be available to researchers. There may be a way to draw a second tube of blood to send for genetic sequencing so that the sequencing is done. That does not address every genetic question that might need to be answered, so some DNA needs to be held in reserve. However, this may be one way to join some larger efforts that are aiming to sequence tens of thousands of patients and make this a part of the solution at the outset.

- The cost for sequencing is high, so it is important not to duplicate that effort.

- For all of the trials that run through NEALS, sample management tends to go through the biorepository, either physically or virtually it is tracked at the site through a barcoding label system. If there are leftover samples after the study, those might become a part of what is shared through NEALS. All of the data from the clinical trial is linked to the sample and shared. There are also studies that are designed just with the purpose of supporting some sort of scientific identification, usually biomarker identification, and that is the main purpose of the study. Patients present to a center to give blood, a lot of biofluids, and a significant amount of clinical information. The biorepository is flexible in that it can store all of those. Thus far, these have been center-based and have been built around one unifying purpose. After those studies are completed, the extra samples are fed into the biorepository for later sharing as well. The centers are not geographically representative, and some centers are more active than others. That is resource-dependent and to some extent, population-dependent. One of the benefits of the National ALS Biorepository is to reach patients who are not near a multi-disciplinary center or are not participating in studies.

- Regarding saving buffy coat and getting DNA from that, if the volume of blood that can be taken is limited, spinning a sample to get the plasma and taking the buffy coat to save for DNA is an efficient use. The amount of DNA from the buffy coat is less than if a tube of blood is set aside. If taking the time to spin a sample and take the buffy coat, in a relatively sophisticated laboratory, it is not that much harder to get a good protocol for cryopreserving cells that can be used for making iPS cells. Though some quality control would be needed.

- One of the tremendous benefits of the National ALS Registry is the biological specimen/risk factor epidemiologic linkage. In any epidemiologic study, the challenge is selection of controls. Depending upon the question a researcher has, there may be multiple ways of identifying a specific control group for a specific project. The control issue is less concerning to some people than others.

- In general for registries, controls are not typically captured.

- It is important to keep in mind that if the bioregistry is ongoing, in theory an investigator could propose a study using all of the cases enrolled starting immediately, and then based on who is identified for that, an appropriate control can be found immediately. However, that will mean finding out who is enrolled in the registry reasonably rapidly in order to identify controls reasonably quickly.

How many collections per participant?

VOTE: Collect Samples One Time Instead of Two	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- Though two samples six months apart have been collected during the pilot study, a series of blood specimens from every participant is not needed. The amount of cost, time, and effort being spent to collect a second specimen from the same people could be invested in different people for a larger sample size.

- Different studies handle this differently. There are reasons to have longitudinal collections and reasons to have cross-sectional collections. The biorepository should be built to the strengths of the registry, one of which is how broad it is. Therefore, a cross-sectional collection may be the most cost-effective method. With a GUID, a research might be able to link a blood sample collected through this study with a blood sample collected through another study. Depending on how specimens are collected, they might be able to create a longitudinal group of samples.

- The real value of the registry is the ability to generate very large numbers of samples. A better use of resource is to increase the sample sizes rather than collecting two samples. Efforts to collect two samples have already been shown to be problematic.

- It is also important to keep in mind that these samples are being captured in different symptomatic windows. The strength of longitudinal data is unknown yet, but having those data should help to understand when they first became symptomatic. That can be lined up and may make the cross-sectional measurements more meaningful.

How many participants per year, at a minimum?

VOTE: 400-500 Participants Is Reasonable	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- The value of the registry is that samples are being captured from people who are not going to clinics. An easy checkbox question could be posed, such as "Are you participating in sample collection elsewhere?" This would help to prioritize those who are remote from a clinic who would not be a sample anyway, and that becomes a more realistic number. This strategy will avoid duplication of costs and will align nicely with other repositories.

- One caveat to that is that because 40% of ALS patients are not seen in referral centers, the registry samples will be biased unless someone chooses to request specimens from more than one location. That model is risky.

- Funders could encourage investigators to collaborate and acquire samples from multiple locations.

- It does not make sense to schedule phlebotomists to conduct in-home collections, particularly given the number of associated challenges, when there are researchers who would be open to collecting a certain number of tubes in the context of a visit to be sent to the Registry. They could also do this using good research practice. The strength of the Registry is the ability to capture people who live distantly from a center, and the bias could be made up for by allowing those who live close to research centers to go to those sites. The hope would be to capture people early while they are still ambulatory and able to attend a clinic site. Then the National ALS Biorepository could include patients who otherwise would not be included in the study. That seems like a money-saving venture, because it focuses the effort on a population that would be under-served and it would not duplicate the efforts that others are already making.

- It is possible that this collection alone could become biased away from people who are seen at centers and toward people who are not seen at centers. If everyone uses a system to link samples, such as GUID, this would allow for collaboration and a total available sample pool that is not necessarily biased. This is somewhat more complicated, but it could be beneficial. Maybe focusing the costs on getting patients involve who otherwise cannot be involved is the best way to use the resources.

- One slight counterpoint regards whether the associated data is the same. That somewhat argues that everyone is going to presumably have pretty much the same associated data within the biorepository.

- In the immediate term, different collections will have different data. Hopefully in the short- to medium-term future, with the GUID that links samples and information, theoretically samples could be requested from people who are in the registry from NEALS or NIH, as well as people in the registry from this collection so that it is ultimately all linked back to the registry data.

- Enrolling in the National ALS Registry is the common denominator. Consideration should be given to having practitioners assist people with signing up for the registry, perhaps through the use of iPads in their offices. Patients who express an interest in being involved in research available through the registry can have samples drawn within the course of their clinic visit. It does not make sense to incorporate fees to send a phlebotomist to a patient's home when the patient is sitting in a clinic that has the capabilities of performing a research blood draw. There would have to be buy-in from the sites, and it would be necessary to determine which ones would follow all of the directions and work out the details with them. But then the biorepository could focus home visits on the under-served.

- It does not make sense to go through all of this for only 100 samples per year. Collecting as many samples as possible, with a minimum of several hundred samples per, year seems reasonable.

- 400 to 500 per year will result in 2500 people collected after five years. Genetic studies probably require the largest number of samples at this point. Those types of studies are seeking tens of thousands of samples globally. This would be making a good dent in that effort. More is always better, and 400 to 500 samples per year seems like a good aim.

Should metal free collections continue?

VOTE: Continue Metals Free Collections	
Support With No Major Concerns or Reservations	0
Support With Major Concerns or Reservations	0
Do Not Support	12

- This is a complicating factor in in-home specimen collection.

- Consideration must be given to how much research has been conducted so far that has pinpointed any particular metal as an agent in the development of ALS.

- There are some studies and some things look potentially interesting and are worth following up. However, there are reservations about continuing to collect the metals free tubes. As reported the previous day, this severely complicates the phlebotomy. People with ALS have already had prodromal issues, so there will be an issue of whether reverse causation issues of the disease are affecting the metal level. This is particularly difficult in terms of finding appropriate controls, because it will necessarily have to be in the past unless a study is being conducted prospectively. If metals free collection is creating problems, perhaps it should not continue.

- It may be worthwhile and may possibly be cheaper to analyze aliquots from the pilot study to assess whether anything stands out, such as elevated mercury or arsenic before committing to the collection of 400 to 500 samples.

- That would be difficult to assess. The differences are not going to be massive, and it would require the appropriate controls. That said, this could be analyzed in the context of progression and whether it affects progression. It is unknown whether disease progression affects these levels, but that potentially could be answered with the existing samples. That would be an interesting initial study with them. In the timeframe of what is in the blood in terms of the measurement of the metals, hair or nails could be collected and that would not complicate the collection of blood and is cheaper to store.

- While the presence of metals is probably the less interesting question at this point, consideration should be given to whether the presence of non-metal free tubes could interfere with any of the assay measurements that investigators may want to perform.

- The metals free tubes are just for the metals.

How much blood (and what type of samples) should be collected?

VOTE	
Support With No Major Concerns or Reservations	**N/A**
Support With Major Concerns or Reservations	**N/A**
Do Not Support	**N/A**

- No vote was taken on this topic.

- Based on the discussions thus far, there seems to be support for collecting three tubes: one tube to set aside for DNA extraction, one PAXgene tube to extract the RNA, and one tube from which to save the red blood cells for fibroblast lines and to get serum.

- Everyone seemed to agree that DNA should be extracted, and that plasma and serum should continue to be collected. The difference between serum and plasma is that all of the cells are centrifuged out of plasma, while serum is allowed to clot and a lot of the proteins gotten rid of. This is a subtle difference to some people, but for some assays this is very important. Ideally, both should probably be collected. The other question regards cells, peripheral blood mononuclear cells (PBMCs), which can be turned into induced pluripotent stem cell lines now. That technology is different even since last year's meeting. Stem cell scientists are now saying that this is working; whereas, before there were a lot of chromosomal abnormalities. Whole blood can be collected in an ethylenediaminetetraacetic acid (EDTA) tube and sent to someone to extract DNA. Then the extracted DNA can be tested in many different ways, including sequencing. However, sequencing does not necessarily pick up on repeats and other things. It seems like there should be one tube of EDTA for DNA. It is not necessary to spin it and take the buffy coat. For good quality DNA, it can be put directly into the freezer. The PAXgene tubes can be treated similarly, either extracting the RNA right away or putting the sample directly in the freezer. The cleanest thing to do is extract the DNA and RNA right away, but they can be frozen and dealt with later. The plasma has to be spun up front, and is probably best collected in an EDTA tube. The reason not to spin an EDTA tube and take the plasma and leave the buffy coat for DNA is that it is extra work. If an extra tube can be collected, it would be better to do one tube for plasma, get rid of the cellular component, and one tube for DNA. This cuts down on processing, and gives more DNA from the tube saved for DNA. What takes some thought is processing cells for iPS lines, which are probably best collected into a cell preparation tube. The cell preparation tube has lithium heparin as the additive, and has a FICOLL™ gradient built in. Those can be spun according to the package insert. The general protocol is to spin them in a centrifuge, take off the buffy coat, wash the buffy coat, and then freeze that in a process for cryopreservation. Basically what that means is re-suspending the cell pellet into a cryovial, the cryovial is placed in a device called a "Mr. Frosty" that is filled with alcohol so that the cells cool down slowly instead of flash freezing and that keeps them from lysing. There are also companies that will do this, and they will save it and send it out to collaborators. It is not that complicated. They have to be overnighted, but it is worth thinking about. To some extent, DNA and RNA can come from a cell line. So there are some benefits to having them. ATSDR should consider this moving forward.

- Of concern is the window of time from collection, to getting it through the mail, to starting it processing. Many things are great when they can be done onsite at the time of collection, but this may not be feasible in remote locations. This was why the buffy coat was the choice in the first place.

- Fisher BioServices routinely performs PBMC harvests. The CPT tube would be ideal, and overnight shipping at room temperature is suitable to get very good yields.

- Having only one serum tube is of concern, because the resource will be used up quickly. For the National Health and Nutrition Examination Survey (NHANES) bank, five 5ml tubes are collected. Serum will be depleted quickly if only tube is collected. At least two should be collected, because that resource is very valuable and people will want serum to perform antibody tests.

Should urine be collected?

VOTE: Continue to Collect Urine	
Support With No Major Concerns or Reservations	10
Support With Major Concerns or Reservations	1
Do Not Support	1

- Over the last 25 years, NHANES has received only 4 or 5 proposals requesting urine but over 100 requesting serum and plasma. Another problem is that it is a large collection to store.

- ALSA is funding a study on urine and based on the data, it looks extremely exciting that there might be a marker in the urine. It is unclear yet whether this will be validated, but it is looking quite remarkable at the moment. With that in mind, if it is not expensive to collect it collection should continue. One thing to keep in mind is the timing of the sample, and that a sample might not be useful if it is not collected correctly. However, this can be adjusted.

- Preliminary results from this study were presented during the International Symposium on ALS-MND in Brussels, and it is worth following up. However, to follow up on a really exciting result like that requires a large urine collection. This is the first urine study that has looked at all promising.

- One reason for not taking urine out is not knowing how useful it might be, but it is cheap and easy. Participants are being asked to do the urine specimen collection themselves and have it ready.

- Metals can also be measured in urine.

- The urine containers used in the pilot study were metals free. The kits were sent several days ahead of time with instructions about how to open the kit, how to collect the specimen, that it should be done first thing the morning of the appointment, and that it should be refrigerated. On the form, the phlebotomist fills in the time the specimen was collected.

Should hair/nails be collected?

VOTE: Do Not Continue Routine Collection of Hair and Nails	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- While these are cheap to collect, but they have to be stored and catalogued.
- There was concern that analytically this is very expensive, but investigators would have funding to do this.
- Rather than just collecting these samples routinely for the biorepository, a call could go out through the registry for a very specific reasons.
- If the existing samples are depleted, collection could be resumed.

Under what circumstances should saliva collections be completed?

VOTE: Continue Saliva Collection	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- Collecting 300 saliva samples per year would be advantageous in terms of increasing the number of people for whom there is DNA. If the primary purpose is to get DNA on more people, a two-tiered approach could be used in which blood is collected on some people so that there also RNA, serum, plasma, and cells for cell lines. Another group of people would just be offered the saliva kit, which could be mailed both ways and is very inexpensive.
- The average yield of DNA from the saliva kit seems to be sufficient.
- The answer seems to be that saliva should be collected when blood cannot be, and that collection of saliva alone from several hundred additional people per year would increase the number of people for whom there are DNA samples.

What quality assurance testing should be done during processing of specimens?

VOTE	
Support With No Major Concerns or Reservations	N/A
Support With Major Concerns or Reservations	N/A
Do Not Support	N/A

- No vote was taken on this topic.
- QA should continue on DNA and RNA.
- The tricky part about this is that most QA is related to what an investigator wants to study.
- Consult with Dr. Bowser about this.
- Dr. Bruijn will provide further information.

Should post-mortem collections continue?

VOTE: Continue Routine Brain, Spinal Cord, & CSF Post-Mortem Collection	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- Post-mortem offers huge opportunities, so it should be continued.
- This is an extremely valuable resource that is quite rare. If there is an opportunity to enhance this collection, it should be done.
- The rationale for not continuing to collect bone and muscle is that it seemed to be an add-on, and it is not clear what investigators might do with this. Metals analyses could be done on the bone.
- The muscle is particularly interesting. If it is an easy biopsy to collect, it certainly is intriguing as a potential biomarker opportunity. Some targets have been identified this way, and though they have not yet been proven to be good, it is worth pursuing. The studies conducted have been on fresh muscle, so it is not clear whether the samples stored in formalin would be useful.
- Collecting bone and muscle does not add a lot of time to the process.
- Though the samples are currently formalin fixed, they do not have to be. They can be frozen or they can be shipped fresh. The question regards whether muscle from end-stage disease is as relevant as muscle biopsies from earlier stage disease.
- Because this is an asymmetric disease that can involve four limbs, so taking one muscle may not be sufficient. It might be beneficial to standardize which muscle and which limb. It may be necessary to take four samples from one person, one from each limb. A muscle from each segment could yield some insight.
- There seems to be agreement to continue to collect brain, spinal cord, and CSF samples routinely and to leave the question open for muscle and bone but not to collect those routinely unless there is some indication to do so.

Should the development of new fibroblast cell lines continue?

VOTE: Continue to Develop New Fibroblast Cell Lines	
Support With No Major Concerns or Reservations	12
Support With Major Concerns or Reservations	0
Do Not Support	0

- Development of new fibroblast cells lines should continue.
- It seems most sensible to aim for creating cell lines from blood.

Maintenance and Distribution

McKing recommended maintaining the specimens in a private laboratory, and integrating distribution of specimens into the biorepository operation, including: facilitating the review process of applications for the specimens, maintaining the inventory of available specimens, and retrieving and shipping specimens to approved researchers. Approval could be sought to charge a minimal fee for retrieval and shipping and custom dissection of brain or spinal cord. Input included the following:

- Adding a marketing component is absolutely critical.
- Create a catalog with links to the catalogs of other biorepositories. That should include samples that could be used as control groups as well.
- NHANES samples are stored with a commercial repository and in CASPIR. NHANES uses 26 laboratories that give results to participants and send them to NHANES as well. NHANES has over a million samples, and charges $8.90 per sample requested. CASPIR has asked whether they can add on a couple of dollars, which is probably possible. *Federal Register* notices were sent to the public to ask for comments, but people did not complain.
- Although CASPIR is not currently charging CDC programs, that is going to change. Wherever the samples are stored, there will be an associated cost.
- In terms of access, it would be very helpful to work closely with other repositories regardless of whether there are charges because NIH, for example, will fund the cost of the assays through grant funding. There has to be a reasonable rationale for how costing is determined.
- In the review process, if an investigator is requesting samples and needs a particular number from another repository, it would be good to be engaged to make sure there are no delays from one group versus another because of processes and systems.
- To ensure effective utilization, it is important not only to have an inventory, but also to have someone who will work very closely with the investigators in helping them to refine their requests. Often, investigators are not sure what they need, what is available, or how to design their study. They need that type of one-on-one interaction before they submit their application, so that should be built into the project.

Closing Remarks

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Dr. Kaye thanked everyone for their attendance, participation, and helpful comments. She indicated that she would probably be calling many of them over the next few months as McKing begins to wrap up and assemble the recommendations. She thought the meeting had been very helpful. She acknowledged the other McKing team members in the room, and stressed that the pilot project has been a great experience and hopefully will be a valuable resource for researchers moving forward.

Dr. Horton expressed appreciation for everyone's time. ATSDR is looking to the experts for input regarding not only the biorepository, but also pertaining to other components of the National ALS Registry. He invited them to reach out to ATSDR at any time. ATSDR values the relationships that they have with each of them and their organizations. He wished everyone safe travels, and officially adjourned the meeting.

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