



NATIONAL ALS BIOREPOSITORY
PILOT STUDY



SUMMARY REPORT

CONTRACT NO. 200-2011-40830
SEPTEMBER 22, 2015



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Introduction

A bill to amend the Public Health Service Act to provide for the establishment of an Amyotrophic Lateral Sclerosis (ALS) Registry (S. 1382: ALS Registry Act) was signed into law on October 10, 2008 by President Bush and became Public Law No: 110-373. The National ALS Registry was developed using existing national administrative data from the Centers for Medicare and Medicaid Services (CMS), Veterans Health Administration (VHA), and Veterans Benefits Administration (VBA). In addition, a self-registration component was deployed in October 2010, which allows persons with ALS (PALS) to self-register into the National ALS Registry through a secure web portal.

Although ALS was first described in the 1800's, little is known about the causes of ALS or how to treat it. There is only one medication for ALS which extends life only a few months. Most "treatment" revolves around supportive care including percutaneous endoscopic gastrostomy (PEG) or feeding tube, ventilatory assistance, motorized wheel chairs, and occupational/physical therapy. More information about possible exposures and genetics are needed to move the understanding of ALS forward as stated in the second purpose of the Public Law creating the National ALS Registry: "examine appropriate factors, such as environmental and occupational, that might be associated with the disease." In many instances this requires biologic specimens. In order to have biologic specimens available for researchers, a biorepository is necessary. The National ALS Registry is the largest cohort of ALS patients in the United States. Although there are other biorepositories, many are limited by clinical practice or treating physician or eligible group, e.g. veterans. Therefore the National ALS Registry is the ideal group in which to collect biologic specimens on a large representative group of persons with ALS in addition to the epidemiologic data currently being collected. The combination of epidemiologic data and biologic specimens/data on such a large group of ALS patients from all over the United States would make it an invaluable resource.

In 2011, McKing was awarded a four year contract by the Agency for Toxic Substances and Disease Registry (ATSDR) to create a biorepository of specimens from persons with ALS enrolled in the National ALS Registry and to conduct a pilot effort of two collection types (blood, urine, hair, and nail clippings) from 300 living ALS patients along with postmortem retrieval (brain, spinal cord, cerebrospinal fluid (CSF), bone samples, and muscle sample) in up to 30 deceased ALS patients. Additionally, a complementary contract was awarded by ATSDR with the purpose of testing the feasibility of development fibroblast cell lines from postmortem skin samples. While McKing retrieved the skin sample as part of its postmortem tissue recoveries, the results and findings of the work to develop cell lines is not included in this report.

Annual Expert Meetings

Throughout this project, McKing activities were guided by the Biorepository Expert Panel. On an annual basis, McKing gathered experts in neurology, biorepositories, laboratory science, and epidemiology to provide recommendations for future activities as well as provide feedback on McKing's activities. Full summary reports of these meetings were submitted to ATSDR within 4 months of each meeting and can be found on the National ALS Registry website.

Year 1: Input into Pilot Study Protocol. The first Annual Expert Panel meeting was held in March 2012 and gathered input for developing the pilot study protocol. Before the meeting, McKing conducted a literature review to identify existing ALS biorepositories and the current research trends among ALS researchers. Based on this information, McKing presented recommendations on what samples to collect, how these samples could be used in research, and how many samples to collect. Following the presentations, a facilitated discussion was held to develop final recommendations on what samples to collect and how many. The final Expert Panel recommendations were to enroll at least 300 participants for in-home specimen collection from living participants which would include blood, urine, hair, and nail samples. Saliva would be collected from those that could not provide a blood sample. Recommendations were made to collect some of the blood using metal-free materials to allow metals testing to be completed in the future. There was also a strong interest in obtaining multiple collections from each participant; therefore, it was decided to conduct two collections from each participant six months apart. The Expert Panel also recommended the collection of tissue samples that would be best collected from postmortem donors. These samples included the brain, spinal cord, CSF, bone sample, and muscle sample. The Expert Panel had a strong interest in the development of fibroblasts from skin samples.

Year 2: Biorepository Governance and Sharing of Samples. The second Annual Expert Panel meeting was held in February 2013 and focused on the development of guidelines for the governance and sharing of samples collected through this pilot study. McKing presented draft guidelines for how researchers would apply for samples and for a scientific review of these applications for samples. These guidelines were based on the National ALS Registry's review process for researcher requests to notify individuals who participated in the Registry through the self-registration portal about a research study. Following the presentations, a facilitated discussion was held to provide input in the development of the guidance document. The following recommendations were made by the Expert Panel.

- Both domestic and international researchers conducting ALS related research should be able to request samples
- The scientific review of applications for samples would determine what analyses are appropriate for the samples available
- ATSDR should make future announcements about availability of the samples following the end of the pilot study.
- Applications for samples should include a summary of the protocol, IRB approval or exemption, and preliminary data related to the proposed assays and/or available statistical data
- A Materials Transfer Agreement (MTA) should be signed by the researcher prior to receipt of samples and should include that researchers are required to acknowledge the sources of samples in abstracts and manuscripts, submit an annual progress report which includes information on presentations and publications, submit results to ATSDR, and return remaining samples at the end of their project.

Year 3: Project Update. The third Annual Expert Panel meeting was held in August 2014 directly following the National ALS Registry Annual Meeting. This meeting presented the current status of the Pilot Study including enrollment numbers, collections completed, challenges

and problems faced to date and solutions that were implemented. Presentations were made by McKing and its key partners. Based on discussions at this meeting, McKing worked with laboratory experts and our laboratory to develop quality assurance testing for a sample of specimens already collected and future specimen collections.

Year 4: Presentation of Project Outcomes. The fourth Annual Expert Panel meeting was held in March 2015. The primary purpose of this meeting was to present preliminary data from the Pilot Study and discuss recommendations for long-term implementation of a National ALS Biorepository. The meeting was held in advance of final data being available in order provide preliminary recommendations on long-term implementation so that ATSDR could potentially move forward this fiscal year with a new solicitation for long-term implementation. Following the presentation of project findings, McKing presented its preliminary recommendations on long-term implementation. These recommendations were discussed among and voted upon by the Expert Panel members. The final recommendations made by the Expert Panel are included in the Recommendations section of this report (page 9).

Options for Long-Term Storage of Biorepository Samples

In Year 3 and 4 of the contract, McKing conducted an assessment of specimen storage options in order to provide recommendations to ATSDR on long-term storage of the specimens collected through this pilot study. McKing collected information on biorepository standards and spoken with other government biorepository programs to understand how they maintain and distribute their samples, and spoke with private and public biorepository programs to better understand the types of facilities and services that would be available to ATSDR. There are two main options available to ATSDR for long-term storage of biorepository samples: 1) the CDC/ATSDR Specimen Packaging, Inventory and Repository (CASPIR) and 2) private biorepository facilities in academic institutions, private for-profit companies, and non-profit organizations. Based on information gathered through interviews with representatives of CASPIR and private repository facilities, McKing recommends that ATSDR continue storage of the National ALS Biorepository specimens in private repository facilities. This recommendation applies to both the biological specimens (blood, urine, hair, and nails) and the postmortem tissues (brain, spinal cord, CSF, muscle, bone, and skin for fibroblast cell lines). McKing's final report to ATSDR on long-term storage recommendations was submitted in March 2015.

Pilot Study

In the following section, we present a discussion of the Pilot Study methods from protocol development to specimen collection.

Study Design

The Pilot Study included two specimen collection components: biological specimens from living participants (in-home) and postmortem specimens. The in-home component aimed to enroll approximately 300 participants, from whom specimens would be collected on two occasions, approximately six months apart. The postmortem component aimed to enroll 30 participants,

who could also participate in the in-home study. In-home collection included blood, urine, hair, and nails. The postmortem collection included the collection of brain, spinal cord, CSF, muscle, and bone. Skin specimens for the creation of cell lines was added part way through implementation of the study.

The Pilot Study was designed to include both individuals specifically recruited to participate and individuals who voluntarily contacted McKing about their interest in participation. To be eligible for the Pilot Study, prospective participants must have been enrolled in the National ALS Registry. The participants in the Pilot Study may not have been representative of ALS patients nationally or of those who enrolled in the National ALS Registry. To assure geographic representation, potential participants were selected for the biospecimen portion of the Pilot Study by state in proportion to state population.

Persons participating in the in-home collection provided informed consent over the phone. There were additional eligibility criteria for participation in the postmortem collection portion of the Pilot Study including verification of ALS diagnosis by a neurologist, evaluation of capacity to consent, and evaluation of family dynamics that might impact a successful donation. Once eligibility was confirmed, those participating in the postmortem collection were visited in their homes and provided informed consent in person. Family members were asked to sign a family authorization form confirming his/her commitment to support and carry out the participant's decision for postmortem tissue donation. When skin specimens were added to the tissue types collected postmortem, a consent addendum was created and approved by the IRB. Participants who consented to participate before the addition of skin specimens were contacted about participating in this additional specimen type and asked to provide informed consent over the phone. For all parts of the consent process that took place over the phone, the participant returned a signed copy of the consent form to McKing before any study procedures took place. Participants were provided a copy of the signed consent form for their records.

IRB and OMB Approvals

This study required IRB review and approval for the protection of human subjects. McKing worked with Western IRB (WIRB), an independent IRB, to conduct IRB review for this project. WIRB reviewed and approved the research and was the IRB of record for this study. McKing requested an Exemption from OMB Paperwork Reduction Act (PRA) review for the Pilot Study, Exemption Category 5 CFR 1320.3(h)(5) sometimes referred to as a Clinical Exemption. The information collected for the Pilot Study was limited to the minimal necessary for the interpretation of biological analyses of body fluids, tissues, or other specimens, or the identification or classification of such specimens. Based on this limited scope of information collected for the Pilot Study, the exemption was approved.

Sample Collection Methods

The methods for specimen collection, processing, and storage procedures differed between the two parts of the Pilot Study.

In-Home Biospecimen Sample Collection

Specimen collection for the blood, urine, hair, and nails was completed in the participant's home by a trained phlebotomist. Specimens were shipped overnight to the laboratory to adhere to the 24 hour window for receiving and processing. The phlebotomists were responsible for returning the completed kit to a FedEx shipping center. Upon arrival at the laboratory, the specimen collection kits were inventoried to determine which samples were collected, data from the TrekView temperature logger was downloaded, hair, nails, and PAXgene tubes went directly to long-term storage, and the rest were transferred to the appropriate lab for processing. Oragene kits were also transferred to long-term storage without processing. The three remaining tubes of blood and the urine were processed and split into aliquots prior to freezing and storage.

Following the third expert panel meeting held in August 2014, McKing developed a specimen quality assurance plan and conducted quality assurance testing on a portion of specimens – both retrospective and prospective. Although most assays have quality assurance measures built in, it is more difficult to identify quality assurance measures for specimens when the future use is unknown. Based on input and guidance from laboratory experts, we decided to extract DNA from a sample of specimens, genotype the DNA, and compare it to our records of sex. As a marker of cell lysis, we tested a sample of serum and plasma samples for hemoglobin. All kits included a TrekView temperature logger to assess the ambient temperature from collection to delivery at the laboratory. Temperature results were monitored throughout the study.

A specimen collection form designed for the Pilot Study captured key data needed by the laboratory and researchers. The completed form was returned in the collection kit. All data collected on the Specimen Collection Form was entered into a separate database that has been submitted along with the final specimen inventory at the end of the contract.

Second collections occurred approximately six months after the first collection. Once the collection was scheduled, the process for scheduling and completing the collection was the same as the first specimen collection.

Postmortem Tissue Collection

For a majority of participants, McKing developed an individualized tissue recovery plan for each participant within 60 days from his/her consent to participate. However, some participants were not ready to make funeral arrangements and donation plans were incomplete until the participant and his/her family selected a funeral home.

The tissue collection plan used by McKing and NDRI included: 1) family primary point of contact (name and contact information); 2) information on funeral plans (name and contact information for funeral home); 3) name and contact information for transportation of the participant's body to and from donation site; 4) name and contact information for the diener who would harvest the tissues; 5) tissue collection site and contact information; and 6) name and contact information for backup diener in case the primary diener was unavailable at the time of the participant's death. The family was provided a toll-free, 24/7 phone number to call when the participant died. The completed donation plan was shared with the participant to ensure his/her family knew the process, points of contact and organizations involved in the donation process.

McKing maintained contact with all participants until death in order to monitor the progression of their disease. Participants or his/her family was contacted once every three months via phone or email. As the participant's disease progressed and he/she appeared closer to death, McKing increased the frequency of contact with the participant or his/her family.

Once the participant died, the body was transported to the designated tissue collection site. The goal was to complete all collections, return the body to the funeral home, and deliver specimens to the laboratories within 48 hours of death. A local diener was contracted to complete the tissue recovery using standard collection procedures which usually took two to three hours. Brain, spinal cord, and CSF were packed into the specially designed collection kit and delivered by courier to the nearest airport for shipment to our Brain Bank. Muscle and bone specimens were deposited in separate formalin-filled vials and were shipped to our primary laboratory. Skin specimen was deposited into a separate vial and shipped to cell line fibroblast contractor.

The brain, spinal cord, and CSF were processed and stored according to procedures already in place for the VA ALS Brain Bank. The muscle and bone samples did not undergo any further process and are stored as whole sections at our laboratory. The skin processing and fibroblast cell line development took place under another contract and results of that activity are not included in this report.

Recruitment and Sample Collection Results

The following section presents the results from the Pilot Study including results of recruitment and separate sections for the biospecimen collections and the postmortem collections.

Recruitment. Recruitment included multiple emails and telephone follow-up of potential participants. There were 1078 emails sent from ATSDR to fourteen groups of potential biospecimen participants. Most groups received three emails from McKing. There were a few groups that received four McKing emails after the protocol was amended and approved by the IRB for a 4th email. The last group did not receive a fourth email from McKing because we the IRB approved study size had been reached (Figure 1). Four hundred sixty-five biospecimen information packets were sent to 458 interested individuals.

One hundred and forty-one emails were sent from ATSDR to six groups of potential postmortem participants. Fourth emails were sent from McKing to the last two mailing groups of postmortem potential participants after the protocol was amended (Figure 2). There were 141 postmortem information packets sent to 97 interested individuals.

Participants. Three hundred and thirty-nine individuals provided consent to take part in the Pilot Study, 202 (59.5%) male and 137 (40.5%) female. Of the 339 biospecimen participants, 221 (64.8%) were recruited and 118 (35.2%) were volunteers. Nine of the 339 participants were unable to schedule an appointment, resulting in 330 participants completing at least one specimen collection.

Thirty individuals provided consent to take part in the postmortem part of the study. Of the 30 postmortem participants, 5 (16.7) were recruited and 25 (83.3) were volunteers. All 30 postmortem participants provided consent for the overall donation of brain, spinal cord, bone,

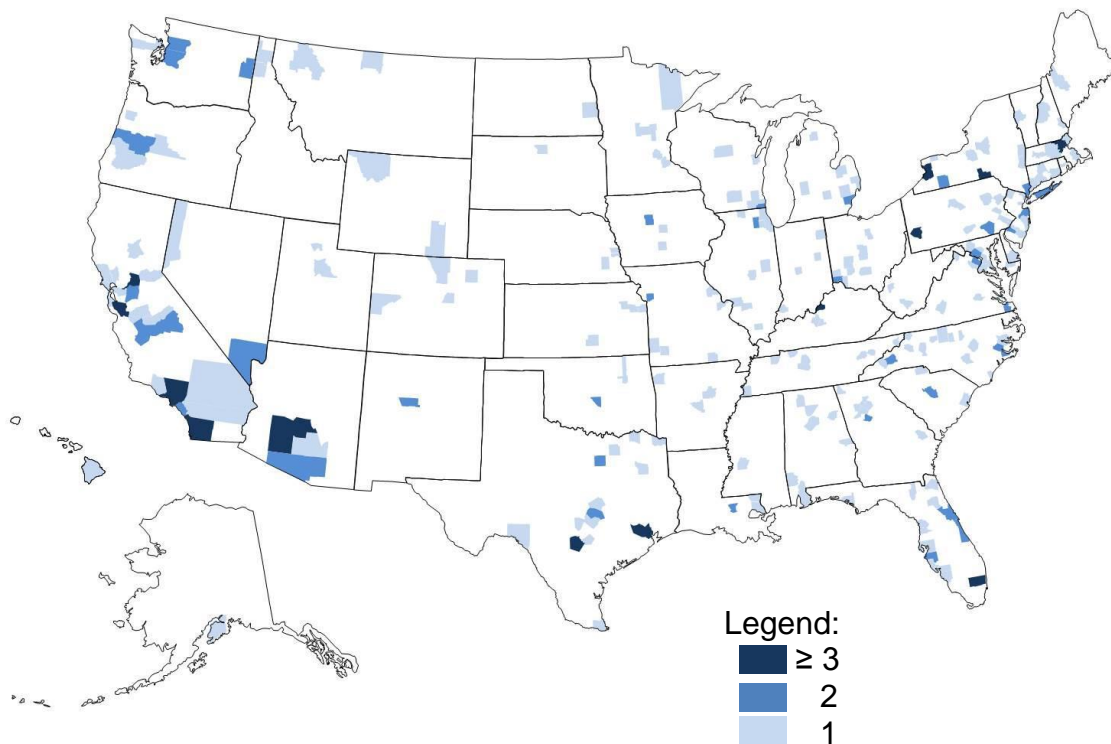
muscle, and CSF). In addition, 27 of the 30 the postmortem participants provided consent for skin sample collection. Three participants did not provide consent for skin sample collection (one died before skin sample collection was added to the protocol, one refused, and one died before the signed skin consent form was received).

In-Home Biospecimen Results

Geographic Distribution. The distance from any MDA and/or ALSA referral center to each participant's residence ranged from 1 to 750 miles. The participants that live in Hawaii or Alaska are more than 2000 miles from a referral center because there are no referral centers in their states. More than half of all of the participants live 50 or more miles from a referral center.

We collected specimens from at least one person in each state.

MAP OF IN-HOME PARTICIPANTS



Specimen collection. There were 330 participants who completed first specimen collections. Three hundred and eleven participants provided at least one vial of blood and 19 were unable to provide any blood for the first specimen collection.

There were 272 participants that completed second specimen collection. Two hundred fifty-five provided at least one vial of blood and 17 were unable to provide any blood during the second specimen collection. Fifty-eight of the participants who had a first specimen collection did not have a second specimen collection. The reasons for not completing the second draw included death (36), too ill or unable to contact (9), and no longer interested or scheduling difficulties (13).

Three hundred and twenty-one participants provided at least one blood specimen and 15 provided a saliva sample. Nine participants were unable to provide any blood; however, eight provided hair samples, nine provided nail samples, nine provided a urine sample, and four provided a saliva sample.

Most participants were able to provide specimens at both collection appointments. The following table shows the number of participants with specified sample pairs.

NUMBER OF SPECIMEN TYPES COLLECTED

Specimen type	No. participants (1 st collection) N=330	No. participants (2 nd collection) N=266
Whole Blood (Plasma, Buffy Coat, RBC)	309	255
Whole blood (metals free)	308	248
Plain blood (serum)	302	246
PAXgene 1 (RNA)	303	248
PAXgene 2 (RNA)	303	247
Urine	321	256
Hair	310	264
Nails	326	271
Saliva	15	0

In-Home Biospecimen Quality Assurance Results. We selected 175 participants' blood samples to undergo quality assurance testing. The samples selected were distributed throughout the study period and were taken from both first and second collections.

Of those selected, there was an appropriate specimen for DNA analysis for 174 participants. We were able to obtain DNA from all the specimens and all genotyping matched our records for sex of the participant. Currently most assays using DNA require less than 2 µg and all excess DNA was frozen for future use. Eighty-two percent of the specimens yielded at least 50 µg DNA and 60% yielded at least 100 µg DNA.

The ratio of absorbance at 260 nm and 280 nm (260/280 ratio) is used to assess the purity of DNA. A ratio of 1.8 is generally accepted as "pure" for DNA and is the industry standard for DNA that can be used in downstream applications such as PCR, DNA Sequencing. Ninety-eight percent of the samples tested met the industry standard for 260/280 ratio.

Free hemoglobin was measured in both serum and plasma. A temperature logger was included in all collection kits to monitor the ambient temperature from collection to delivery at the laboratory. Temperature results were monitored throughout the study and based on high temperature reports during collections in summer 2013, shipping procedures were changed to include a chilled gel pack rather than a room temperature gel pack in the returned collection kit. Following this change in procedure, we did not have any further high temperature readings.

The overall quality assurance testing showed that the specimens were generally of good quality. However, not all specimens will be appropriate for all analyses. It will be important to work with researchers to determine their assay specific specimen requirements so that this information can be used to identify the appropriate specimens for their study

Postmortem Results

Geographic distribution. The distance from postmortem participant's residence to MDA and ALSA referral centers ranged from 1 to 365 miles. More than half of the postmortem participants lived 50 or more miles from a referral center.

Deceased participants. Fifteen postmortem participants have donated specimens as of September 22, 2015. The length of time in the study for these participants from date of providing informed consent to date of death ranged from 1-22 months. As of September 22, 2015 the length of time in the study for participants that are still living, ranged from 18-24 months. The age at death for the deceased participants ranged from 43-76 years of age. Two additional participants are deceased but withdrew from the postmortem part of the study and did not provide postmortem donations. However, these participant did take part in the biospecimen part of the study.

Specimen collection. Fifteen postmortem participants provided brain, spinal cord, CSF, bone and muscle specimens. Skin samples were obtained from 13 participants.

Recommendations

Upon completion of the Pilot Study, McKing has proven that nationwide collection of biospecimen and postmortem samples from PALS is feasible and can be done in a cost-effective manner. The Expert Panel that we convened in March 2015 recommends that ATSDR continue to collect biospecimen and postmortem samples for the National ALS Biorepository. Based on our experiences and lessons learned from the Pilot Study, McKing recommends the following changes to procedures and samples collected.

Recruitment. As previously discussed, McKing experienced a slow start to recruitment and then often did not have any response from the multiple recruitment emails that were sent to participants. We feel that this was due to the separation of enrollment in the National ALS Registry and contact to introduce the opportunity to participant in the National ALS Biorepository. In order to better connect these activities, we recommend that PALS are given an opportunity to indicate their interest in participating in the National ALS Biorepository as they complete the initial enrollment into the National ALS Registry. Individuals already enrolled should be notified of the opportunity to participate in the biorepository. This will also give ATSDR an opportunity to collect additional contact information, such as phone number and physical mailing address, in order to follow-up with interested enrollees. McKing recommends that ATSDR continue to select a geographic representative sample of interested enrollees to participant in the National ALS Biorepository. Based on interest in participation during the Pilot Study and the approximate number of enrollees into the Registry each year, McKing recommends collecting biospecimen samples from 500-600 individuals per year and consent up to 30 interested participants each year for postmortem tissue donations.

Biospecimen Collection and Processing. In March 2015, the Expert Panel extensively discussed the type of samples and what processing and quality assurance testing should be done with the specimens collected. The recommendations of the Expert Panel included:

- Continue blood collections
 - Conduct only one collection per participant
 - Discontinue the metals-free collection (if there is a high demand for metals-free collection, re-start metals free collections)
 - Include a blood tube to allow for the creation of cell lines from blood rather than from postmortem skin fibroblast
- Extract DNA and RNA from blood tube during initial processing of specimens
- Continue urine collection
- Discontinue hair and nail clippings (if there is a high demand for hair and/or nail clippings, re-start this collection)
- Continue saliva collections for those that have difficult blood draws
- Expand saliva collections to individuals not selected for a biospecimen collection to increase the number of DNA samples the Biorepository receives
- Conduct quality assurance testing of the biospecimen samples during initial processing

The Expert Panel also recommended that ATSDR conduct regular market research to determine what type of specimens ALS researchers are interested in using based on current research and technology. This research should drive what types of samples are collected and how they are collected. In future years, ATSDR will be able to analyze the demand for samples in order to determine what specimens should be collected each year.

Post Mortem Collections. At the March 2015 Expert Panel meeting, the post mortem collections were also extensively discussed. The Expert Panel strongly recommended that ATSDR make arrangements to follow through with postmortem collections for participants in the Pilot Study but who are still alive at the end of the McKing contract. At the time, we estimated this may be 10-15 participants. For future postmortem collection, the recommendations of the Expert Panel included:

- Continue the brain, spinal cords and CSF collections, including the metal-free CSF collection
- Continue to analyze and process the brain, spinal cord and CSF to match the procedures of the VA ALS Brain Bank
- Discontinue the muscle and bone collection; however, if there is a high demand for the muscle samples, re-start this collection
 - If the muscle collection is continued, freeze samples instead of storing samples in formalin and collect one muscle sample from each extremity instead of the one sample from the back
- Discontinue skin collections for fibroblast development, develop cell lines from blood draws instead

Marketing of the National ALS Biorepository. The most important aspect of the National ALS Biorepository is its ability to share samples with researchers to further research investigating risk factors for ALS and potential new treatments for ALS. The Expert Panel discussed at length, the need to market and promote the availability of samples through the National ALS Biorepository to researchers across the U.S. Those experienced in running biorepositories emphasized the need

for a catalogue of samples included in the National ALS Biorepository that would be easily accessible to researchers. It was also recommended to cross-promote the National ALS Biorepository with other ALS biorepositories, such as NEALS and the VA, to encourage researchers to use samples from all ALS biorepositories.

Distribution of Samples McKing also recommends that the distribution of samples be merged into operations of the collection process as one Biorepository program. If ATSDR elects to contract out the collection process, that contractor should also facilitate the review of applications for samples, manage the sample inventory, and distribute the samples to ATSDR approved researchers. This will allow for the sample inventory to be up-to-date at all times. Along with this, the organization that is managing this process should also be available to researchers to answer questions or assist them with refining their applications based on the types of samples available through the National ALS Biorepository. At the Year 2 Expert Panel meeting in February 2013, it was recommended that ATSDR charge a nominal fee to researchers for samples to cover the cost of retrieving and shipping of specimens.

Along with the specimens, researchers should receive data that can be used to assess the quality of the specimens such as the time elapsed from collection to processing of biospecimens and if a high temperature alarm occurred during the shipping of the biospecimens. For post-mortem tissues, data to be provided should include the RNA Integrity Number (RIN) and the elapsed time from death to processing of the tissues. Researchers should also receive standard data about the participants. This should include the age at diagnosis, age at death, sex, and state of residence and if available race, family history of ALS, family history of other neurodegenerative diseases, ALS FRS closest to collection, age at first symptoms, and survival time. Therefore, ATSDR should prepare this limited dataset ahead of time to be able to provide this information with the samples. In addition, in order to be able to quickly prepare requested epidemiologic data to accompany samples, ATSDR should prepare a data file that contains all survey data for the individuals that participated in the Pilot Study. If ATSDR elects to continue the biorepository, adding the limited data to the file should occur on a monthly basis. Addition of the survey data should occur yearly as the data are cleaned and finalized.