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

## National ALS Biorepository Pilot Study Update

**August 14, 2014**
**Summary Report**

## Table of Contents

## 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 |
| BCBC | Beta Cell Biology Consortium (NIH) |
| CDC | Centers for Disease Control and Prevention |
| CSF | Cerebrospinal Fluid |
| DP | Dementia Pugilistica |
| FTLD | Frontotemporal Lobar Degeneration |
| HIRN | Human Islet Research Network |
| IRB | Institutional Review Board |
| JDRF | Juvenile Diabetes Research Foundation |
| MDA | Muscular Dystrophy Association |
| NCI | National Cancer Institute (NIH) |
| NDRI | The National Disease Research Interchange |
| NEALS | Northeast Amyotrophic Lateral Sclerosis Consortium |
| NEI | National Eye Institute (NIH) |
| NHGRI | National Human Genome Research Institute (NIH) |
| NHLBI | National Heart, Lung, and Blood Institute (NIH) |
| NIAID | National Institute of Allergy and Infectious Diseases (NIH) |
| NIAMS | National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIH) |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases (NIH) |
| NIH | National Institutes of Health |
| NIMH | National Institute of Mental Health (NIH) |
| NOK | Next of Kin |
| ORDR | Office of Rare Diseases Research (NIH) |
| ORIP | NIH Office of the Director |
| PFP | Periodate-Lysine-Paraformaldehyde |
| PTSD | Post-Traumatic Stress Disorder |
| QA/QC | Quality Assurance/Quality Control |
| SOP | Standard Operating Procedure |
| UAGA | Uniform Anatomical Gift Act |
| 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 Update**

**Minutes of the Meeting**
**August 14, 2014**

## Purpose

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

## National ALS Biorepository Pilot Study: Progress to Date

**Wendy E. Kaye, PhD**
**Senior Epidemiologist**
**McKing Consulting Corporation**

Dr. Kaye thanked everyone for attending the meeting, including those who stayed following the annual Amyotrophic Lateral Sclerosis (ALS) meeting. The hope was that it was helpful to coordinate the two meetings so that people only had to fly to Atlanta once.

As a reminder, Dr. Kaye 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 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.

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:

NATIONAL ALS BIOREPOSITORY PILOT STUDY

**Biorepositories in ALS Research**

| 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 was to correlate biomarkers with extensive epidemiologic data collected by the 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 Registry is a rich data source that will allow for the linkage of analyses that may be performed on specimens to the epidemiological data. The Registry enrolls a nationally representative population-based sample of participants. That is, while ATSDR does not have any control over who registers, everyone in the United States (US) is eligible so the registry is not limited to a specific clinic or certain types of services such as being served by the Department of Veterans Affairs (VA). This will increase the number of specimens available for research on patients with ALS in the US.

The goal of this study is to pilot methods for collecting and banking biological specimens from participants in the Registry in order to assess the potential for developing a comprehensive, national research resource associated with the Registry. The objectives of the pilot study are to maximize scientific potential, given the 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 such things as the sample size, 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 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:

NATIONAL ALS BIOREPOSITORY PILOT STUDY

## 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.

Participant input resulted in the priorities being blood, urine, nail clippings, hair clippings, and saliva from people for whom blood cannot be obtained. Blood was the highest priority for specimen collection, and 5 tubes of blood will be collected in the order shown in the following table:

NATIONAL ALS BIOREPOSITORY PILOT STUDY

## In-Home Collection

| Collection priority | Sample preservative | # tubes | ml / tube | Fractions | Potential analyses (examples) |
|---|---|---|---|---|---|
| **Blood** | | | | | |
| **1** | $K_2$EDTA | 1 | 10 | White cells (buffy coat), red cells, plasma | DNA, proteins, red blood cell lipids |
| **2** | $K_2$EDTA | 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 are to be drawn in the home, which is why skin biopsies are 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, to take part in the study, participants must be enrolled in the Registry. A potential participant must have agreed to be contacted about ALS studies through the Registry, and must be willing to have someone come to his/her house for specimen collection. Any stage of disease progression was permitted.

Because ATSDR was trying to test the feasibility, recruitment was proportional to the population. Individuals were selected from Registry based on state. An email announcing the project was sent by the 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.

A package of information was sent to anyone expressing interest in the project, with follow-up with potential participants about one week later to answer questions and go over the consent form. Originally it was thought that this would take two calls, but most people had read the package and understood it. Therefore, this was done all in one process. 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 orders 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 filled in a 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. The form was to be sent along with the kit. However, there were a number of problems with this, including the following:

- ❑ All dates were not filled in
- ❑ Forms were cut into pieces and attached to the tubes
- ❑ Answers on the form sometimes did not agree with the specimens received
- ❑ Questions were misinterpreted (When did you last drink? 2009)
- ❑ Questions on hair and nails were skipped

Some suggested revisions to the form have been made and it will be revised if the project goes forward.

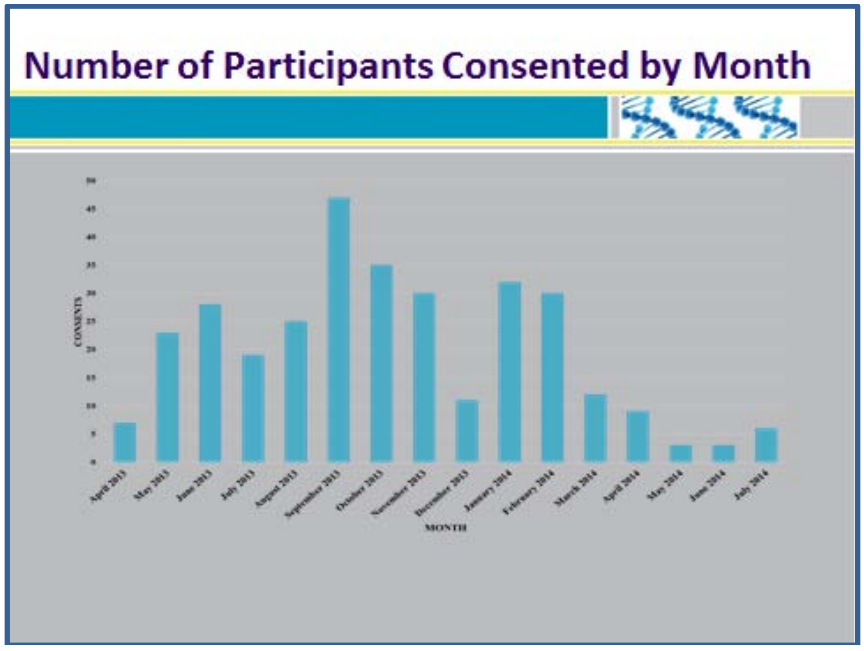
Collections were only supposed to be done Monday through Thursday, because they were to be done in time to be shipped by FedEx the same day for next day delivery. McKing sent letters to confirm appointments, and calls were made to phlebotomists to answer questions. Phlebotomists were also

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. They thought that there would only be problems with this in more rural areas, but that was not the case. In some larger metropolitan areas, like Los Angeles for example, people do not want to deal with traffic and want to stay within their part of town. Multiple providers were used in order to find and get coverage in rural areas and some cities. Each phlebotomist had to meet minimal standards, and McKing provided training materials for collecting and shipping specimens. The companies went over these with the phlebotomists and attempted to make sure that they understood the project.

As noted, collections were supposed to occur on Monday through Thursday only, and specimens were supposed to be shipped overnight to the lab. Once in the lab, blood and urine aliquots were created and hair and nails were stored ambient on the shelves. But, what can go wrong does go wrong. Some phlebotomists confirmed the night before, but still did not go to the appointment. Some phlebotomists rescheduled appointments without consulting McKing or the phlebotomy company they worked for, and rescheduled appointments to a day or time when next day FedEx delivery to the lab was not possible. One phlebotomist changed two appointments to Saturday because his work schedule changed, which was against all of the policies. Some phlebotomists took specimens home and placed them in the refrigerator instead of shipping them to the lab. At least they put them in the refrigerator unlike one phlebotomist who left the specimens at the facility and did not call for pick up. They were found a week later behind someone's desk in an assisted living facility. Some phlebotomists acted unprofessionally, some were not well-trained, and others did not include or did not accurately complete the specimen collection form.

There were a number of other challenges as well. At first, there was a slow response to recruitment emails and the Registry only has emails. Therefore, the Institutional Review Board (IRB) protocol was amended to make emailing more frequent because people were not checking their emails regularly. Some potential participants did not want people coming to their homes. Finding reliable phlebotomists across the country was, and remains, a challenge. High summer temperatures required changes in packing/shipping procedures.

The following graph shows the number of people consented by month, but it is important to note that consents do not always turn into someone who provides a specimen:



There was a large group of consents last September, but the reason there are now so few is that the end of collection is drawing near. Only 5 to 10 more people are needed for the study to be full. Recruitment began in April 2013, with the following collections made as of July 31, 2014:

- 320 consented
- 303 have had their first draws completed
- 192 have had their draws completed
- 29 withdrew after their first draw (21 deceased, 8 not interested)

Participants have now been consented from every state. Dr. Kaye reminded everyone that since a geographic distribution of the population is needed, she wanted a map with pins in it the old fashioned way so that she could walk by it every morning to see the status. Pins are inserted into the map when the bloods are processed and are back in the lab, and:


There were some eligibility criteria for postmortem collection that were above and beyond having to be in the Registry. There was an effort to recruit people whose disease was more progressed, but who were cognitively able to sign a consent form. To be eligible for postmortem collection, persons had to be enrolled in the Registry, they had to agree to be contacted by the Registry about research studies, and they had to sign a Health Insurance Portability and Accountability Act (HIPAA) Authorization to permit McKing to contact their neurologist. There were also discussions with treating neurologists to determine whether they thought there may be any family dynamic issues that would not make someone a good candidate. The family member takes on a major roll in the project because they have to call McKing to put this in motion when a person passes away, so they have to be on board. A signed family authorization is collected, which is non-binding, but states that the family member understands what their family member wanted and what they are supposed to do.

Recruitment was similar, with an email sent by the 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

the alert on the ATSDR website. 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 schedule an in-home appointment with the participant and the family member to obtain informed consent. Consenting of participants for this study component was done in his/her home. A call is made quarterly to assess disease progression, and an effort is made to time those calls with their visits to their neurologist. An attempt is made to be proactive in terms of when a collection may need to be done. The original protocol included brain, spinal cord, CSF, muscle, and bone. Skin specimens were added at a later date. Therefore, it was necessary to re-consent participants for skin donation, which was done over the phone. Some of the people who were recruited for the in-home collection portion decided they wanted to be involved in the postmortem component as well and vice versa, so everybody who is in enrolled in the postmortem component is also enrolled in the in-home collection component. Therefore, there will be blood, urine, hair, nails, and postmortem specimens for these participants.

The following is a picture of the postmortem collection kit:


One of the challenges with postmortem collection was assessing eligibility and obtaining consent quickly. At least one or two people were interested in the project passed away before they could be consented. Having to re-consent everyone for the addition of a skin specimen was time-consuming and somewhat complicated. Working with participants to make final arrangements has challenges as well. It is helpful to know if the collection can be done at the facility, or if another facility is needed for the collection. Funeral arrangements must have been made in order for McKing to know who to contact. A few participants have not made any arrangements. It can also be challenging to find dieners in remote locations, and assure that they are available at all times. Designing kits for additional specimen types also posed challenges.

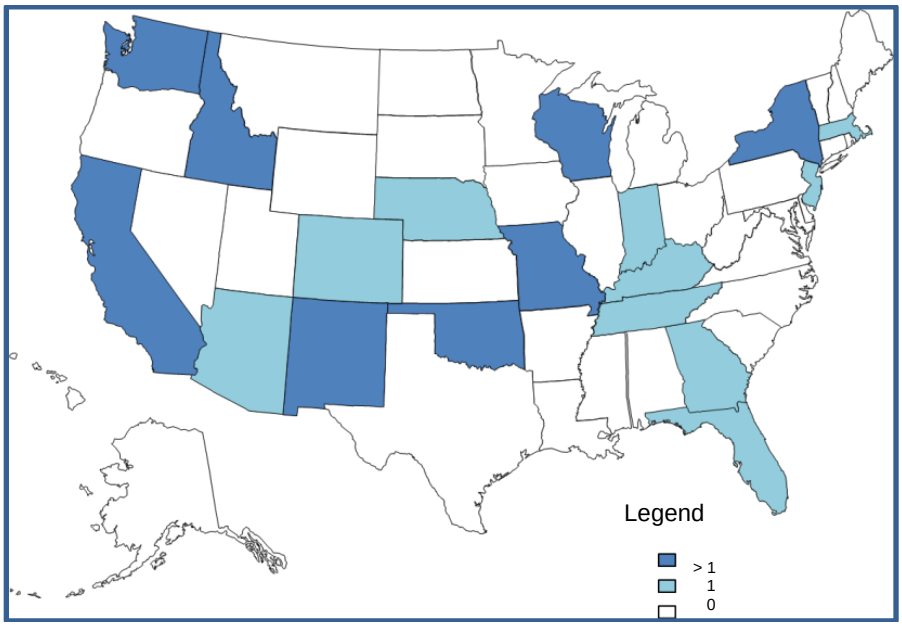
Recruitment for this component of the project began in April 2013. As of July 31, 2014, 30 participants had been consented and 5 collections had been completed, which resulted in the following specimens:

- ❑ 5 brains and spinal cords
- ❑ 5 muscle samples
- ❑ 5 bone samples
- ❑ 5 CSF samples

- ❑ 3 skin samples (0 cell-lines)

In terms of the skin samples, the first one grew cells in the lab but then succumbed to some type of mold and had to be destroyed before the specimen could cross contaminate other specimens in the rest of the lab. The group instituted a more rigorous cleansing process before trying to isolate the fibroblasts; however, the cleansing process went a little too far and killed all the cells. The dieners are cleaning the specimens before they put them in the kits, and they are going to divide the next couple of sets in half and try two different cleaning methods on them to determine what works best.

The distribution of participants throughout the US shows that not all participants are from the same state or from cities. The following map shows a distribution of who is participating in this project, with more than one participant in the darker blue states and one person in the lighter blue states:




In terms of other project activities, governance of specimens is an important issue. The goals of governance are to 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. All of those need to be finalized so that they are available as soon as the specimens are available. The idea was that there would be a review panel to assess the requests to use the specimens that would be very similar to the procedures now being used to evaluate research proposals for sending out emails about a research study. Membership and procedures would be based on guidance for the National ALS Research Committee established by ATSDR, and the members could vary depending upon the type of research. Additional paperwork must be developed to deal with investigator obligations such as Confidentiality and Data Use Agreement, Material Transfer Agreement, and project updates.

The study team was also asked to assess long-term storage options. This project is just to test the feasibility of collecting and processing these specimens. It does not include long-term storage or filling requests for specimens. The Project Manager, Maggie Ritsick, and another Public Health Advisor are in the process of conducting a number of interviews to try to obtain additional information that can be written up about this. So far they have spoken with representatives from biorepository programs at CDC and the National Institutes of Health (NIH). They are contacting various organizations that have biorepository capabilities, which include the CDC repository, commercial

organizations, academic institutions, and other organizations. The idea is to determine and make recommendations about what type of organizations are best-suited to store National ALS Biorepository samples. This is not meant to recommend a particular institution. It will be to recommend the types of institutions and the pros and cons of using a private laboratory versus storing them in the CDC biorepository facility.

Next steps are to continue to recruit participants, continue to complete second collections, continue quarterly contact with postmortem participants, maintain contact with subcontractors to assure implementation of the study, and develop the final report and recommendations for long-term implementation of a biorepository. There are currently 303 participants and the study team is permitted to collect 600 specimens under its contract. Because such a large number of people have been unable to do their second collections, a request was made of the IRB for permission to increase the participation for the purpose of having more pairs. Basically, there will be 600 specimens, but there could be 330 for which there is one specimen and 300 for which there are two specimens on due to attrition. Therefore, a few people are being recruited to fill in behind those. In essence, every time two people cannot do their second draw, one more person can be added to the study.

The team members are as follows, and Dr. Kaye requested that those present stand to be recognized:

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

The ALS Biorepository contact information is as follows:

http://wwwn.cdc.gov/als/ALSBioRegistry.aspx
or call 1-855-874-6912

**Discussion Points**

Dr. Bradly requested additional information about how the postmortem specimens are being prepared and stored.

Dr. Kaye indicated that Dr. Stein would give the details of this during his presentation.

Dr. Mitsumoto wondered if there was any value in separating the plasma prior to overnighting it and having it done in the lab.

Dr. Bowser agreed that this is an issue, and he asked whether records are kept at least on the time from collection to freezing (e.g., how many hours).

Dr. Kaye responded that it would always be at least 24 hours. They know when the specimen was drawn.

Dr. Bowser pointed out that research-wise, they would not be able to use these specimens for proteomic, metabolomic, and other studies. Having the time information is critical, because that would at least allow the researcher the opportunity to say he/she could not use the specimen because the time was too long.

Dr. Mitsumoto said that was the reason they decided not to do blood, because someone has to spin and separate the plasma ideally.

Dr. Bowser said that he and others who have published on this and looking at stability for proteins and the metabolome over time determined that it is about two hours. If it is not processed and frozen within two hours, artifacts will be created in all studies.

Dr. Kaye pointed out that in some of the places she had been, she could not get to a centrifuge in two hours.

Dr. Bowser stressed that the problem will be if a scientist receives 100 samples from this repository and another 100 samples from the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS) repository that has standard operating procedures (SOPs) that everyone follows in a certain way, they will get different data. The end result of their study will be unclear, and they may not know why.

Dr. Kaye reminded everyone that these issues were raised during the last meeting, and there will have to be continued discussions about what additional information will need to be made available about the specimens and what additional data needs to travel with the specimens.

Dr. Mitsumoto noted that CNS collection and autopsy are probably separate. For example, the patient and family consent. He wondered if that was enough. In New York State, for example, a spouse or first degree relative has to sign after the patient dies.

Dr. Kaye said they have found the first person consent trumps everything.
Dr. Bowser said there are state laws for these issues. He has done autopsies in about nine states, and different states have different rules even after death.

Dr. Kaye said she understood that, but no autopsies are being done on these participants. They are asking for consent for participants to give their brain to the study when they die.

Dr. Bowser emphasized that an autopsy has to be done to collect a brain, and that different states have different rules on consenting for such a procedure. Some states are fine with a signed consent prior to death, others require next of kin consent after time of death, and some states require next of kin after death that is witnessed by somebody. He cautioned being careful about how they are doing this around the country.

NDRI who is coordinating the postmortem collection keeps track of all state requirements and makes sure they are met for each donation.

Dr. Mitsumoto said they decided not to collect plasma because there is so much involved and it costs more, but he wondered if they could ask the study team to collect samples for their patients through the ALS Repository and get access to those samples for their study.

Dr. Kaye said she did not think so, and while it might be possible to figure out whether they shared a participant, the study is now full and will take only 5 to 10 more people. If the biorepository goes forward, there may be a way to leverage it to get blood drawn through the biorepository and then link it.

Dr. Horton added that the pilot study ends in the fall of 2015. If ATSDR moves forward with a full-blown biorepository, they could discuss this with Dr. Mitsumoto.

Dr. Boylan inquired as to how much time there is between the first and second blood draws. He also wondered whether there had been any interim analyses to determine where in the disease stage

participants are in terms of whether they are getting people who are more advanced or late-stage disease, which could explain attrition between the first and second draws.

Dr. Kaye indicated that there are six months between blood draws. For the in-home portion, anecdotally she said she thought that a number of people have been happy to be able to participate because they are not eligible for other studies due to advanced disease. Many people have also been happy with the in-home component because mobility is an issue, so she is thinking that they may be at a later stage.

Dr. Boylan suggested taking disease duration into consideration for the future in order to ensure that the second collection is obtained, and because it would provide a somewhat more uniform set of samples in relation to the disease process. He said he also remembered that the centrifuge issue was raised about the samples. In a clinical trial that was done, the contract phlebotomists had portable centrifuges with them and were spinning at the site and doing the cool down at that time specifically because of that issue. These were relatively portable, table-top centrifuges.

Dr. Kaye said that for the 303 people, she would guess that they had 280 phlebotomists.

Ms. Bledsoe asked what types of quality assurance/quality control (QA/QC) studies are being done on the samples for the in-home collection, and who is doing the consenting for the postmortem collection.

Dr. Kaye indicated that McKing has a Social Worker who goes to everyone's house to consent them for the postmortem study. No QA/QC studies are being done with the specimens. All that is being done is processing and freezing. The contract did not incorporate or provide funds for anything beyond this. There are temperature loggers and the data collection forms, but no analyses are being done on any specimens. In this case, unlike the NEALS model, one lab is doing all of the specimen processing of the blood and urine.

Ms. Bledsoe expressed concern about what happens before the samples get to the lab facility, and was surprised that funds were not built in for that. It is going to be critical to know whether the samples are being collected in the right way before scaling up.

Dr. Bowser agreed that having some QC would be critical. It is a great study in that feasibility to collect samples in homes throughout the country has been demonstrated. Because that by itself is quite challenging, it is good to know that it can be done. However, before ramping up to conduct a large study, it is important to know the quality of the samples. They do not want to end up with "garbage in/garbage out" to the scientists. There are some ways to do QC and that would be important to know.

Dr. Kaye responded that unfortunately, nobody mentioned that when the protocol was being developed during the meeting in 2012, so it was not included.

## Partner's Progress and Challenges

### Collection Kits, Processing Samples, and Storage

**Scott Hixon**
**Area Director, Technical Services**
**Fisher BioServices**

Mr. Hixon indicated 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 in 50 countries. The

company has four premier brands: 1) Thermo 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 is the leader in critical biological material management; has biorepositories in several countries, a number of which are in the US; and specializes in cold chain logistics. Fisher BioServices can provide global market solutions, including biobanking and biorepository, cell therapy solutions, clinical trial sample management, biological API management, clinical trial kit production such as those for the ALS Bioregistry, laboratory processing, qualification/ validation, and cold-chain logistics. Currently, Fisher BioServices has over 1000 liquid nitrogen shippers in the field, so they are probably are one of the largest liquid nitrogen shippers in the world. They can ship everything from ambient temperature to liquid nitrogen temperatures.

Fisher BioServices has an information management system that 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, including custom temperature. Fisher BioServices provides a large distribution channel through which samples and kits can be distributed, and all of those can be linked together.

Fisher BioServices has a global footprint, with 170 million+ samples stored globally. Fisher Clinical Services Depot locations are used for Fisher clinical services as a means of getting material in and out of countries. Biorepositories are located in the US, Switzerland, the United Kingdom (UK), South Africa, and Singapore.

Fisher BioServices manages some very large clinical and cohort studies, one of which is the City of Hope. This study expands the original study that included over 133,000 female active/retired public school professionals in California. The biobank is estimated to include 300,000+ samples. Similar to the ALS study, the City of Hope study is conducting in-home collections. Samples require laboratory processing within 24 hours after collection, and clinical sample collection kits have been produced for phlebotomists. Those samples are being sent back to Fisher BioServices for processing as well.

Another example is the National Children's Study, which is a national longitudinal birth cohort observational study that is the Congressional law part of the Children's Health Act of 2000. This is a study of more than 100,000 children across the US to examine the effects of environmental factors on health (e.g., air, water, diet, sound, family, community, culture) that will track these children for 20 years. A variety of samples are being collected, processed, and stored (e.g., environmental, urine, cells, blood, dust, carpet, and other samples), which will be sent to Fisher BioServices.

Fisher BioServices' role in the ALS Biorepository study is to create collection kits at the direction of McKing in terms of acquiring the supplies and assembling and distributing the sample collection/shipping kits. As Dr. Kaye mentioned, there are various types of kits for this study. The kits contain collection supplies, pre-paid air bills, collection forms, and phlebotomist guidelines. The air bills that are included in the kit are partially filled for ease of shipping.

Laboratory services are also provided once the collected kits arrive at Fisher BioServices, including the following separations:

Blood Processing

- ❑ 10 ml EDTA: plasma, red blood cells, buffy coat
- ❑ 6 ml EDTA: plasma only (CDC tubes)
- ❑ 10 ml red top: serum

Urine Processing

- ❑ 10 x 1ml in CDC screened vials
- ❑ 1 x 4.5ml with mercury additive (CDC vials)
- ❑ 3 x 10ml (CDC vials)

Repository services for the ALS Biorepository study include secure storage and management at -80 degrees of the samples collected (e.g., blood, urine, saliva, bone and muscle, nail, hair). The Inventory Management System captures, stores, and manages all data associated with the samples related to collection, processing, and storage.

Some of the challenges faced in Kit Production, the first set of kits produced were ID-specific, and had a bar code associated with them but do not get assigned to the patient until they are 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 visits 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 of between the two draws. 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.

In terms of challenges faced during the receipt and inventory of samples, inconsistent information was provided by phlebotomists as Dr. Kaye 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 no dates are 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, by making some changes to the field 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, there were two different 2mL vial types (Nunc and Nalgene) 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. Currently, this requires splitting the processing into separate functions. Additionally, the 2mL Nalgene tubes come with the caps separated. If they were together, it would save time in the lab when processing samples. In addition, CDC pre-screened transfer pipettes were used for creating some of the samples and these can only be processed manually. Currently, the processing of one kit takes approximately 40 to 45 minutes, with each additional kit adding 5 minutes. If the 5mL serological tips were per-screened by CDC and used for all aliquots, it would save approximately 10 minutes for the first kit and 2 minutes each additional kit. The transfer pipette does not allow for accurate measurements. Volume has to be estimated by graduations on vials, which makes it necessary to go in and out of the parent container more times. If pipette tips were prescreened, Fisher could aliquot all samples on its automated Hamilton platform. This could significantly reduce time and labor.

This is a pilot project and when it is over, Fisher will have to figure out how to close out the project. After the termination letter is received, Fisher will supply final reports regarding shipping, receiving, and processing. A determination also must be made about what to do in terms of material disposition. There will be CDC supplied kit components and the Biorepository samples stored with Fisher. The

options are for Fisher to continue to store them, ship them to another facility, or destroy them. Final invoices will be issued, and any additional activities will need to be discussed with the Project Manager and may incur additional costs.

## Discussion Points

It seemed to Dr. Brady that pre-screening and metal in the tubes was adding a number of steps. He requested some background about whether this was standard for blood collection and why the concern about metals was so high. He recalled discussing this previously, but could not remember the rationale.

Dr. Kaye reminded everyone that during the first meeting in 2012, the people interested in being able to study environmental contaminants wanted to ensure that the materials were prescreened. Therefore, the tubes and anything near the tubes have to be pre-screened for metals. CDC screened for 15 metals and certified them. These are done in lot batches, and they have an arrangement that they purchase x number of tubes, they receive 100 out of the lot, and if the lot passes CDC testing, CDC buys the lot. If the lot does not pass testing, they go back into the general population. CDC has been doing this with tubes, tourniquets, gloves, wipes, pipettes, needles, et cetera.

Mr. Hixon added that Fisher is currently doing this for three other studies for which CDC is conducting pre-screening, and maintains an active lot that can be used for processing in addition to some retention samples of tubes that have been pre-screened for future analyses if necessary. For example, 100 vials may be set aside in Fisher's retention facility that will never be used. The other three studies are primarily automated processing, though some steps are manual for these. Everything is being pre-screened.

## Postmortem Collection

**John Lonsdale, PhD**
**National Disease Research Interchange (NDRI)**

Dr. Lonsdale indicated that NDRI's role is to acquire fresh, fixed, or frozen tissues from living and diseased donors and provide them to approved investigators as quickly as possible. NDRI is not a traditional biorepository and does not want to build up huge banks of tissues. To that end, NDRI's mission statement is as follows:

> *To advance disease research through the procurement, preservation, and distribution of human cells, tissues, and organs.*
>
> *To provide researchers on the cutting edge of disease research with the biomedical resources that are essential for their work.*

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. Since 1980, the organization has served more than 5,500 discrete investigators with over 300,000 biospecimens. The work of these investigators is reflected in acknowledgements that NDRI has received in over 2,700 peer-reviewed publications.

For over 30 years, NDRI has been funded primarily by NIH. Approximately 65% of NDRI's total funding comes from the federal government, most of which is in the form of a grant from the NIH Office of the Director (ORIP). Independent funding also comes from a variety of institutes and centers within NIH, including:

- National Cancer Institute (NCI)
- National Eye Institute (NEI)
- National Heart, Lung, and Blood Institute (NHLBI)
- National Human Genome Research Institute (NHGRI)
- National Institute of Allergy and Infectious Diseases (NIAID)
- National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
- National Institute of Mental Health (NIMH)
- Office of Rare Diseases Research (ORDR)

Additional funding comes from the corporate from the pharma/biotech industry. In the last 5 years, NDRI has provided tissues to about 330 for-profit organizations. That is allowed for in the various consent forms that NDRI uses.

Examples of special initiatives in which NDRI has been involved include the following:

- ATSDR/Boston VA ALS Projects
- Beta Cell Biology Consortium/Human Islet Research Network (BCBC/HIRN) Islet Project
- Cystic Fibrosis Foundation Collaboration
- Genotype-Tissue Expression Project
- Joslin Medalist Diabetes Project

With appropriate consent, NDRI can collect every tissue in the human body. Various types of biospecimens are collected, including:

- Normal
- Diseased (common and rare)
- High quality, low-PMI organs and tissues
- All tissues, including brain/CNS
- Cancer/normal adjacent
- Cord blood/placenta/umbilical cord
- Isolated normal /T1D/T2D pancreatic islet cells

NDRI acts as a matching service for donor referrals that come in through a massive procurement network of partners comprised of 240 sites that are then matched for the researchers in NDRI's database. The breakdown of the 240 sites is as follows:

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

These sites are strictly regulated, so the consent forms that NDRI and they use all follow the hierarchy of the Uniform Anatomical Gift Act (UAGA) and any state and local regulations.

NDRI began working on the ALS Bioregistry project in April 2013, and engaged in a weekly project teleconference with the McKing team. Early on there was a lot of effort in starting to build up a bank of consented participants. In terms of the donation plan, McKing tells NDRI about prospects prior to consenting and NDRI does 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 contracted recovery specialist.

There is a lot to put in place prior to the recovery, so the more than can be put into place prior to a participant's death, the greater the chances of success. NDRI contacts the recovery specialist and funeral home to discuss and arrange recovery logistics, including equipment availability, and NDRI sends them kits. McKing and NDRI developed a long and complex SOP, which drives the entire process from the dissection through to the packaging, preservation, and shipping. Periodically, NDRI re-contacts the recovery specialist and funeral home to touch base because potentially 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. That donor is basically in the bank at that point.

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 polystyrene 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 cool and cushioned in the bucket. On top of that, 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 gets 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 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.

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 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, 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. 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.

Currently, there are 30 consented donors and 25 pending recoveries. There are 25 assigned recovery techs, 2 pending kit shipments, and 20 confirmed funeral homes (5 have not been provided by the family yet). From the 5 completed recoveries, there are 5 whole brain and spinal cords; 4 CSF, bone, and muscle (kits were not available for 1); and 3 skin (later addition to recovery protocol, needed re-consenting).

## Discussion Points

Regarding preparation of the brain and spinal cord, Dr. Bradley requested clarification regarding whether the whole brain is frozen or if any is prepared for histology directly.

Dr. Lonsdale replied that what goes in the NDRI box is fresh and not preserved.

Dr. Bowser noted that Dr. Lonsdale nicely described how the brain is carefully placed in the box and maintained in a cool position for shipping, but it was not clear to Dr. Bowser where the spinal cord is placed.

Dr. Lonsdale clarified that once the final round piece of foam has been placed on top of the brain, the spinal cord is coiled in a bag and that sits flat on top of the last round piece of foam. It basically sits inside at the top of the bucket. The key is to make sure that there is no crimping and no pressure.

Dr. Mitsumoto asked what a cool pack is and the temperature of the cool packs, noting that the brain is a huge substance and it takes a long time to cool the core.

Dr. Lonsdale responded that the cool packs are basically large gel bricks that are pre-cooled to

-20degrees for at least 3 hours before going into the bucket. The brain is basically being cooled at the fastest rate possible given the temperature dissipation from the cool packs. The kit has been validated to ensure that fresh brains are delivered within temperature parameters without getting anywhere near the alarm temperatures.

Dr. Bradley inquired as to whether they had any data on how rapidly the center of the brain is cooled down to below -10.

Dr. Lonsdale said that he did not.

Dr. Boylan inquired as to where the spinal fluid is obtained.

Dr. Lonsdale indicated that the spinal fluid is collected from the cervical region just before the brain is recovered.

## Brain, Spinal Cord, and CSF Processing and Storage

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

Dr. Stein discussed what occurs when Boston University School of Medicine receives postmortem tissue (e.g., brain, spinal cord, and CSF). Boston University has a number of brain banks through a number of affiliations, including the following [*nationwide referral base, most cases couriered from distant sites]:

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

Functions of the ALS biorepository in Boston include assignment of a code number, collection of some basic information (e.g., age, sex, postmortem interval), and then immediately processing of the specimen, 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 somewhat easier to see 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:

## Regions frozen and fixed

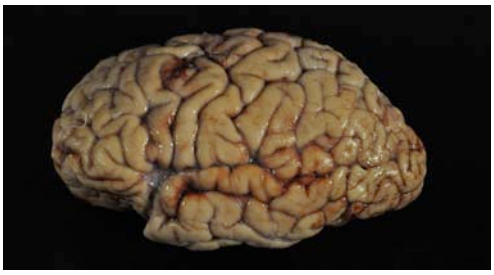
1. Olfactory bulb
2. Midbrain at level of red nucleus
2A. Midbrain at superior cerebellar peduncle
3. Precentral, postcentral cortex (BA 4,3,2,1)
   - 3A Leg
   - 3B Arm
   - 3C Face
4. Inferior parietal cortex (BA 39,40)
5. Anterior cingulate (BA 24)
5A. Superior frontal (BA 8,9)
6. Inferior frontal cortex (BA 10,11,12)
7. Lateral frontal (BA45,46)
8. Caudate, putamen, and accumbens (CAP)
9. Anterior temporal (BA 38)
10. Superior temporal (BA 20, 21,22)
11. Amygdala, with entorhinal cortex (BA 28)
12. Globus pallidus, insula, sub. innominata
13. Anterior hippocampus
14. Hippocampal formation, lateral geniculate
15. Superior temporal posterior (BA 41,42)
16. Thalamus with centromedian nucleus

16A Thalamus with subthalamic nucleus
17. Posterior cingulate (BA23,31)
18. Calcarine cortex (BA 17,18)
19. Superior parietal cortex (BA 7B)
20. Upper pons (level of locus cœruleus)
20A. Lower pons at Vth cranial nerve
21. Medulla oblongata( including inferior olives)
22. Spinal cord
   - A-B Cervical
   - C-D Thoracic
   - E-F Lumbar
23. Cerebellar vermis
24. Cerebellum with dentate nucleus
25. area 19
26. Cerebellar tonsil
27. Pituitary
28. Anterior spinal roots
29. Posterior spinal roots

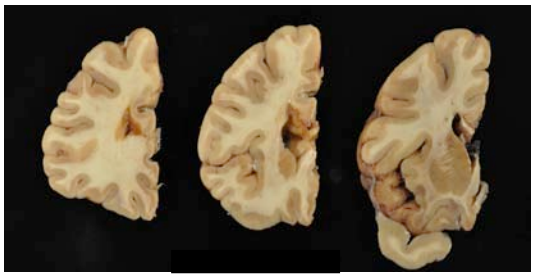
*Frozen sections & paraffin blocks; Remaining coronal slabs frozen

These are all made into paraffin blocks. 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.

Dr. Stein then reviewed their first case to illustrate what they do and the pathology that they see. 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 1200 to 1400 grams). His brain 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:

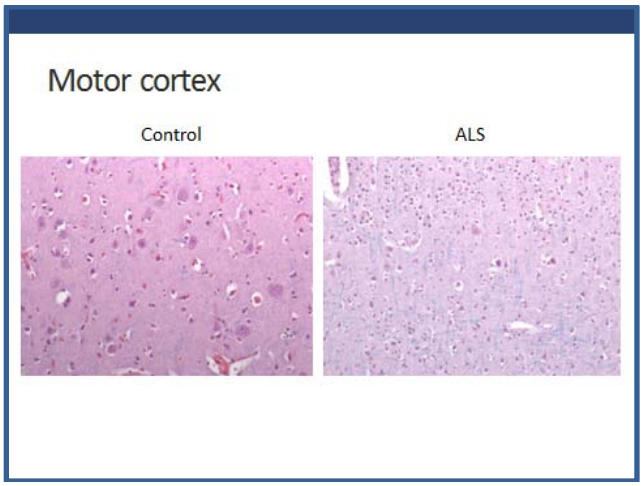


The coronal sections show an area consistent with a hemorrhagic stroke:

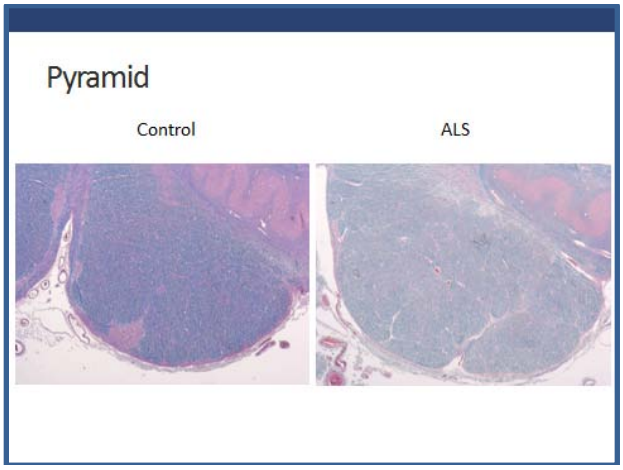


In the brainstem, there was a mild pallor in the nigra which can be seen in Lewy body disease. The spinal cord showed the classic picture of ALS, with marked degeneration of the anterior spinal nerve roots.

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

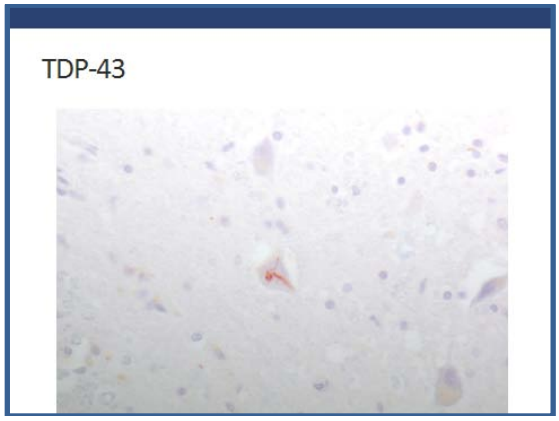



The following shows that the tracts coming down from the motor cortex are degenerated. This is the pyramid. In ALS, it is pale and degenerated:




In the spinal cord, the tracts are also degenerated, which is where it gets the name lateral sclerosis:


Almost all sporadic forms of ALS will have inclusions that are immune-positive for 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 neuropathological 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 semi-quantitative measures generated on various parameters (b-amyloid burden, NFTs, vascular disease, Lewy bodies, TDP-43) using standard published neuropathological criteria. The same is done for all of the Boston University brain banks, which allows for comparison across cases.

Returning to the case, this individual, like many cases, had a number of pathologies. He had the classic findings of ALS with upper and lower motor neuron degeneration with TDP-43 positive

inclusions. He also had significant vascular disease, neuropathological changes of Alzeheimer's disease, and Lewy body disease. This illustrates that it is rare to have a case with just one pathology, especially the older the individual is, and it is important to screen for additional pathologies.

The types of diagnoses Boston University has seen in its ALS Brain Bank include the following: ALS (or other), vascular disease, frontotemporal lobar degeneration (FTLD), Lewy body disease, a few cases of chronic traumatic encephalopathy (especially in the Veteran population, many of whom have had severe and repeated head injuries), and Alzheimer disease. For the five cases received so far for the ALS study, four have pathologically confirmed ALS and one is still being worked up. The average RIN is 5.4 and the average pH is 6.2.

In terms of future directions, a lot can be done and some of what is being done for some of Boston University's other brain banks can be leveraged. For the VA ALS study, a TissueMetrix database was used to track all of the specimens, their locations, and the various endpoints that are associated with them. Boston University is in the process of upgrading to the next version of TissueMetrix, which is a web-based, scalable database with storefront option for researchers that allows barcode labeling of specimens. Also for that study, all neuropathological data are recorded in a Microsoft Access database of semi-quantitative neuropathological measures in the event that an investigator is interested in knowing how many cases have TDP-43 pathology, for example. In addition, Boston University will engage in tissue distribution.

## Discussion Points

Dr. Bradley said he had been involved in both personally collecting brain and spinal cord specimens and also setting up brain banks, so he was very well aware of all of the problems of collection, preservation, and so forth. He congratulated Dr. Stein, emphasizing that this was really state-of-the-art work. As he understood it, the brains arrive to Boston University cool not frozen, and he wondered what their temperature was on average upon arrival.

Dr. Stein said that when NDRI initially implemented this, they included temperature monitors in some and he thought the temperature was approximately 6 to 9 degrees. Boston University has not monitored the temperatures. There have been some issues, so he and Dr. Kaye plan to monitor to see if they can get some better data.

Dr. Bradley noted that the RIN and pH were excellent, so the brains seemed to be coming through very well to Boston University thus far. In terms of the spinal cord, it is always a problem for dieners to get at the whole spinal cord. He asked whether Boston University was receiving the whole spinal cord or only portions.

Dr. Stein said that the amount of spinal cord they were receiving had been variable. The biggest problem has been to get cervical cord. In all cases, they have received lumbar and thoracic. Some dieners are better than others at handling spinal cords. The spinal cord is very delicate and has a toothpaste effect if it is squeezed at all. In a couple of the cases, they have received parts of the cord.

Dr. Mitsumoto asked whether the spinal cord was taken from the posterior or anterior approach.

Dr. Stein said he thought it depended on who did it. For most of these cases, it is just brain and spinal cord so that would be a posterior approach. If they were to do a full autopsy, an anterior approach would be used.

Dr. Mitsumoto noted that when a patient is deceased and a death certificate has to be filled out, it includes a question regarding whether an autopsy is done or not. He wondered how they were

dealing with this. If the patient's family wants an autopsy and brain samples, he asked whether the primary physician is contacted who is supposed to write the death certificate.

Dr. Kaye replied that while they do not contact the primary physician, they do tell families that if they want the results they may have them. Drs. Stein and McKee have offered to speak to the patients' families. So far no one has requested this.

Dr. Mitsumoto asked whether there was any specific histology for the chronic traumatic encephalopathy diagnoses, or if it was all based on history such as contusion in the past.

Dr. Stein responded that it was based on the pattern of tau pathology. It basically required pathology in characteristic areas, such as depth of the sulcus, perivascular or around vessels, often includes abundant tau-positive threads, and sometimes there is more white matter involved. The same characteristic pattern is seen as is described in Dementia Pugilistica (DP).

Dr. Bowser said he was curious about how well the cord cut after sitting cooled for a day, because that is always somewhat challenging to slice without having the toothpaste problem. He also asked how many frozen pieces are saved.

Dr. Stein responded that the number of pieces saved depends upon what they receive, but they try to freeze the majority and save what is needed for diagnosis for paraffin. On average, they get probably about 6 to 8 cervical sections, about 10 to 15 thoracic sections, and a handful of lumbar sections. The same for the motor cortex. They take block sized sections. They try to do upper, middle, lower, so 5 to 10 from each region if possible.

Dr. Brooks said there were potentially two ways of looking at feasibility for this bio repository of autopsy tissues. One is in terms of the RIN and pH and what the tissue is capable of producing in terms of neurochemical studies, and the other is emphasized by the case presented, which is ALS-Plus. He wondered if they were going to stage these for Lewy body disease, AD, and ALS. An overall value of such an autopsy tissue repository is to be able to pull out the ALS-Plus cases. Brad Schneider thinks there is a fifth stage with much more tau in the midbrain. The issue is that there needs to be an ability to look at pure ALS and some of these other components.

Dr. Stein replied that they are staging these for tau and Lewy bodies, and they are taking all of the areas so that they can do the TDP-43 staging as well. All of that is in the report.

## Discussion and Questions: Long-Term Implementation

**Wendy E. Kaye, PhD**
**Senior Epidemiologist**
**McKing Consulting Corporation**

During this session, Dr. Kaye invited participants to engage in a discussion regarding long-term implementation.

### Discussion Points

Dr. Mitsumoto asked whether patients in the ALS study are told that an autopsy is available, and whether there is any expense involved in terms of patients and their families. It is an incredibly important process to get the brain tissues, and he was curious how they could go about this process.

Dr. Kaye responded that participants were actively recruited out of the Registry to ask them if they would be interested in participating. ALS Registry members who volunteered were also included. It was not part of their registration, and there is no cost to the patients and families. The study covers all of the expenses of getting the body to the facility, getting it back to the funeral home, the dieners, the pathology reports, storage, et cetera.

Dr. Bradley asked what the success rate was with regard to people who agreed to participate, and he noted that when ALS physicians who have relationship with patients ask for donation, they get about 75% positive responses. If they asked people with no connection, he would speculate about 10%.

Dr. Kaye said she would say very high because a large portion of the people were volunteers. Perhaps there is some pent up demand in the ALS community where people have been thinking about this and when they got the opportunity that someone was willing to take postmortem samples, they jumped at the chance. The emails did not necessarily result in that many takers, but probably 90% of those who called in to express interest ended up being consented. She considered one of the success stories to be someone who really wanted to participate, but who they did not get consented in time. The funeral home called McKing because they thought the individual was in the study. With the help of John, they were able to get him into another ALS study. Dr. Kaye explained to the wife that he could not be included in the ALS Biorepository study, but could be in another. The wife agreed, and within 30 minutes he was enrolled in another project.

Dr. Bradley noted that the Vermont Mental Hospital had a 100% autopsy rate. He could never understand this, so he went in to speak with the Medical Director, who said that he says to the families, “You know, if I really try hard, I might be able to get it done for free for you” and in that case they agree.

Dr. Mitsumoto said he was also interested in ALS-PLS and they have not had a good number of autopsies. Very few cases have been reported. This is a good mechanism to get an autopsy of these cases who are usually loss to follow up.

Dr. Kaye replied that there is a lot of follow-up and that they carry a phone for this study 24/7. Contact is also made on a quarterly basis. For any study with longitudinal follow-up, it is important to keep up with participants to learn whether they have moved, if they are getting sicker, if they have moved into hospice, et cetera. There is a lot of work to ensure that in the end it all works out. With the PLS, there would be an even longer period of time for doing this. If the mechanism was built in, there would be no reason why this could not be done. She will be curious to see if all of the participants in the ALS Bioregistry study have ALS, because a lot of them will have self-registered and will have gone through the validation. It is known based on medical records that those six questions are about 93% accurate, so out of the 30 people in the study, one or so should slip by who is not really ALS. It will be interesting to see how well the validation questions are really working.

Dr. Brady thought that the VA bank may have one PLS case and one other diagnosis out of over 149, and the remainder are ALS. They do follow their cases for a long time from enrollment. They have been tracking participants since 2006, in some cases with up to 44 years of duration. It is true that a lot of time must be spent on the phone with them. When they approached the original VA ALS Registry folks about enrolling in the VA Brain Bank, they had about a 56% success rate from cold calls.

Dr. Brooks asked whether the feasibility study would continue until they had the bodies and autopsies on all 300 participants, noting that the accrual rate was 5 cases in 2 years.

Dr. Kaye clarified that the in-home blood and urine specimens would be on 300, and that the postmortem would be for 30 people. The study is due to end on September 22, 2015. At that point,

the contract is up, but they are in the process of figuring out a way to move any remaining participants to other projects. If the project is continuing, this will not be an issue. There are some veterans in the sample, so they have talked to Dr. Brady about working with the VA to move them into his project. There are also other projects at NDRI that other researchers are conducting. So the ALS Bioregistry study team will work with anyone who is still alive and interested to move them into another study. Recruitment for the postmortem study began April 13, 2013 so the accrual was 5 people in one year. Where people were progression-wise, she was surprised that there were not more the first year. She tried to scale it more toward people who were more progressed based on them having completed the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) in the Registry. However, by the time the emails were sent, half of those people were deceased. She tried to lag this, but it was hard to predict.

Dr. Brooks inquired as to whether part of the feasibility measurement was the metric of obtaining these autopsy specimens at a certain rate, or if there was a large issue of what they are used for and getting them out to researchers.

Dr. Horton replied that a large part of the feasibility study was just to determine logistics alone. They know they can get samples from large referral centers or large metropolitan areas, but it was unknown whether it would be possible to get brains or biospecimens from hard-to-reach areas.

Dr. Kaye added that in essence, another part of the question pertained to whether people will want these specimens and whether they are being used.

Dr. Horton said that when they started this initiative, they were clear to state that they did not want to duplicate efforts. Maybe what NEALS has is good enough. But, he thinks they owe it to patients to see if it makes sense to try to fold a biorepository into the Registry and be able to pair the epidemiological data with the biospecimens. They will wait to see what the report says. If it concludes that current efforts are sufficient, ATSDR will focus its resources elsewhere. However, he thought they had to explore whether this is something ATSDR should do for the sake of the patients.

Dr. Kaye added that NEALS is located in referral centers and some of the other studies are all referral centers. The ALS Bioregistry participants are from a more diverse population base. It may be interesting to see if they are somehow different than those who are being seen in referral centers.

Dr. Brooks said he was also thinking of the potential for targeting on a national scale.

Dr. Mitsumoto noted that they are dealing with a disease of an unknown mechanism. This is still not settled. He thought if they could get tissue out, he had a very strong conviction that they have to deal with the patient to find the mechanism. Animals are great, but the human body itself is so important. At the moment, it is not enough. If there are specific risks related to ALS, autopsy tissues are essential.

Dr. Bradley thought one important parameter to assess would be the national nature of the collection. It is very difficult to get patients to be donors when they move away from their practitioners. To have this nationally, what NDRI has been set up, is a tremendous attribute. However, one of the outcomes should be how many cases they failed to collect who died and were signed up.

Dr. Kaye agreed, acknowledging that everything that could go wrong did go wrong with one of the cases. The patient called McKing's office 800 number instead of the 24-hour number on a Saturday, so the message was not found until Monday, the denier was on vacation, et cetera. However, they decided to move forward and the tissues came in in good condition because the funeral home was very cooperative and had followed the protocol.

Dr. Mitsumoto pointed out that with regard to the death certificate and the box regarding whether an autopsy was done/not done, though no issue has arisen, ATSDR should have this all spelled out. They need to talk to lawyers about what the practice should be.

Dr. Muravov noted that the value of this project, if successful, would be to have tissues on a national level that are linked ideally to the risk factor surveys completed prior to the collection of the tissue. No one else would have this.

## Closing Remarks

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Dr. Kaye thanked everyone for staying, recognizing that it had been a long couple of days. Contact will be made with everyone probably in the fall to begin talking about dates and availability for the final meeting. At least 90% of the data will be available at that point, and there can be discussion about next steps, moving forward, and a report date for the report to ATSDR.

Dr. Horton expressed his hope that everyone found this meeting to be informative. The ALS Biorepository has a lot of potential. It is important to have buy-in and not to duplicate existing efforts. If there is a gap that ATSDR can fill through the biorepository, the agency wants to be helpful to the scientific community at large.

With no further comments raised or questions posed, Dr. Kaye wished everyone safe travels and officially adjourned the meeting.

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