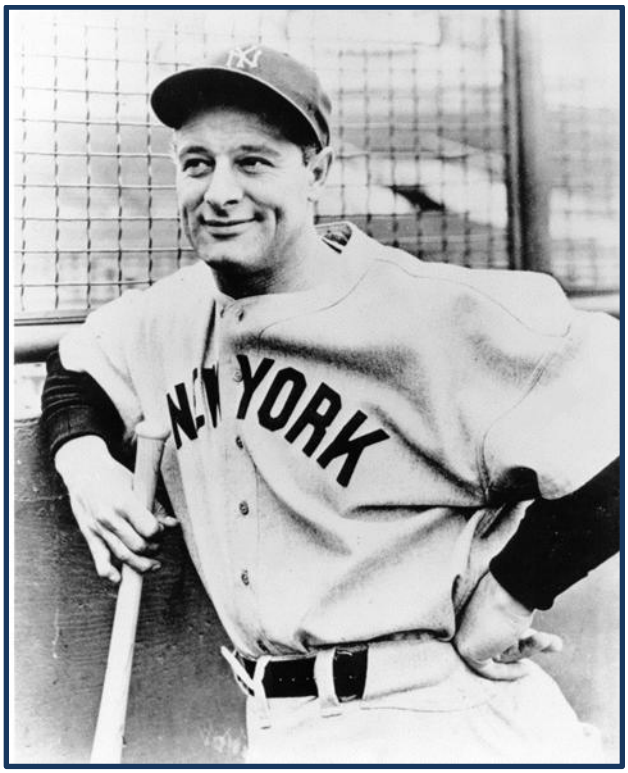
Department of Health and Human Services
Centers for Disease Control and Prevention
Agency for Toxic Substances and Disease Registry

The National Amyotrophic Lateral Sclerosis (ALS) Registry First Biorepository Pilot Study Expert Panel Meeting



March 26-27, 2012
Summary Report

This document has not been revised or edited to conform to agency standards. The findings and conclusions in this report are those of the meeting presenters and attendees and do not necessarily represent the views of the Agency for Toxic Substances and Disease Registry.

Acronyms

Acronym	Expansion
ACES	ALS Consortium of Epidemiologic Studies
ALS	Amyotrophic Lateral Sclerosis
ALS RG	Amyotrophic Lateral Sclerosis Research Group
ALSA	Amyotrophic Lateral Sclerosis Association
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised
APOE	Apolipoprotein E
ATSDR	Agency for Toxic Substances and Disease Registry
C9ORF72	chromosome 9 open reading frame 72
CAG	Community Advisory Group
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFS	Chronic Fatigue Syndrome
ChIP	Chromatin immunoprecipitation
CLIA	Clinical Laboratory Improvement Amendments
CSF	cerebrospinal fluid
dbGAP	Database of Genotypes and Phenotypes
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eMERGE	Electronic Medical Records and Genomics (Network)
ESAB	Ethics and Security Advisory Board
FDA	Food and Drug Administration
FFPE	Formalin Fixed Paraffin Embedded (tissue)
FTD	frontotemporal dementia
FTLD-U	Ubiquitin-positive Frontotemporal Lobar Degeneration
FUS/TLS	FUsed/Translocated in LipoSarcoma
FY	Fiscal Year
GARB	Genetic Alliance Registry and BioBank
GENEVA	Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis
GINA	Genetic Information Nondiscrimination Act
GTEx	Genotype Tissue Expression Project
GWAS	Genome-Wide Association Studies
HHS	(United States Department of) Health and Human Services
HMO	Health Maintenance Organization
iPS	Induced pluripotent stem
IRB	Institutional Review Board
MAVERIC	Massachusetts Veterans Epidemiology Research and Information Center
MDA	Muscular Dystrophy Association
MESA	Marshfield Epidemiologic Study Area
MND	Motor Neuron Disease
NCI	National Cancer Institute
NCS	National Children's Study
NEALS	Northeast Amyotrophic Lateral Sclerosis Consortium
NHANES	National Health and Nutrition Examination Survey
NHGRI	National Human Genome Research Institute

NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
OMB	Office of Management and Budget
PALS	Persons with Amyotrophic Lateral Sclerosis
PBMC	Peripheral Blood Mononuclear Cell
PMI	Post-Mortem Interval
PMRP	Personalized Medicine Research Project
pNF-H	phosphorylated Neurofilament H
RNA	Ribonucleic acid
RIN	RNA Integrity Number
SACTL	Southern Arizona Core Tissue Laboratory
SOD1	Superoxide Dismutase 1
SOP	Standard Operating Procedure
TBI	Traumatic Brain Injury
TDP-43	Transactivation Response (TAR) DNA binding protein-43
UBQLN2	Ubiquilin-2
VA	(United States Department of) Veterans Affairs
VAB	United States Department of Veterans Affairs Biorepository
VBA	Veterans Benefits Administration
VHA	Veterans Health Administration
WHI	Women's Health Initiative

Centers for Disease Control and Prevention (CDC)
Agency for Toxic Substances and Disease Registry (ATSDR)
The National Amyotrophic Lateral Sclerosis (ALS) Registry Biorepository
Pilot Study Expert Panel Meeting

Minutes of the Meeting
March 26-27, 2012

Purpose

Purpose: Discuss on-going work in biorepositories and Amyotrophic Lateral Sclerosis (ALS) research and provide comments on a proposal for a biorepository of tissue samples to complement the National ALS Registry.

Welcome and Introductions

Robert Kingon, Facilitator
Atlanta, Georgia

At 9:00 a.m., Robert Kingon welcomed the group to the meeting. He asked that those present introduce themselves. Following the introductions, he offered housekeeping notes and reviewed the day's agenda.

Background and History of the National ALS Registry

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

Dr. Horton added his welcome and presented an overview of the National ALS Registry, explaining that over the last 5-6 years, a number of people have worked very hard to bring the registry to fruition. They have made a great deal of progress and are now seeking ways to enhance the registry, including the creation of a bioregistry. He encouraged the group to speak freely as they offered feedback.

The Agency for Toxic Substances and Disease Registry (ATSDR) is a federal agency of the US Department of Health and Human Services (HHS) and is a sister agency of the Centers for Disease Control and Prevention (CDC). ATSDR focuses on environmental health issues, especially as they relate to exposure to toxic substances. The agency is located in Atlanta, Georgia and engages in a number of activities, including health studies; emergency response; applied research; and health surveillance and registries. ATSDR operates several registries.

The National ALS Registry was created for a number of reasons. ALS is a devastating disease. Approximately 80% of cases die within 2-5 years of diagnosis. Therefore, it is critical to have a registry to understand not only who has ALS, but also why people get ALS. The incidence and prevalence data on ALS are not reliable, as they come from small-scale studies and are extrapolated to the US population. The National ALS Registry allows for national-level incidence and prevalence of ALS.

Congress enacted the ALS Registry Act in October, 2008. The act directs ATSDR to create and maintain the registry, but does not make ALS a reportable disease. The purpose of the registry is to describe the incidence and prevalence of ALS; describe the demographics of ALS patients, and examine risk factors for the disease.

To evaluate the feasibility of using existing data to create the National ALS Registry, ATSDR conducted 4 pilot projects to identify individuals in the national databases who have been treated, or would have been treated, for any motor neuron disease (MND) by a health care provider in the local catchment area; review medical records from local data sources to determine correct diagnosis; determine which ICD9 and procedure codes in the national databases are most reliable for identifying ALS cases; and develop algorithms to identify true cases of ALS.

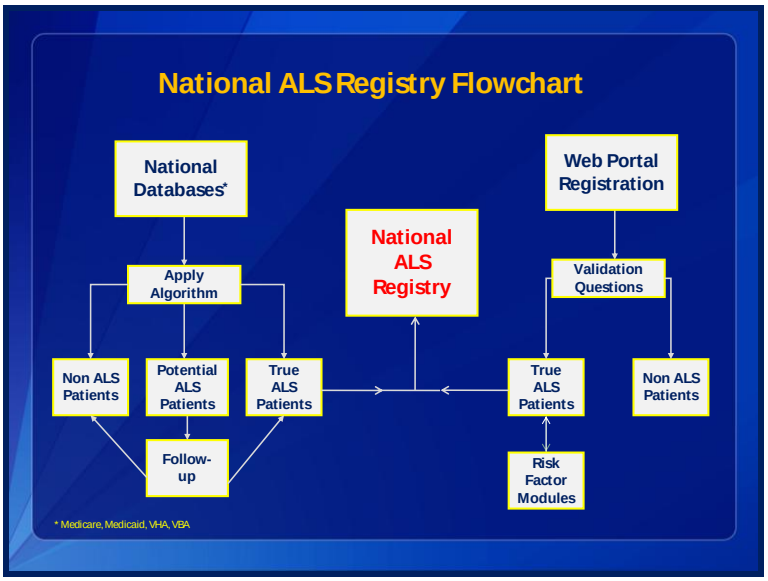
Using medical record review as a gold standard, ATSDR built algorithms to identify true cases of ALS. The creation process was trial and error and, with changes, the ultimate algorithm yields the best sensitivity and specificity. Several attributes were identified: ALS in more than one year; RILUTEK®, although not all ALS patients take Rilutek; and frequent visits to a neurologist. Records were ruled out if there were no visits to a neurologist and no ALS visits.

ATSDR secured databases from Medicare, Medicaid, and the US Department of Veterans Affairs (VA) and applied the algorithms to the databases, allowing them to tease out true ALS cases, cases that are clearly not ALS, and cases with unclear ALS status. The VA databases are the Veterans Health Administration (VHA) and the Veterans Benefits Administration (VBA) databases.

A Web-based portal for self-identification is hosted on the CDC server, which is highly secure. This portal is critical, given that all ALS patients are not reflected in the Medicare, Medicaid, and VA databases. Further, there is a time lag of up to 3 years or more associated with those databases, where the Web portal allows for efficient, real-time capturing of ALS cases. The portal allows ATSDR to capture epidemiological information through brief risk factor surveys. It also provides other educational material for physicians and health professionals. As publications emerge, they will be made available through the portal.

The current methodology for the National ALS Registry includes applying the algorithm to the Medicare, Medicaid, and VA databases, which represent approximately 90 million people in the US, and self-registration through the Web portal. All ALS patients are encouraged to access the Web portal, even if they are included in one of the larger databases. Self-enrollment improves efficiency and also allows participants to answer risk factor questions. The Web portal was launched in 2010, and the response has been very good so far, with thousands of risk factor surveys already completed. The challenge with any registry is to sustain momentum and awareness about it, especially for those who are newly diagnosed.

The National ALS Registry receives information as follows:



Potential ALS patients are re-evaluated when subsequent years of data are available to determine whether they are true ALS cases. The validation questions in the Web portal are the same questions used by the VA when they operated their ALS registry in the early 2000s. ATSDR worked with Stanford University to develop the risk factor modules and to convert them to an electronic, self-administered format. These modules are noted in the literature as potential associations with ALS:

- ❑ Demographics
- ❑ Military History
- ❑ Lifetime Occupational History
- ❑ Smoking and Drinking History
- ❑ Physical Activity
- ❑ Family History of ALS and Neurodegenerative Diseases
- ❑ ALS Functional Rating Scale-Revised (ALSFRS-R), self-administration version

The ALSFRS-R assesses disease progression and quality of life, so participants in the registry are asked to complete that module twice a year. The other modules are completed only once.

New components are planned for the National ALS Registry. Additional risk factor surveys are in development and should be available in late 2012 or early 2013. A clinical research notification system will aid researchers who seek participants in studies. Under this system, researchers will submit proposals for studies or clinical trials to ATSDR. The application process will be online and will require appropriate Institutional Review Board (IRB) and Food and Drug Administration (FDA) approvals, where applicable. If the project is approved, ATSDR will contact members of the Registry who fit the study population and inform them about the project, provide its recruitment materials, and contact information for the researchers. Patients can then contact the researchers if they are interested in participating. This feature of the Registry is expected to be live in April 2012.

Other new plans for the Registry include state- and metropolitan area-based surveillance projects, which will help test the completeness of the registry. These active case-finding approaches to ALS include direct contact with neurologists in the catchment areas. ATSDR will be able to compare that information from catchment areas with information in the National ALS Registry to assess the completeness of the Registry data. If cases are not being captured, then ATSDR will adjust the methodology. These surveillance projects are in areas that over-represent different demographic groups and should provide insight into how ALS affects various sub-populations such as African Americans, Latinos, and Asian Americans. The addition of a bioregistry would make the National ALS Registry world-class. ATSDR has expertise in registries, but not necessarily in bioregistries. Dr. Horton expressed his hope that the group would share their expertise to help build an excellent bioregistry for ALS.

Discussion Points

- Dr. Brujin commented on the family history module in the self-enrollment portal, noting that family history and genetics can change over time. The questionnaire includes general questions about MNDs, as opposed to ALS specifically.

- Dr. Horton said that some of the questions mention specific MNDs, including Parkinson's disease and ALS.

- Dr. Brujin asked whether the clinical research notification system will allow researchers to access participants for work on chromosome-9.

- Dr. Horton replied that the Registry will be available for such work and can provide subsets of populations for recruitment.

- Dr. Kamel said that the Medicare database will include mostly older people, which is the population with the most ALS cases. However, some of the more interesting cases are in younger populations. She wondered how ATDSR would reach a more diverse age population.

- Dr. Kaye clarified that persons with ALS become automatically eligible for Medicare, regardless of age. The algorithm takes this eligibility into account. The VA automatically provides service-related disability related to ALS at any time.

- Dr. Horton added that the registry merges the Medicare and Medicaid databases, matching Social Security Numbers to eliminate duplicates.

- Dr. Kasarskis pointed out that the ALSFRS-R is a measure of function and not a measure of quality of life per se. He asked whether specific quality of life measures, such as depression, were included in the questionnaires.

- Dr. Horton acknowledged that the ALSFRS-R does not address quality of life questions and said that ATSDR is always interested in entertaining new ideas for modules. They have worked with the ALS Research Group (RG), which has created clinically-based questions for ALS patients to answer. The Office of Management and Budget (OMB) is concerned that participants in registries are not overburdened by questionnaires, but ideas from researchers, physicians, and patients are welcomed.

- Dr. Kasarskis asked whether a relative or friend of an ALS patient can interact with the Web portal on behalf of the person with ALS.

- Dr. Horton responded that ALS patients can receive assistance in completing the questionnaires, but it is important that they read the consent form and that their assistants not complete the questionnaires for them, but with them.

- Dr. Horn asked whether information from the registry could be reported back to participants, emphasizing that sharing information can help engage participants and improve participation rates.

- Dr. Horton said that it is important to analyze and publish the data that they collect so that they can share information with their patients and stakeholders. They are not yet at a point where they have information to share, but they will keep participants informed via the Web portal and possibly through emails and Webinars. The Registry is a collaborative effort, and they rely on their partners who interact with ALS patients on a daily basis. They are working with the ALS Association and the Muscular Dystrophy Association (MDA) and their affiliates to spread the work about the registry.

Purpose and Goals of the Meeting

Wendy Kaye, PhD
Senior Epidemiologist
McKing Consulting Corporation
National ALS Registry Program
Agency for Toxic Substances & Disease Registry

Dr. Kaye expressed her pride in the National ALS Registry and its progress in a relatively short amount of time. She reminded the group that this meeting would focus on the best way to create a biorepository in association with the National ALS Registry. A draft protocol was created to generate conversation, and the group's input would be critical, particularly regarding which specimens should be collected and the population size for a pilot to test the feasibility of the biorepository. She stressed that rather than focusing on logistics and how the specimens will be used, they should focus on the scientific aspects of the specimens that should be collected and processing for storage.

Why Do We Need an ALS Biorepository?

Advances in ALS Research

Amelie Gubitz, PhD
Program Director, Neurodegeneration
National Institute of Neurological Disorders and Stroke (NINDS)
National Institutes of Health (NIH)

Dr. Gubitz thanked ATSDR for the opportunity to present exciting new discoveries in ALS research, highlighting the importance of doing research using human biospecimens. ALS research is a shared mission across multiple institutes at the National Institutes of Health (NIH). NIH includes 27 different institutes and centers, at least 10 of which have investment in ALS

research, extramurally and intramurally. The National Institute of Neurological Disorders and Stroke (NINDS) is the lead institute for ALS research at NIH, but the contributions of other institutes are important and tend to focus on specific aspects of the disease. In fiscal year (FY) 2011, NIH's overall investment in ALS research was approximately $44 million.

NINDS supports investigator-initiated, peer-reviewed ALS research. The research community is in the best position to identify scientific opportunities and to design research projects that take advantage of those opportunities. Project ideas are vetted by peer reviews, study sections, and the NINDS Advisory Council. Approximately half of NINDS's investment in ALS is focused on projects that explore the molecular pathogenesis of the disease. They are also interested in the etiology of ALS, studying genetics and epidemiology. NINDS invests in preclinical therapy development for ALS, which includes biomarker research as well as clinical trials.

NINDS also supports resources for ALS research, including the NINDS Repository, which is a biobank of DNA samples and cell lines with associated clinical data for multiple neurological disorders, including ALS. In collaboration with the scientific ALS community, NINDS developed and launched Common Data Elements for ALS Clinical Research. The goal of these Common Data Elements is to standardize clinical data sets to facilitate data mining and for meta-analysis. NINDS has a dedicated investment in the exciting research area of the development of induced pluripotent stem cell strategies for ALS.

Advances in the molecular pathology of ALS have also been exciting. It has long been known that intracellular inclusions of ubiquitinated protein in affected tissues are a hallmark of ALS and other neurodegenerative disorders. In terms of ALS, the protein content of these inclusions remained enigmatic for a long time. In 2006, a landmark study from the University of Pennsylvania assessed autopsy tissue from patients who suffered from sporadic ALS and ubiquitin-positive Frontotemporal Lobar Degeneration (FTLD-U). The researchers were able to show that the protein in these inclusions is Transactivation Response (TAR) DNA binding protein (TDP)-43. This discovery came as a surprise to the ALS field, as TDP-43 had previously not been associated with neurodegenerative disease, and all that was known about the protein is that it likely has important roles in cellular RNA metabolism.

Since the original study, this finding has been confirmed and replicated by research teams across the world. This pathology is now referred to as TDP-43 proteinopathy. TDP-43 inclusions are present in spinal cord tissue and brain tissue of most cases of sporadic and familial ALS as well as FTLD-U. The inclusions are absent from familial ALS that is linked to mutations in the superoxide dismutase 1 (SOD1) gene. This is important, as recent ALS research has focused on SOD1-linked ALS. It seems that there are important molecular differences, although this work is evolving.

Evidence is accumulating that ALS and FTLD-U are disorders on a clinical and pathological spectrum with overlapping molecular pathogenesis. This may have important therapeutic implications. Further, the presence of TDP-43 inclusions suggests that cytoplasmic protein aggregations and/or defects in RNA metabolism may play important roles in ALS and FTDL-U.

Several milestones have been achieved in ALS gene discovery. ALS is largely a sporadic disease, with only 5% to 10% of cases showing clear familial inheritance. The first breakthrough in ALS genetics research was attained in 1993, when mutations in SOD1 were found to be causative in about 15% of familial ALS cases. Since 2000, over 15 additional familial ALS genes have been identified. When the TDP-43 positive pathology was discovered, researchers found mutations in the gene that encodes TDP-43 in some ALS families, as well as

mutations in a highly-related gene, FUsed/Translocated in LipoSarcoma (FUS/TLS). The most recent additions to the list of ALS are Ubiquilin-2 (UBQLN2) and chromosome 9 open reading frame 72 (C9ORF72). Since ALS is a largely sporadic disease, researchers have used genome-wide association studies in order to search for genetic risk factors for the disease. This approach has not revealed major risk alleles for ALS, but in some populations, the studies identified a "hot spot" for linkage on chromosome 9. It is now known that this is the locus of the C9ORF72 gene.

In the summer of 2011, Teepu Siddique and colleagues reported that mutations in UBQLN2 cause dominant x-linked ALS. Although the mutations are rare, this finding is important since UBQLN2 is involved in cellular protein degradation and turnover. The finding suggests that defects in protein recycling leads to the death of motor neurons. This pathway of disease is plausible, as there are protein aggregates in affected tissues.

In October 2001, two back-to-back articles were published in the journal *Neuron*. One article was authored by an international consortium of scientists led by Bryan Traynor, and the other was authored by Rosa Rademakers and colleagues. Both teams were independently able to pinpoint the genetic mutation on chromosome 9. Previous studies had suggested that there was an association between ALS and a genetic lesion on the short arm of chromosome 9, but for a long time, the nature of this lesion remained elusive. Only through elegant detective work were both teams able to show that the nature of the lesions is a long repeat expansion of a sequence motif in an intronic region of an unknown gene called C9ORF72. The C9ORF72 repeat expansions are the most common cause of ALS and frontotemporal dementia (FTD) identified to date. This type of genetic abnormality is enriched in families where ALS and FTD co-occur or where individuals suffer from symptoms of both disorders. The link between the two diseases is very important. The nature of these repeat expansions is similar to other diseases, including myotonic dystrophy. This commonality could be important for therapeutic purposes.

Regarding disease mechanisms of ALS, when mutations in SOD1 were found to be a genetic culprit of the disease, research teams developed mutant SOD1-based animal models. These models have been extremely valuable in delineating the molecular pathogenesis of SOD1-linked ALS. Mutant SOD1-mediated toxicity in ALS is non-cell autonomous, which means that it affects cells beyond motor neurons: "the neighborhood matters." Further, disease is not caused by loss of enzymatic activity of the SOD1 protein, but by a toxic gain-of-function mechanism. It appears that mutant SOD1 disrupts multiple cellular processes in multiple cell types. Once mutant SOD1 begins to misfold, cells experience a multi-pronged crisis. It is not known whether the cellular pathways of disease run in parallel, whether there are 1 or 2 important pathways, or whether there are important points of convergence upstream of motor neuron degeneration.

A major question in the field is whether the suspected disease mechanisms of SOD1-linked ALS are also relevant to other forms of the disease, especially sporadic ALS. This question needs to be addressed by research that employs human biospecimens, including post-mortem tissues, cell lines or primary cells, and biofluids. The scientific community recognizes this need. Brian Kaspar and his colleagues derived astrocyte cells from fresh autopsy tissues from ALS patients who had suffered from sporadic ALS or familial SOD1-linked ALS. These astrocytes are toxic to normal motor neurons in cell culture, and the toxicity is dependent on the expression of SOD1. This finding is surprising in the context of sporadic ALS. The research community is discussing the finding, and it warrants confirmation. This type of study showcases the importance of conducting research using human biomaterials.

There are a number of opportunities and challenges for ALS research in 2012 and beyond. Genetics and neuropathological research have provided clues about the molecular pathogeneses of ALS. There are likely a number of complexities and heterogeneity, which presents a major challenge for the field but it also offers multiple points for therapeutic intervention. The community seeks therapeutic targets were there is convergence between the different forms of ALS. Additionally, the etiology of sporadic ALS remains unexplained. It is anticipated that environmental, behavioral, and genetic risk factors may be involved. Another challenge is the urgent need for better and additional biomarkers in ALS to expedite diagnosis, to help define prognosis, and to improve clinical trials. Such biomarkers can be derived from human biofluids, which highlights the need for high-quality and accessible biofluid collections. While animal models are important research tools in the field of ALS, the models do not fully recapitulate human disease, especially sporadic ALS. Alternative model systems that are based on human biomaterials are needed.

It is clear that research using human biospecimens has greatly advanced the understanding of ALS. Therefore, there is a continued need for high-quality and broadly accessible biofluid samples and post-mortem tissue for ALS research. The ALS field has several biobanks, but many of them are relatively small and are typically housed at academic institutions. They may only have limited accessibility. With a few exceptions, they also often lack the funding or capacity for broader sample distribution. The National ALS Registry Biorepository would address a critical need in the ALS research field and complement the existing ALS biobanks.

Discussion Points

- Dr. Vaught asked how “high-quality” is defined in this context.

- Dr. Gubitz replied that “high-quality” refers to following standard operating procedures (SOPs) for the collection and storage of biosamples. Many research labs work on these issues actively. In particular, the Northeast ALS Consortium (NEALS) Biorepository has put a great deal of effort into ensuring that the samples are collected in a homogenous fashion, that the quality of the samples is preserved, and that the samples are tracked correctly so that distribution and retrieval is transparent and done in an informative way. It is also preferable to associate the biosamples with clinical data sets. The NINDS Common Data Elements help to harmonize the clinical data sets that are associated with samples.

- Dr. Vaught said that the answers to those questions will help them decide whether to collect blood, saliva, or both.

- Dr. Gwinn asked how the use of the Common Data Elements is promoted throughout the clinical community.

- Dr. Gubitz answered that the elements were developed in collaboration with ALS clinicians. The elements are accessible through the NINDS website. A team in their clinical office is charged with raising awareness about the elements, but it is a challenge. The elements are only helpful if they are adopted by the community. They include a great deal of detail, which can be a burden.

- Dr. Gwinn asked whether any journals were promoting meta-analysis.

- Dr. Gubitz replied that there have been efforts in that arena. Common Data Elements have been developed for a range of diseases, and the effort is active.

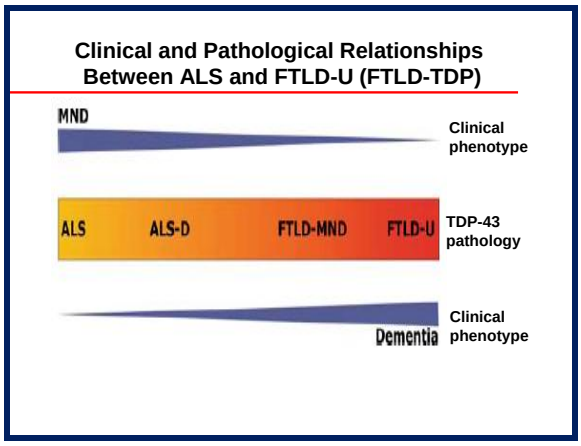
Rationale for ALS Biorepository

Lucie Bruijn, PhD
Chief Scientist
The Amyotrophic Lateral Sclerosis Association

Dr. Brujin expressed her gratitude for being invited to participate in the meeting, and her excitement about the potential for a National ALS Biorepository. She offered an overview of the importance and value of the biorepository. It will be critical to integrate and combine with existing resources, as it is unlikely that one biorepository will be able to do everything. There are well-established networks in the ALS community, and a great deal of work has already been accomplished on SOPs.

The discovery of new genes has changed the field of ALS significantly, and the discoveries would not have been possible without biorepository collections. There are challenges in long clinical trials, and the ALSFRS is a good indicator of change over time. Survival is still the key endpoint, and the trials are very long and expensive; therefore, it is difficult to incentivize industry participation. Biomarkers and repositories are critically important for discovery, diagnostics, and clinical trials. Understanding the disease mechanism is critical for finding better treatments.

The NINDS repository represents a successful partnership with many ALS Association (ALSA) - certified centers, MDA chapters, and persons with ALS (PALS). At one time, the NINDS repository only included 4-5 ALS samples. Now, the repository includes 2000 samples, which reflects the support and energy of the ALS community which is committed to using the samples. When a repository is built, it must be useful. ALSA supported work to search for the Apolipoprotein E (APOE) for ALS, and that work has been a testament to the complexity of ALS and the diversity of samples in the repository. Perhaps small gene changes in sporadic ALS cannot be discerned by current technology. It is important to collect appropriate similar data. ALSA has been able to work globally to look at samples, and there have been indications of an interesting locus that determines onset of ALS. The critical studies of chromosome 9 would not have been possible without biorepositories. Chromosome 9 has major implications for familial as well as sporadic ALS. Human brain and spinal cord samples are critical to ALS research. ALS is a spectrum, as illustrated below:



This mapping work was possible through work with brain tissue and the identification of various pathologies. The question of where SOD1 fits into the picture is still open. The chromosome 9 cases indicate different localizations of pathologies where FTLD dominates and where ALS dominates. Having well-characterized tissue from these populations will add to understanding of chromosome 9 mutations.

Another study assessed the SOD mutation in mouse models. The study found that phosphorylated Neurofilament H (pNF-H) is a good marker for ALS disease progression, as levels in blood increased over time. The question was then applied to human tissue through a partnership with Dr. Kevin Boylan at the Mayo Clinic. In ALS patients, there is a higher level of pNF-H as compared to controls. Other studies have confirmed the relevance of pNF-H. It may be important to study a combination of markers for ALS, rather than a single marker. pNF-H and C3 correlate strikingly with ALS compared to other disease areas. These examples highlight the importance of sharing samples among groups to validate findings and to make comparisons between animal models and human tissue.

A range of excellent animal models is available to contribute to the understanding of the ALS disease mechanism. The model systems allow researchers to examine many aspects of ALS. In particular, the animal models allow for work in real time, where human tissue is an endpoint. Without combining the work in animal models with work in human tissue, it will be difficult to prove that the pathways discovered are important to ALS. An abundance of interesting pathways are discovered in animal models, and the research community is challenged to validate those pathways in human tissues so that they can hone in on the pathways that are meaningful and potentially therapeutically relevant.

A new study by Piera Pasinelli and her colleagues focuses on the question of whether SOD1 may also play a role in sporadic ALS, as opposed to only in familiar ALS. This question is critical for the field, as clinical trials are on-going which shut down the production of SOD1 in mutant cases and familial ALS. If SOD1 is relevant in sporadic cases as well, then such therapies can be expanded. The Pasinelli study could not have been completed without well-defined human tissue. The over-oxidized forms of SOD1 were not found in all ALS cases, but were found in bulbar cases in particular. The study is interesting, but needs to be replicated using clearly-defined and well-characterized samples. The work was conducted in lymphoblasts.

Good sources of tissue include cerebrospinal fluid (CSF), plasma, lymphoblasts, brain and spinal cord, and fibroblasts. Collaboration with existing collections is critical, and it is also important to assure standardization and appropriate clinical and environmental data. Otherwise, studies may be fragmented. Access to the biorepository is vitally important because many researchers may not be adept at collecting specimens, but may have good ideas.

Discussion Points

- Dr. Bouzyk asked about environmental components and methylation signatures.
- Dr. Brujin replied that there is growing interest in this area in the field, although ALSA has not funded any work in it. NIH may have seen more approaches to it. The right tools are needed to address those questions.

Brief Presentations on ALS Biorepositories

Northeast ALS Research Consortium

James Berry, MD, MPH
Neurology, Massachusetts General Hospital, East
Charlestown, Massachusetts

Robert Bowser, PhD
Director, Gregory W. Fulton ALS and Neuromuscular Research Center
Professor, Divisions of Neurology and Neurobiology
Barrow Neurological Institute and St. Joseph's Hospital and Medical Center
Phoenix, Arizona

Dr. Berry pointed out that biobanking is important because of the potential to increase the quality and number of samples that are available, and to link clinical information. Biomarker discovery has numerous potential benefits as well, including facilitating clinical research by hastening diagnosis and entry into studies. A biomarker can potentially and ideally serve as a surrogate endpoint, and biomarkers can inform research into ALS pathophysiology. A biomarker serving as a surrogate endpoint does not necessarily need to speak to the underlying pathophysiology of the disease; rather, the biomarker could be something that changes over time in a predictable manner.

The overarching problem with earlier biobanks and biorepositories is that patient accrual for sample collection is slow and can limit biomarker studies. As a result, a number of concessions are made as collection schemes for biomarker research are created. Sample sizes in these studies tend to be small, and the sample collection and storage techniques have not been uniform, which can degrade the quality of the samples. There can be a lack of adequate clinical information and disease mimics. Therefore, there is often a lack of validation studies.

Biobanks can be built with these concerns in mind, utilizing strategies to overcome the limitations. For instance, a large biobank can be built to hasten access to pre-collected samples. Multicenter studies can be used to boost the number of samples in the biobank. Rigorous SOPs can increase procedural quality. Clinical information can be included with samples in the database, and appropriate disease mimics can be included. Samples should be shared with ALS scientists, allowing for validation of research and opportunities for new thinking. Alzheimer's disease researchers have worked hard to build multicenter studies and to standardize collection procedures. This work resulted in the identification of CSF biomarkers. It took a large, concerted effort on the part of the field to confront the challenges in Alzheimer's disease research, many of which are similar to the challenges that ALS research faces.

The NEALS Biorepository was built with the goal of creating a large, high-quality, standardized biofluid repository. Its principal aims are to reduce pre-analytical sample variation through the application of SOPs, link clinical data, identify and validate biomarkers, share samples, and maintain and expand the biorepository.

The first study that was actively designed to recruit patients and collect samples is called "BIO 001." Four groups of people were recruited for the study:

- ❑ Persons with ALS
- ❑ Persons with a pure upper or lower MND that could not yet be categorized as ALS
- ❑ Diseases mimics
- ❑ Healthy controls

The study built in a 7-day medication washout for non-essential medications to further reduce pre-analytic sample variation. At the first visit, demographic and clinical information was collected, and the ALSFRS was administered and databased. The samples included blood for serum and plasma, and whole blood for DNA and CSF, from those patients who agreed. Patients in the motor neuron groups had follow-up visits at 6, 12, and 18 months for ALSFRS and serum and plasma collection. There was no longitudinal CSF collection. A telephone follow-up was conducted every 6 months thereafter.

Thirty centers were involved in this study, and it was clear that technical factors could introduce enough variability to wash out potential disease variability. Strict and formal SOPs were put into place for sample collection and storage. The study was administered through the NEALS Coordinating Center in the same manner as a clinical trial would be run (e.g., the study structure, including training, communication, and monitoring, was similar to a clinical trial). Materials were managed centrally and shipped to the study sites to reduce variability. SOPs governed the storage of samples, which were stored locally and then batch-shipped to the Coordination Center, where they are stored in a central repository. Quality measures were not built into BIO-001, but have been included in subsequent studies.

The contents of the biorepository include almost 16,000 cryovials of plasma; 1200 vials of serum; almost 5000 vials of CSF; and almost 300 DNA samples. The patients include the following:

Banked Specimens	Baseline Plasma	6mo Plasma	12mo Plasma	18mo Plasma	CSF	DNA
ALS	200	127	78	43	98	115
UMN/ LMN	49	29	14	10	24	39
Mimics	105	-	-	-	43	71
Healthy	104	-	-	-	52	52

The study is not complete, as follow-up visits are on-going.

The NEALS Biorepository also includes legacy samples from completed NEALS studies and clinical trials that incorporated blood draws for plasma or serum. There is value to these samples, and different tracking mechanisms are used for them. The SOPs for collecting these samples are not standard, but they are recorded.

Clinical data are linked to the samples in the NEALS Biorepository. Information is collected on demographics, concomitant medications, compliance with washout, medical history, and disease information for motor neuron patients, including symptom onset date and location, date of diagnosis, family history, gene testing history, El Escorial Criteria, and ALSFRS-R. The repository does not include more extensive testing of respiratory function or strength because of a trade-off between time and complexity versus a measure of disease progression.

Regarding identifying and validating biomarkers, Dr. Bowser explained a number of on-going efforts using high-scale or high-level approaches looking at specific patterns or signatures within plasma or CSF. Metabolomic studies assess metabolic patterns and signatures within the sample. The medication washout period is important because the high-end approaches can identify where samples came from. NEALS tested a number of tubes from different manufacturers to consider the stability of the proteome within samples at given temperatures and over time. The results were built into the NEALS SOPs. Some of the first metabolic signatures were a byproduct of RILUTEK®, so a great deal of work was devoted to the specifics of the medication washout and the SOPs. Other on-going work includes targeted ELISA and gel-based techniques to validate protein biomarkers in blood and CSF. This work also validates other published work, and has revealed inconsistencies in published research. It is important to validate discoveries that have been made and published.

The NEALS Biorepository has also been moving in new directions. New studies include RNA, so those samples are being collected. Targeted analysis considers particular proteins and signatures reported in the literature. Longitudinal studies are also being conducted. NEALS has been engaged in blood-based longitudinal studies and is now looking toward CSF.

To support its goal of sharing samples, NEALS created a Biofluids Repository Committee. This large group of individuals includes representatives from the clinical arena, research, nursing, biostatistics, and other areas. An online application process allows researchers to request samples from the biorepository. Samples and linked clinical information are sent to researchers de-identified. The committee oversees the process and reviews the sample requests. About 20 requests for samples have been submitted recently, and most applications have been approved. The committee occasionally asks for additional information on the applicant's assay to demonstrate that they can reproduce it. In many cases, NEALS provides test sets to researchers to ensure that the assay is reproducible. NEALS wants to ensure that the samples will yield meaningful data and new information for the field.

The Biorepository is expanding in its centralized form and as a virtual repository. Samples are barcoded, and each site has a barcode reader. The central site then has access to details about each sample and each participant. New sample types are being collected to respond to new approaches in the field. NEALS serves as a national resource, given that international sample sharing is challenging. Other expansion considerations include longitudinal collection of CSF and blood. Post-mortem tissue is being collected as well, but SOPs and logistics of this collection can be challenging. Potential additional sample types include fibroblasts, saliva, urine, and imaging.

NEALS has engaged in a longitudinal biomarker collaboration with 6 sites. This work utilizes the NEALS SOPs, except a medical washout is not included. Fasting is encouraged, but not required. Atraumatic needles are used to reduce potential side effects. The virtual repository component of the database is being used, and the samples will be available to share with the ALS community. Some of the funding for this project came from NINDS, and requests for the samples are being received. It is important to remember that these samples are being collected for the entire community.

Department of Veterans Affairs Biorepository: Presented on Behalf of the VA ALS Registry

Edward Kasarskis, MD, PhD
Co-Principal Investigator
Cynthia Shaw Crispen Chair for ALS Research, Department of Neurology
University of Kentucky
Lexington, Kentucky

Dr. Kasarskis indicated that the VA ALS Registry is a longstanding collaborative effort. Its genesis lies in the question of whether military personnel who were deployed to the Persian Gulf War I carry an increased risk for ALS compared to military veterans who did not participate in the first Persian Gulf War. The formal study of this phenomenon lasted until 2002 and strictly focused on the epidemiological question of whether deployment to the Gulf War theater carried an increased risk of ALS. The publications from this study remain controversial.

Following and overlapping with that study was the VA's recognition that there may be a more significant problem with ALS. The VA funded the VA registry from January 1, 2003, until December 31, 2005. During the funding period, it was recognized that collecting blood for a parallel component would be desirable; thus, the VA ALS Registry DNA Bank was created.

The DNA bank collected DNA samples for the purposes of research on genetic and environmental interactions as a genesis of ALS. The veterans who came forward with diagnoses of ALS were not examined by the leaders of the DNA bank. Instead, a team of individuals from the VA ALS Registry examined their medical records to determine diagnostic certainty. The screening questions for the ATSDR registry are based on questions that were developed in the context of the VA registry. The core of cases examined by several neurologists formed the gold standard. Participation in the DNA bank required a separate consent form from the registry.

Collecting samples was challenging for a number of reasons. The individuals lived all across the country. The sample was a prevalence sample, not an incidence sample. Some participants were too ill to travel to their nearest VA location to donate blood for the purposes of a DNA bank, so the strategy was to go to them. A nationwide visiting nurse service was contracted by the VA to collect these samples. The nurses were accustomed to providing clinical care, not collecting research samples. Therefore, they had to be trained to perform a different function and to handle samples appropriately. Contractual logistics were challenging.

All VA ALS Registry participants were asked to participate in the DNA bank via a separate consent form, which was mailed to them. The DNA is saved only for use in ALS studies and cannot be shared with researchers who may wish to use the samples as a disease control. These limitations were imposed by a variety of organizations that reviewed the consent form.

The effort was successful because samples were collected in individuals' homes. Very ill individuals were given priority. The ALSFRS indicated which patients were more advanced, and they were reached for samples first. The logistics for prioritization required an on-going dialogue with the nursing service. The contracted service standardized the training program, but because the company's mission is clinical service provision, they needed to be reminded that their function in this case was research-based.

The DNA collection proceeded smoothly, in general. Venous blood samples were desired, and the backup sample was buccal scraping cells for people who had poor venous access. Supply kits were provided to the nurses, and samples were shipped to the Boston VA, where the DNA was extracted. The Boston VA is the physical repository for the samples. The DNA Coordinating Center maintains the link between the clinical and genetic data. The arrangement follows VA protocol.

Of the approximately 2000 individuals who were contacted, the vast majority agreed to receive a consent form for the DNA Bank via mail (n=1993). Approximately 1600 consent forms were received, and approximately 1200 samples were collected. Despite the logistical challenges associated with collecting samples, the response was very good and is indicative of working with a prevalence sample.

The DNA Bank was supported by $2.1 million from 2003 through 2005. The cost per patient is approximately $1400. Thus far, the primary end user of the bank has been Silke Schmidt, PhD, Duke University, for her Genes and Environmental Exposures in Veterans with ALS (GENEVA) study. The VA has imposed restrictions limiting the sharing of DNA samples outside of the VA, but those access restrictions have been relaxing so that other researchers may be able to use the collection.

Dr. Kasarskis observed that in general, ALS patients want to contribute samples to this effort. People who have ALS may not be able to participate in clinical drug studies because of their location or because of a lull in the flow of clinical trials, but every ALS patient can participate in the DNA Bank. The VA DNA Bank was built via telephone contact with the veterans, and there was a high degree of enthusiasm among participants even without a face-to-face relationship. He receives frequent phone calls from patients who want to donate their bodies for research purposes. Financial and logistical support is needed to help them make those contributions.

The bank is a research activity and is funded as such. There have been attempts to include quality-of-life measures from questionnaires completed by clinicians, but those unfunded duties have not been successful. Further, the DNA bank must be user-friendly for patients and families, who work extremely hard to meet the physical and emotional needs of ALS patients. While the patients may want to contribute, there are limits on what they can do.

Academic recognition for clinicians who assist with the blood samples and who provide the phenotypes is important. The phenotype is critical, and it might be worthwhile to consider an online repository to reference the academic contributors to the samples. These approaches will expand the community and build buy-in.

Carolinas Neuromuscular / ALS Center Blood Tissue Biorepository

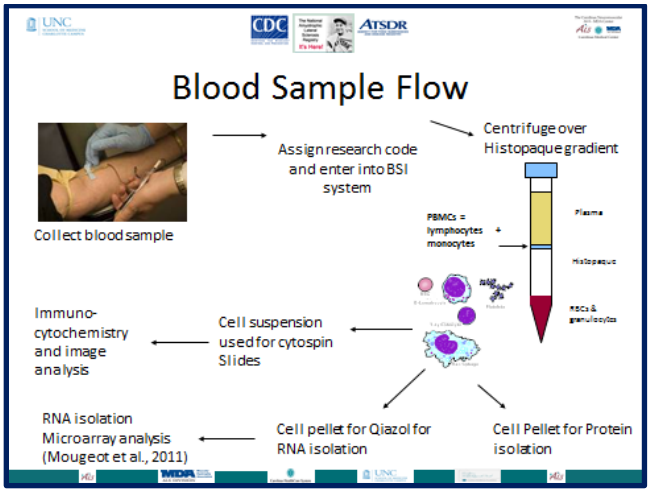
Benjamin Rix Brooks, MD, Director
Carolinas Neuromuscular/ALS-MDA Center
Department of Neurology, Carolinas Medical Center
Adjunct Professor of Neurology, University of North Carolina School of Medicine
Charlotte, North Carolina

Dr. Brooks explained that the purpose of a biorepository is to collect a plurality of different samples that will allow the development of a biomarker. A biomarker is necessary to identify information with regard to diagnosis, rate of progression, or response to treatment.

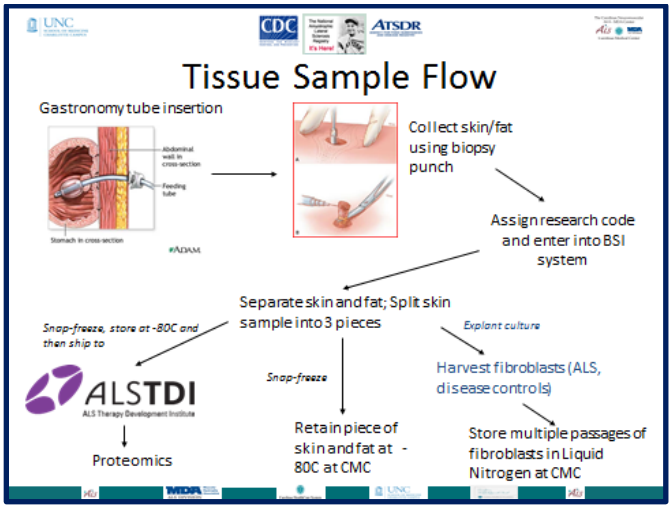
The Carolinas Neuromuscular / ALS Center Blood Tissue Biorepository collects many of the same samples collected by NEALS. In addition, they collect peripheral blood mononuclear cells (PBMCs) that contain lymphocytes and monocytes that may demonstrate different reflections of the pathogenesis ongoing in ALS. Lymphoblasts can indicate different aspects of the pathology represented in the peripheral blood compartment. CSF is important, and in addition, the biorepository collects skin and muscle samples from controls looking at genome expression data.

Samples are collected under IRB protocols and SOPs that are identical to NEALS. Clinical data are collected via self-report questionnaires that are completed by all participants. All subjects with ALS are characterized clinically in terms of a variety of phenotypes. Disease controls and healthy controls are also included.

A Biological Specimen Inventory system maintains the repository and tracks all samples. The Biological Specimen Inventory system is used for all biological specimens at the Carolinas Medical Center and gives staff the ability to track all systems. Data entry is feature-rich and can be completed at locations throughout the Center. There is a tool for managing requests and tracking workflow of samples internally and potentially externally, as well as for tracking shipments. An example blood sample flow is depicted below:



An example tissue sample flow is depicted below:



Discussion Points

- Dr. Bouzyk asked for more detail regarding the collection of information, especially epidemiological information, about patients who will be included in the biorepository. The ALS Consortium of Epidemiologic Studies (ACES) questionnaire could be a good start to collect material and to subset cases for future research not only on demographic information, but also on environmental exposure, lifestyle, and other factors.

- Mr. Tison said that the ALS Association has a list of 22 sites for brain and spinal cord banks. At least a few of these sites are specifically ALS banks. He asked whether cooperation or replacement, and not competition, should be expected with existing banks and registries.

- Dr. Horton replied that part of ATSDR's effort includes learning about the registries and banks that already exist. He expressed his hope that their efforts would complement on-going work in the field and noted that it was too early in the process to think about integration.

- Regarding the potential for people with ALS to be included in multiple biorepositories, Dr. Sowell suggested adding a module asking if the participants have already participated in a biorepository. Then it might be possible to identify specimens in other biorepositories and to create a mechanism for linking data in the registry to specimens that are already in various biorepositories. Further, the approach could help with the problem of de-duplicating specimens from the same people that are in various biorepositories. The community can address this issue in a coordinated fashion with the appropriate interactions and data collection.

- Dr. Corriveau agreed that people should be asked if they are participating in another bank or registry. Initially, it would be beneficial to acquire DNA from whole blood as well as saliva. Genetic material can then verify whether samples are banked in other biorepositories.

- Dr. Brujin added that it is important that the national registry ensure that patients feel that they can be part of other, on-going efforts without conflict. She wondered how to help promote the VA registry, as the constraints for using that registry seem to be changing.

- Dr. Kasarskis answered that the changes in the VA registry are dependent on the VA approval structure. When it is appropriate to advertise the registry and to promote the mechanism for accessing samples, it will be important to do so.

- Dr. Bowser said that while linking samples in the national bioregistry to samples that have already been collected via other mechanisms will present logistical problems, the linking should be done. It is important to demonstrate to patients and the field that the different repositories and researchers are working together. Linking federal information to clinical information at sites around the country should be addressed proactively and early in the process. He felt that most people who have been involved in collecting samples would be supportive of linking information.

- Dr. Horn said that it will be important to discover duplication, especially in familial cases. In rare diseases, reference and validation populations often overlap, which does not advance the science. The patient community and patient advocacy groups will be important partners in building ways to link information. Regarding the national biorepository, issues of governance, sharing, and access are critical issues. The science of sample collection is important, and many great scientists are engaged in that work. In creating the biorepository, they must not forget about the people with ALS who contribute samples, who would be disappointed if their contributions were not used by the field.

- Dr. Horton agreed, noting that ATSDR is not conducting its mechanism for using the registry for clinical trials in a vacuum. A panel of internal and external experts is advising them in that work, and he envisioned a similar structure for a national biorepository.

- Dr. Berry said that if samples are to be cross-linked, input from a number of IRBs from different institutions will be needed. Different ethics review boards could take different viewpoints. He suggested sampling various IRBs around the country early in the process to learn about potential conflicts and differing opinions. Scientifically, genetically identifying samples is a good idea; however, the idea is very complicated from an ethics perspective. IRBs may have concerns with identifying samples in de-identified pools of biofluids, linking them, and then re-de-identifying them.

- Mr. Tison asked how far 1 blood donation, 1 CSF sample, and 1 skin sample can be spread across separate studies over time. He also asked whether it is realistic for families of deceased individuals to want study feedback.

- Dr. Bowser answered that in the case of blood and CSF, a few milliliters of fluid are collected. Those samples are split into different tubes with various amounts of fluids. A study could use 1 of those tubes, so a patient could provide sufficient material for anywhere from 10 to a few dozen studies. DNA and postmortem tissue are relatively stable and can be stored and used for some time; however, depending on the type of analysis, biofluids have a lifetime. He preferred an approach that ensures that fluid samples are used while they are viable, and stressed that allowing access to samples for studies is important.

- Dr. Cross added that the use of samples also depends on the data sharing agreement of the institution. For instance, her repository requires that all data from a sample is shared with the repository for use in other studies.

- Dr. Berry said that fibroblasts from skin biopsies can be used indefinitely, and is important that the consent process makes that clear. Regarding sharing information with patients and families, he said that banks are usually built in a de-identified way. It is therefore difficult to link specific samples to specific results. At the same time, results from samples from the bank can be tracked so that patients and families can understand the importance of what the bank is accomplishing.

- Dr. Brady added that brain banks can give specific feedback by providing neuropathology reports to participating families. His brain bank also provides consultation with a neuropathologist to explain the report. Newsletters and websites are general ways to share information with patients as well. He noted that well-written and broadly-conceived consent forms are a crucial piece of their work.

- Dr. Kasarskis added that IRB consent can be a significant issue, especially when working with multiple institutions. If institutional buy-in is present, then a centralized IRB could be used for the purposes of contributing to a national effort such as the biorepository. The Neuro Next Clinical Trial Network applications require institutional agreement to use a centralized IRB. Institutions may need to be educated about this approach.

- Dr. Corriveau said that cross-disease relevance has been important for ALS research, and consent and sample use should take these approaches into consideration.

Lessons Learned from Biorepositories

Marshfield Clinic Biobank

Catherine McCarty, PhD, MPH
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Dr. McCarty shared her experience with the Personalized Medicine Research Project (PMRP) at the Marshfield Clinic in Marshfield, Wisconsin. The ultimate goal of the PMRP was to collect information to truly personalize healthcare in the long-term. Its short-term goal was to establish one of the first population-based biobanks in the US to conduct research in genetic epidemiology, pharmacogenetics, and population genetics. The bank includes samples from 20,000 individuals and is part of the Electronic Medical Records and Genomics (eMERGE) Network, funded by the National Human Genome Research Institute (NHGRI).

Marshfield is a town of 20,000 located in the center of Wisconsin. The Marshfield Clinic is a large healthcare system. The Marshfield Epidemiologic Study Area (MESA) is centered in Marshfield. Periodic validation studies have shown that nearly everyone in MESA seeks all of their healthcare through the Marshfield Clinic, and a shared electronic health record system with the hospital captures inpatient and outpatient information. Several generations are represented in the biobank. The system of care includes all specialties and subspecialties, save whole

organ transplant, and has had internally-developed electronic health records since the late 1960's. A research foundation has strong programs in genomics and clinical research. More than half of the Marshfield population, and about two-thirds of the Medicare-age population, belong to the health maintenance organization (HMO), allowing for capture of health events that occur outside the system.

Creating the biobank required a great deal of consultation and education at multiple levels. The consultation and education process included a Community Advisory Group (CAG), a Scientific Advisory Board (SAB); an Ethics and Security Advisory Board (ESAB); focus group discussions; community talks; media releases; a health message video for waiting rooms; and an employee newsletter. Planning began in 2001, and recruitment began in September of 2002. The focus group process included opportunities for commentary on the materials and for feedback on the large-scale DNA biobank. Separate focus groups were created for employees. Half of the working-age adults in Marshfield are employed by the clinic or the hospital. Overarching themes that emerged from the focus groups included the following:

- Trust in the Marshfield Clinic
- Opposition to human cloning, which was an issue in the lay press at the time
- Concern about insurance discrimination, especially given the Marshfield Clinic-owned insurance company
- Confidentiality of data, particularly clinical data (employees were especially concerned about this)
- Some would never participate regardless

The focus groups recommended clarifying data security procedures and keeping the materials very simple. The biobank's security provisions included one-way data encryption and a number of physical and non-physical security measures. The eMERGE network focuses on data security and on the level of data that is shared with the smallest risk of re-identification; however, the most significant risk for re-identification is internal. Every healthcare system conducts regular audits to determine who is looking at medical records. Under the Marshfield policy, anyone with access to the medical record could not have access to the linked genotypic and phenotypic data. Wisconsin also had strict data confidentiality laws in place. The project also obtained a Certificate of Confidentiality from NIH.

More focus groups were conducted in 2003, after recruitment for PMRP had been on-going for about 1.5 years. It was important to assure the IRB that the groups were not intended to recruit for participation in the biobank. The focus groups addressed non-participants in the biobank. The main reason given for non-participation was a lack of interest. Two primary concerns emerged from those groups, 1) the materials for the biobank were dense and discouraged people from participating; and 2) confidentiality concerns, especially from employees. Additionally, some people were not aware of PMRP. The groups suggested making the materials more concise and offering more financial incentives to participate. The participants were compensated $20 for their time. PRMP responded by creating simple newspaper inserts and posters with information about the program, with input from the CAG.

The CAG includes 20 members who represent a cross-section of the community. They meet about 3 times a year to provide feedback on all aspects of PMRP, such as protocol changes and newsletter content. Early in the process, when NHGRI had provided funding for participation in the eMERGE network and the Database of Genotypes and Phenotypes (dbGAP), a broad consent form was needed to allow for the sharing; however, dbGAP did not exist when the PRMP consent form was created. The CAG expressed initial hesitation because

the government would be responsible for keeping the data confidential, but overwhelmingly, the group supported sharing the data. They suggested creating a newsletter article on the subject and asking for response. The newsletter is sent 3 times a year to about 12,000 homes. Only 1 person did not want to be included in dbGAP and withdrew from the project. That response validated the decision not to re-consent all participants in the project, so a waiver of informed consent was obtained through their IRB.

CAG discussed stored pathology samples as well. When there is broad consent to access the medical record, the access does not extend to stored pathology samples unless specifically stated. Access is available to the pathologist's interpretation of those samples. PMRP could either apply for a waiver of informed consent or re-consent the 20,000 participants. The CAG was initially surprised that the samples were saved for so long, and expressed a desire to have those samples used for good science. The group recommended against committing time and financial resources to the re-consent process. The information was shared in the newsletter, and the response to the newsletter was positive. A waiver of informed consent was obtained.

A joint meeting was held with the CAG and the ESAB. The meeting focused on the issue of access to prospective residual clinical blood samples, especially for sequential samples of serum and plasma. The initial consent form did not address this issue. The CAG was supportive, and the ESAB was concerned and felt that re-consent was necessary. Another newsletter article was generated, and the group also suggested sending a specific letter to participants about the protocol changes and ongoing studies. A small percentage of participants elected to withdraw from the study for various reasons.

The study logistics included active recruitment and consent of MESA residents aged 18 and over. The newsletter includes a letter of introduction and a reminder to have blood drawn. The reimbursement includes $20, and an additional $10 for an extensive dietary history questionnaire and Baecke physical activity questionnaire.

The process for accessing biobanks should not be tedious. An initial feasibility request is made, and the project undergoes scientific merit review, either through a funding agency or internally at the Marshfield Clinic. IRB approval is obtained for all studies. An Oversight Committee reviews the project as well, as the biobank is a relatively limited resource. Funding is required for phenotyping and identification and retrieval of samples. Phenotypic and genotypic data are returned to the PMRP within 6 months after completing analyses.

Approximately 20,000 people are currently enrolled in the PMRP, aged 18 through 102; 57% of participants are female, and 98% are European-American. The biobank includes DNA, plasma and serum samples as well as information on dietary intake and physical activity. Participants have consented to allow sharing of de-identified data and samples. There is access to the extensive Marshfield Clinic Electronic Health Record. The project has the ability to re-contact subjects. Genome-Wide Association Studies (GWAS) data are included for approximately 5000 subjects, and a molecular fingerprint project has been conducted for all subjects. Whole genome sequencing has been completed for 24 subjects.

Lessons learned from the PRMP include the following:

- Multidisciplinary collaborations are key.
- Informatics research and support is critical and must keep pace with genetic technology for discovery and translation.
- On-going community consultation at all levels is vital.
- Regular direct contact with all subjects should be planned.
- It is more efficient to collect information up-front rather than to reach participants after they have enrolled.
- Process samples under Clinical Laboratory Improvement Amendments (CLIA), which is important for returning results and using the samples for healthcare purposes.

Information about the project, including consent forms and ongoing projects, is available at www.mfldclin.edu/pmrp.

Genetic Alliance Biobank

Liz Horn, PhD, MBI, Director
Genetic Alliance Registry and BioBank
Washington, DC

Dr. Horn commented on how technology, informatics, and other advances and factors have changed the landscape of research. The single-condition, siloed collections of the past will not advance science. Cross-disease, multi-condition collections are needed. It is important to have shared infrastructure, group governance, and protocols for standardized data. Further, data are needed from multiple sources. A relatively new phenomenon is the emergence of the "citizen scientist" movement, with dynamic involvement of consumers, advocates, and citizen scientist. Technology drives this movement, and there are many opportunities. In the past, drug development progressed in a linear fashion. Today, translational science operates like a network. Registries and biobanks are critical to advancing science. Despite challenges in funding and other areas, great opportunities exist for sharing and collaboration.

The Genetic Alliance Registry and BioBank (GARB) was created in 2003 with the goal to provide a mechanism to collect well-characterized and well-annotated biospecimens with the necessary clinical data. This work is conducted in the realm of rare diseases. It is challenging to find participants, and scientists are located in different places. Sharing can be limited. GARB created an infrastructure and logistics to collect data and biospecimens and to secure research collaborations, assemble cohorts, and provide expertise. Different advocacy groups administer registries and biobanks through the system. In this model, the groups own, govern, and steward their own samples. Often, staff scientists and members of academic collaborate with the groups.

Specimen collection for the biorepository, which mainly consists of blood collection for DNA, is logistically straightforward. Tissue collection can be more complicated. The system includes different mechanisms for donation, as different groups need different materials. The system operates as a "research institute without walls," providing opportunities to collect samples and data, including provider-entered data and patient-entered data. The cost of provider-entered data can be a challenge. GARB has its own IRB and broad consent forms with the ability to re-contact participants. Participants can agree to share samples only for their condition, other related conditions, or broad conditions. Prioritization of samples is important, particularly in rare samples. Participants are active. The GARB model is unique because it is able to be nimble

and to address the needs of different groups and their research interests. Flexibility is an important part of the approach.

When building a biobank, considerations of collection, processing and storage, and access are important. It is also important to think early in the process about how the samples will be used.

Collection considerations include:

- ❑ Types of samples needed
- ❑ Kinds of donors needed, and inclusion and exclusion criteria
- ❑ Timing of the collection, which can be critical at the point of care
- ❑ Where samples will be collected
- ❑ When will samples be collected
- ❑ Who will collect the samples
- ❑ How often will samples be collected
- ❑ Whether other similar sample collections exist, and how samples can be shared

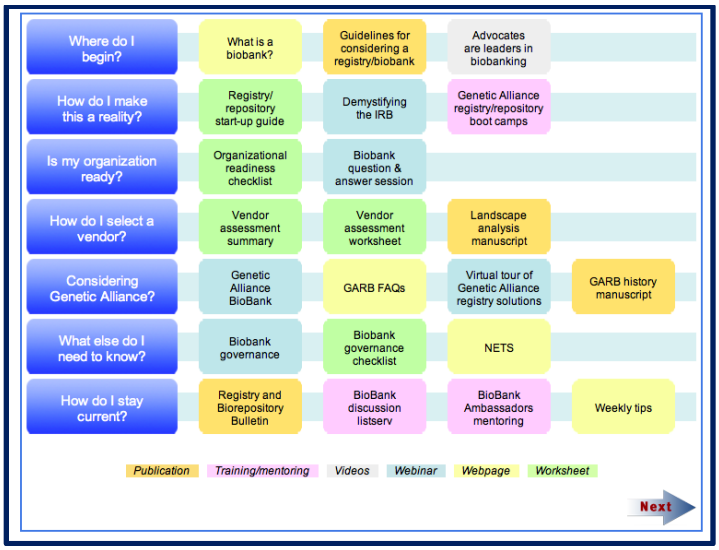
Processing and storage considerations include:

- ❑ Where samples will be processed
- ❑ How samples will be processed
- ❑ Where samples will be stored
- ❑ How samples will be accessed
- ❑ Types of experiments samples will be used for
- ❑ SOPs for extraction for storage

Access considerations include:

- ❑ Who has access to the samples
- ❑ Who determines access to the samples
- ❑ What the protocols will be (Through the Alliance's IRB protocol, all data are de-identified. The patient advocacy group that serves as the steward of the data has the opportunity to recontact as needed)
- ❑ What the protocols are for distribution
- ❑ What the governance mechanism will be
- ❑ How results will be returned, at least aggregate results
- ❑ How will samples be used

GARB created a criteria and scoring scheme for vendor assessment. They conduct frequent evaluations of registries and biorepositories, and they have moved their biobank multiple times. The scheme assigns points to scalability and clinical data, which is sometimes more important than the biospecimen. Data standards and linkage, as well as the samples and quality control, are assessed in the scheme. Very few vendors have both registries and biobanks, and it is important to link data to samples. The following toolbox is helpful in starting a registry or biobank:



GARB generated a Webinar on demystifying the IRB as well as other tools and tips, including a startup guide of 53 questions.

Dr. Horn shared the following lessons that GARB has learned:

- ❑ Do not duplicate efforts, especially given that biobanks are expensive and administratively burdensome.
- ❑ Develop partnerships to share data and resources where possible.
- ❑ Ensure that the biobank is sustainable over time.
- ❑ Use common data fields and controlled vocabulary to allow comparison with multiple data sets.
- ❑ Follow best practices.
- ❑ Retention is critical, because it is harder to recruit new participants than to keep those that are participating.
- ❑ Prior proper planning prevents poor performance.
- ❑ Stewardship is vital.
- ❑ Good partners are essential.
- ❑ People matter.
- ❑ Involve advocacy organizations and the community.

Advocacy organizations understand the unmet needs of the community and can develop trust within the community. They can leverage scarce resources and facilitate collaboration between stakeholders. Community members and advocacy groups are committed to the cause and serve as good stewards of the collection.

Discussion Points

- Dr. Bouzyk commented on the regulatory aspects of the biobanks, which will be important in the future.

- Dr. Boylan said that his institution's IRB has permitted his team to provide DNA test results to patients who contributed DNA in a research study. Those collections were not conducted in a CLIA facility. This work raises the issue of what constitutes healthcare, and what

constitutes information. Patients who are not necessarily being treated or managed are made aware of the results of the research, and they tend to be appreciative of the information. A staged process gives patients an opportunity to agree to receiving the information, and if interest is not expressed at the point of collection, then the information is not offered. More commonly, the patients ask for follow-up information and genetic counseling, when appropriate.

- Dr. Bowser said that collecting samples for research purposes as opposed to diagnosis may preclude using a CLIA setting. He asked Dr. McCarty about the letters that were sent to participants regarding participating in ongoing studies. Few participants opted out of the project, and he asked how it was ensured that the participants read and received the letter.

- Dr. McCarty answered that the ESAB discussed whether a response would be required. A stamped, addressed postcard was included with the letter to facilitate response, but there is no guarantee that the letter was read.

- Ms. Bledsoe asked about the types of research data have been received by PMRP and how the varying formats and documentation of research protocols were handled.

- Dr. McCarty responded that external collaborators do have access to de-identified phenotypic data, which comes from the electronic medical record and is processed internally and well-documented. They do not have direct access to the electronic medical record. External investigators share genotyping data, which comes from different platforms. The bioinformatics group developed a template for how that information is documented.

- Dr. Cross added that questionnaires as well as serum and plasma data could also be received. The serum and plasma data are documented according to its platform and the testing that was conducted.

- Dr. Corriveau asked how many full-time employees (FTEs) are associated with the PMRP. Data sharing in the proposed national biorepository will take a different form from the contact conducted by the PMRP.

- Dr. McCarty answered that the initial recruitment into the project included 2.5 FTEs in the laboratory, 10 FTE research coordinators, and 1 part-time genetic counselor. Their infrastructure does not include direct return of information.

Technology for Sample Collection and Processing

Scott Hixon
Associate Director, Technical Services
Fisher BioServices
Rockville, Maryland

Mr. Hixon explained that Fisher BioServices is one of the world's largest biorepositories. They manage a number of commercial and governmental biorepositories. Many biorepositories began in single labs and the samples may not have been monitored properly. The inventory system may be inadequate, and samples may be improperly labeled. Frequently, access to samples was not controlled. Emergency procedures may not be in place, and back-up power

may not be available. Fisher is often contacted by these small biorepositories after a freezer has malfunctioned, and the infrastructure is not present to support the biorepository.

Mr. Hixon reviewed the "basics" of biorepositories, pointing out that redundancy is needed in freezer and refrigerator storage space and emergency back-up power. Service plans or on-site engineering should be in place. Equipment should be validated and serviced on a regular basis. An inventory system for sample management should be able to support the project, and if the work has clinical implications, then the system must be compliant with Title 21 of the Code of Federal Regulations (CFR). The inventory management system should allow on-line sample request and destruction. The facility should have security measures to include 24-hour-a-day, 7-day-a-week monitoring. The repository should have an escort policy. Back-up storage capacity should be available for all temperature ranges. Temperature should be monitored at all times. Data should be collected at least once per day, and an electronic data system can collect continuous alarms.

There are many different types of repositories in the US. Private repositories are built for internal use and for the company's internal needs, such as a pharmaceutical company. Some commercial biorepositories are created for local communities, while others have an international presence with multiple locations. Different inventory systems are used by commercial biorepositories, and they should be capable of integration with other systems. Commercial repositories tend to be more flexible than other types, and they have a wide range of storage capabilities. Some commercial biorepositories have complementary services such as laboratory, kitting, storage, distribution capabilities, clinical trial support, direct billing to investigators, and drug packaging. Some commercial biorepositories are self-supporting. Examples of government repositories include the National Cancer Institute (NCI) Clinical Repository in Frederick, Maryland; and repositories maintained by the Women's Health Initiative (WHI) and the National Children's Study (NCS). Government repositories may or may not be open for other uses.

Repositories in the future will likely be fully automated. There are limitations to using an automated system, as systems are limited in certain conditions such as liquid nitrogen and -81s. So far, the automated systems only handle liquid samples. Future repositories are likely to utilize automated processing. These processes can sometimes be integrated with samples for biorepositories. Manual interaction is still required for some processes.

Storing samples is only half the battle. Collecting, transporting, and processing samples is just as important to the long-term success of a repository. The design of the repository should consider what will be collected and what the downstream applications of the samples will be. Understanding the study will have an impact on how samples are collected, such as central versus random collection; one-time versus multiple collections per subject; and local, central, or multiple site processing. Other considerations include how data will be connected to the sample, and labeling samples correctly.

Collection inconsistencies can be avoided by providing standardized collection kits, especially if collection will take place at participants' homes. The kits should include all supplies for collection and shipping. Collection forms or electronic data capture can be used. Electronic data capture is easier at a collection site, as opposed to at a participant's home. The collection kits should include pre-labeled tubes, containers, forms, and well-written collection procedures.

The physical collection is more likely to be successful if the kit includes detailed instructions. Samples should be collected in a specific order. A butterfly apparatus is used to collect samples with liquid preservatives, and the patient's positioning is important. The collection form should be completed as soon as the samples are collected. The form can double as a shipping manifest, and it should be pre-labeled and specific to the kit.

Collection kits should be designed to support the project correctly. Patient specific-kits could be used to link data in advance, or kits could be linked at a later time. Stock kits can also be used, or "just in time" kits can be created at the time that it is requested. Kits should have the flexibility for large or small projects. Because the samples will be collected at participants' homes, some of the processing cannot occur at the site. The samples must be sent to a central processing facility, and this approach limits the types of samples that can be collected. Shipping supplies and instructions should be included, and air bills should be pre-filled.

A number of processes can be applied to the samples, including blood fractionation and aliquotting. If buccal cell or saliva samples are obtained, then limited processing is required. DNA and RNA samples can be processed when the sample is received, or samples can be stored and processed later. Unless they are going to be stored, samples should be processed on the day they are received by the laboratory. After processing, the samples should be placed in permanent storage. The inventory should be updated, and online access is important. Data can be made available via automatic uploads or interaction through Web-based services or other systems.

Discussion Points

- Dr. Brooks asked about data on failure and whether samples were lost.
- Mr. Hixon answered that Fisher has data on how long their generators have had to run. They have operated for 48 hours. Samples can be monitored, and thus far, no samples have been lost. Freezers do fail, and mechanical freezers are more apt to fail than liquid nitrogen freezers.
- Dr. Horn said that GARB uses a commercial repository vendor because of the important logistics associated with collection and storage. She noted that when kits are shipped to participants' homes, a contact person should be available to answer any questions that participants might have.
- Mr. Hixon agreed, noting that depending on the nature of the study, repository staff can serve as a point of contact, or the sponsor of the study can fill that role. Dr. Horn added that the protocol includes a chain of command.
- Dr. Horton commented that it has not yet been decided whether mail-out kits would be used for the National ALS Registry Biorepository. They are exploring all options.
- Dr. Corriveau asked about samples that would be suitable for collection at home, other than buccal swabs.
- Mr. Hixon replied that blood samples can be collected, but they should be sent back the same day that they are collected. It is important that at-home collection should be as easy as possible for the participants. For instance, all samples should be sent at the same

temperature. Fisher participates in studies that are nurse-based, in which participants draw their own blood, and other studies include on-site phlebotomy services at homes.

- Dr. Horn added that her group has conducted outreach events with an on-site phlebotomist and has sent a phlebotomist to a participant's home.
- Dr. Horton asked whether finger-pricking kits could be used. Mr. Hixon said that Fisher does not usually provide finger-pricking kits, as larger volumes of are usually needed. Dr. Sowell noted that in order to be successful, a blood spot needs to be collected properly by a trained person.
- Dr. Bouzyk asked whether Fisher has conducted research into evidence-based biobanking. Mr. Hixon said that Fisher does not conduct research, but they partner with others to do that work.
- Dr. Vaught reported that the Office of Biorepositories and Biospecimen Research at NCI funds biospecimen research into methodologies and evidence-based practices. They hold an annual symposium on these issues and they fund contractors to conduct studies for evidence on how to best collect, process, store, and disseminate samples. Much of the work has not been published or widely disseminated.
- For transparency, Dr. Horn noted that Fisher is not GARB's vendor.

Presentation of the Biorepository Protocol

Marta Gwinn, MD, MPH
Senior Consultant for Epidemiology
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Dr. Gwinn reminded the group that the meeting's goal was for the expert panel to provide input into the draft biorepository protocol and to provide advice on key aspects of the protocol, such as the types of samples to be collected and the sample size. Although their focus should not be on logistics, it is impossible to completely separate logistics from scientific objectives.

The goals of the National ALS Registry Biorepository Pilot Study are to maximize the potential utility of the samples that are collected for scientific research, given registry parameters. This project is being constructed within the context of the existing registry program. The pilot study should maximize cost-efficiency, which is crucial to the sustainability of the project. The pilot study will make recommendations for long-term sustainability and will recommend a process for providing research access.

No single study or database can provide all the answers about ALS, even basic data such as how many people have ALS, or the underlying genetic and environmental causes of the disease. Other questions pertain to how understanding those causes can lead to prevention and treatment, and the biomarkers that are useful for predicting disease progression and treatment response. An integrated approach is needed to answer all of these questions, bringing together findings from epidemiologic, clinical, and basic research.

The National ALS Registry Biorepository could add to on-going efforts. The project presents the opportunity to correlate biomarkers with the extensive epidemiologic data that are collected with the registry. The project also presents an opportunity to enroll a nationally representative, population-based sample of people with ALS. The sample will not be selected by geographic area, exposure, or clinical characteristics. The biorepository could add to the total number of biological specimens available for research on ALS.

Dr. Gwinn presented classes of environmental factors that have been proposed for studies of ALS susceptibility:

Proposed Risk Factor	Example / Potential Biomarkers
Infectious agents	enterovirus PCR for enterovirus RNA in CSF, brain or spinal cord
Pesticides	organophosphates paraoxonase enzyme activity, *PON1* genotype (blood)
Metals	lead, mercury brain, blood, CSF levels of metals and organic compounds
Drugs, chemicals	formaldehyde cytogenetic biomarkers
Injury	head trauma A-beta protein deposition in neurons, *APOE* genotype

New research findings in the genetics of ALS have changed the way the field thinks about the disease. These breakthroughs have been possible because it is relatively easy to study genetic variation in relation to health outcomes.

Dr. Gwinn presented a table based on a recent article that described a “roadmap” for biomarkers in ALS research. Skin and muscle samples are not included on the table because the Bioregistry proposes to collect samples from participants outside the clinical environment. CSF will not be collected onsite. Each sample has comparative logistical and scientific advantages and disadvantages. Some samples are uniquely valuable for certain purposes. It is important to create a proposal that balances the types of samples with the types of anticipated analyses. The panel should discuss the relative merit of these samples.

Characteristic	Blood*	CSF	Urine	Saliva
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	+++	+	+	++
+++ strong; ++ moderate; + weak				
*Plasma versus serum needs to be specified; serum may have advantages for the stability of some proteins, e.g. immunoglobulins. EDTA sample will be needed for DNA or RNA studies.				

Dr. Gwinn shared a proposed minimum specimen collection, and noted the potential for self-collected specimens. Some collected specimens could be desirable for certain analyses and as fallbacks if other samples are not possible:

Collection priority	Sample preservative	ml	Fractions	Examples of potential analyses
Blood				
1	EDTA	9	White cells, red cells, plasma	DNA, proteins, red cell lipids
2	Clot activator	8	Serum	Clinical biochemistries, metabolic products, other small molecules
3	Acid citrate dextrose	6		DNA, RNA, immortalized lymphocytes
Urine		9	--	Electrolytes, environmental chemicals, metabolic products, gut microbiome
Potential self-collected specimens				
Saliva		2	--	DNA, others?
Urine		9	--	Electrolytes, environmental chemicals, metabolic products
Nail clippings		--	--	Metals
Hair clippings		--	--	Metals

The main goal of the pilot project is to develop the National ALS Registry Biorepository as a research resource. The priorities are to collect biospecimens that complement the types of data that are in the registry. For example, the registry does not include imaging data, but does include occupational and environmental data. The pilot project should allow for comparisons or combinations with other studies in order to maximize scientific utility. The collection should be "future proofed" as much as possible to anticipate technologies or research priorities that could develop in the near future.

Presentation of Brain Bank Protocol

VA Cooperative Studies Program VA Biorepository Brain Bank (CSP501)

Christopher "Kit" Brady, PhD
Director, Scientific Operations
VA Biorepository (151C), VA Boston Healthcare System
U.S. Department of Veterans Affairs
Boston, Massachusetts

Dr. Brady described his experience with the VA Biorepository (VAB) Brain Bank, a national ALS brain bank. He reminded everyone that the VA became involved with ALS and brain banking because ALS has been linked to deployment to the Persian Gulf and military service in general. In 2006, the Scientific Advisory Committee of the VA ALS Registry requested that the VAB Brain Bank be initiated by the VA Cooperative Studies Program to collect brain and spinal cord tissue from Veterans in the ALS registry. The VAB ALS Brain Bank (CSP 501) is coordinated at the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC) at the Boston VA. MAVERIC is responsible for enrollment and patient follow-up, as well as

coordination of tissue recovery throughout the US. Tissue samples are shipped to the Southern Arizona Core Tissue Laboratory (SACTL) in Tucson, Arizona. Diagnostic neuropathological analyses are conducted at the VAs in Bedford and Boston, MA. Those reports are made available to patients who are interested in receiving it.

The brain bank received names from the VA ALS Registry and contacted participants during the time that the registry was open. Subsequently, the brain bank has developed a new initiative with the Boston VA neurology department and has started to work with other VA neurology departments across the country. Enrollment begins with a description of the study on the telephone. Interested patients are mailed a consent form, which is reviewed over the phone. The patients and next-of-kin sign the form, and next-of-kin buy-in from the beginning of the process is very important. When the consent form is received, medical history is collected, including a baseline ALSFRS. The ALSFRS is administered semiannually in order to collect longitudinal ALSFRS data. Frequency of follow-up calls are scheduled depending on the severity of the disease state, with more frequent calls given to those with advanced ALS.

Tissue recovery is critical. Each enrolled patient is connected to their local VA. If the VA is not able to conduct the recovery, then the brain bank contracts with the local medical examiner's office or diener service. Those contracts are important to have in place, as recoveries may be conducted after-hours or on weekends, when VA pathology departments are closed. Another important partner in the process is the family's funeral home in order to coordinate transportation. The brain bank also pre-positions boxes and has the goal of receiving the boxes in less than 24 hours.

Persons with ALS and their families are extremely invested in the process. Of the VA ALS Registry participants who were contacted, 232 patients, or 55%, agreed to participate in the brain bank. Of those who agreed, 142 are undergoing regular follow-up and data collection. Of the 90 participants who have died, tissue has been successfully recovered from 84 patients. The losses were due to pager failure or due to family members changing their mind about participation at the time of death. Participants have been consented in 47 states, and the brain bank has developed a national network of VA hospitals, diener services, and medical examiners to perform tissue recoveries.

The bank in Tucson operates under standard brain banking procedures with respect to specimen grossing, digital imaging, quality control, storage of samples, and software development. The neuropathology report is prepared by the staff at the Tucson and Boston. The Tucson bank oversees tissue distribution. The Tucson site also collects and stores control samples, and it is important to think about how to collect and store a national sample of controls.

Different brain banks and biorepositories use different software. The VAB Brain Bank uses Tissue Metrix Biospecimen Management Software. It is important to consider whether different types of software are compatible and whether data from one can go into another. Tissue Metrix allows for tracking events about samples, including data from the donor, results of grossing, results of diagnostic neuropathology, collection, gross digital images, the sample location, tracking sample distribution, and feedback from end users.

Clinical data collection is critical to the usefulness of the samples. The VAB Brain Bank has access to pre-existing clinical data from the VA ALS Registry. The brain bank also continues to collect longitudinal data, including updating medications, disease progression, and the VA electronic medical records.

Tissue distribution is currently conducted via a paper-based process. Requests are reviewed for feasibility, approval, and funding. The VA Central Office assembles a review committee to consider the request and makes final determinations regarding who gets tissue, and how much tissue is released. Tissue and clinical data are then released as needed by the investigator.

Informed consent is important, given that the tissue can only be used as the donors consented it to be used. The process should be broad-based and should allow for on-going collection and re-contact. Levels of consent are extremely important. In addition to the person with ALS, participation and involvement from the next-of-kin is critical to the success of the work. Consent with the next-of-kin is reconfirmed at death, and establishing understanding early in the process creates low levels of distress. Consent should be clear regarding disclosure, confidentiality, and Genetic Information Nondiscrimination Act (GINA) language. A Certificate of Confidentiality from NIH adds additional confidentiality protection to the process.

Other critical elements of the brain bank include informatics and data capture, which will help ensure that the tissue is useful in the future. Tissue processing expertise, infrastructure and data management, governance, and distribution are other issues that must be considered. The cost of administering a brain bank is significant.

For the proposed pilot study, Dr. Brady suggested utilizing one of the brain banks in Boston, Massachusetts rather than the Tucson bank. The Boston bank is serving as the brain bank for ALS, Alzheimer's Disease, and a number of other diseases, conditions, and initiatives. This pre-existing infrastructure has a history of collecting both veteran and non-veteran tissue samples. Using this bank would also ensure that the VA bank and the ATSDR bank would use identical procedures, measures, and structures. ALS brains are a precious resource, and distributing those resources across banks would protect them against complete loss in case of failure. Utilizing pre-existing infrastructure and expertise would also reduce start-up costs. Dr. Brady acknowledged the VAB Brain Bank staff, which focuses on personal contact with participants. The personal contact facilitates their success rates.

Discussion Points

- Dr. Belay asked about reimbursement for conducting autopsies. The system for neurodegenerative disease charges $3000 to $5000, but other issues are associated with that work, such as infection control. Dr. Brady replied that most of the time, the VA departments will complete the autopsy at no cost. The average fee for other services is approximately $1000.

- Dr. Bradley commended Dr. Brady on the national network of dieners, funeral homes, and pathology departments. That network is key to the effort, as collections occur at any time of the day or night. He asked whether samples are still being collected and whether VA funding for the collection is ongoing. He also asked about the population of the US that is covered by veterans and the capacity for expanding to the general population, given that veterans constitute 10% of the population, and there are approximately 10,000 cases of ALS per year in the general population.

- Dr. Brady replied that the VA funds the brain bank on a year-to-year basis, and the VA has been generous and supportive in funding and providing for the project.

- Regarding veterans and the general population, Dr. Kasarskis said that they could acquire that data. He noted that autopsies are "a lost art" and that autopsy rates are currently low (e.g., performed on only 1% to 3% of deaths). Engaging the family in the process is extremely important, given that the donor's survivors ultimately make decisions and give legal permission to pathology departments to acquire the tissues. It is important that the process is very simple for the legal next-of-kin, especially given that the legal next-of-kin could be, for instance, an estranged spouse who may not have been involved in the initial discussions.

- Dr. Brujin asked how many requests for tissue have been received, and how many have been granted. Dr. Brady replied that the brain bank has released 4 samples, and 12-16 requests have been made. Some requests are still under review.

- Dr. Brujin asked how the availability of the bank is disseminated and promoted. Dr. Brady answered that the review and request process had been burdensome in the past, but was now resolved. They are revisiting their procedures based on early experience with releasing tissue and will make a large-scale announcement about the availability of tissue.

- Ms. Bledsoe added that the process is being streamlined in order to facilitate access to the specimens.

- Dr. Gubitz asked whether samples were available only to VA researchers. Dr. Brady replied that the tissues are released to VA and non-VA researchers. The Technical Director of the Tucson bank is the point of contact for initial requests from the VA Biorepository Brain Bank.

Discussion of ALS Biorepository and Brain Banking Protocol

Wendy Kaye, PhD
Senior Epidemiologist
McKing Consulting Corporation
National ALS Registry Program
Agency for Toxic Substances & Disease Registry

Dr. Kaye opened the floor for suggestions regarding any of the discussion topics and subjects that had been introduced. She emphasized that they were hoping for suggestions pertaining to:

- ❑ The number of specimens that might be reasonable for a feasibility study
- ❑ What, if any, additional data should be collected along with the specimens or in addition to specimens
- ❑ What specimens should be collected
- ❑ The potential for self-collection option and long-term implementation
- ❑ Brain banking
- ❑ Collection of tissue samples

Discussion Points

- Dr. Brooks suggested that one of the principles of the biorepository might be to bring existing repositories together. The biorepository could have a component of new specimens as well as a component of curating the existing specimens.

- Dr. Kaye agreed that collaboration between existing resources and documentation of retrieving samples from more than one resource for a project should be pursued, but not as part of this endeavor, which was to collect new specimens attached to the National ALS Registry.

- Dr. Brooks agreed, but noted that the protocol should address potential issues associated with persons contributing to more than one repository. Dr. Kaye said that the specimens can be "fingerprinted" and de-identified, and it will be possible to determine whether they are in multiple registries.

- Dr. Corriveau said that the NINDS Repository checks a set of 6 marker satellites of DNA for every sample to make sure that when 2 tubes come in, they are from the same person. They also check the gender of the donor.

- Dr. Bidichandani stressed that community involvement, education, outreach, and similar work should be included in the protocol design. Dr. Kaye replied that those issues would be addressed in the logistics development of the project. Only persons who are engaged in the National ALS Registry will be included in this biorepository.

- Dr. Horn asked who the users of the proposed collection will be. It is important to understand who the users would be, what they want, and what they need. Dr. Kaye said that a process will be created for researchers to access the specimens.

- Dr. Gubitz asked about control pools. This aspect of the bioregistry may be dependent on the specimens that are collected. Dr. Kaye said that controls would not be collected as part of this project.

- Dr. Brujin wondered whether the pilot project should attempt to collect as much as possible rather than determine the specimens based on feasibility. Dr. Kaye agreed that one approach could be to capture as much as possible in the pilot, and then whittle down the list for the full implementation.

- Dr. Kasarskis referred to the table of relative advantages of different specimens. He encouraged adding skin and muscle biopsies to the table. He also pointed out that the ease of sampling should be taken into consideration. Some advanced ALS patients may be better suited to a skin biopsy than a venous sample. Additionally, associated side effects of sampling should be considered. There is a defined complication rates for spinal taps, for instance, including headaches that can persist.

- Dr. Corriveau suggested that the pilot collect as much as possible while utilizing best practice. Data is available on best practices for biomarker discoveries and on the impact of following them or not following them.

- Dr. Boylan agreed. Regarding "future-proofing" the repository, he pointed out that medically viable samples are not necessarily research-viable samples. There can be sensitivity to temperature exposure and time between actual collection and processing. These factors can compromise the utility of the specimens. DNA and RNA are less of a concern in this case than other protein biomarkers.

- Dr. Bowser concurred that DNA and RNA can be collected in specific vials and shipped at room temperature, but depending on the downstream research questions, especially protein-related investigations, collection may be more sensitive. Timing, processing, and storage are critical for that work. To facilitate protein-based or biochemically-based markers, those important points should be taken into consideration. Collecting as many samples and as many kinds of samples in the pilot is important, because the pilot is a feasibility study. If 100 samples will be collected, should they collect 10 each of 10 types of samples, or more in order to properly assess feasibility?

- Dr. Weisskopf added that processing time varies when collecting biochemical markers from large cohorts. One approach is to collect volunteer samples. When requests are made for samples from the biorepository, a series of pilots of the assay can be performed to determine whether the assay works, does not vary, and is not sensitive to factors such as processing time.

- Dr. Bradley said that control groups are needed for any research.

- Dr. Traino commented that if it not feasible to acquire control samples, it might be possible to collaborate with a group that collects healthy tissue for research purposes, such as the NIH Common Fund's Genotype Tissue Expression Project (GTEx).

- Mr. Tison asked about historical examples of correlation to environmental exposures to biosamples with neurodegenerative diseases to indicate that certain sample types are more useful than others.

- Dr. Weisskopf said that from an environmental perspective, certain tissue types are more useful than others in yielding information about past history. For example, bone tissue and teeth samples offer the possibility to consider past exposures as opposed to "what's on board now," which can be assessed by urine and blood.

- Dr. Bouzyk agreed and suggested considering hair and nail clippings in the biorepositories. Regarding controls, they might consider collecting families or trios in order to add extra value to the studies.

- Dr. Weisskopf observed that people in the National ALS Registry are likely to have caregivers or partners who might be interested in participating as a control. While hair and fingernails are useful for determining past exposure, they still have a window of about 1 year.

- Dr. Horton said that the National ALS Registry does not collect control information. That work is typically conducted by researchers for individual studies. He understood that bioregistries are different, however. CDC's National Health and Nutrition Examination Survey (NHANES) collects certain biospecimens and he wondered whether it would be feasible to use that resource as a control.

- Dr. Gwinn said that NHANES samples have not been fully exploited for use as controls in studies such as this one, but they could explore NHANES as a potential control group.

- Dr. Horton explained that NHANES is an on-going, national survey that CDC administers yearly. The survey includes a wide variety of questions and the collection of biological specimens.

- Dr. Bradley commented that spouses may not be ideal controls because they have shared the same environment as the ALS patient for a certain number of years, which could pose a problem for environmental research.

- Dr. Kamel said that not very many samples are useful for environmental measurements, as most samples only provide a picture of recent exposure. Often, ALS researchers are interested in exposures throughout the lifetime. In addition to clinical data, environmental exposure data should be collected and linked to the samples. A modular questionnaire was developed at Stanford University that addresses occupational history and other sources of environmental exposures. The modules are designed not to be burdensome and to be used in contexts such as this one. This information will be critical in the future.

- Dr. Horton said that the 7 modules currently used in the National ALS Registry were developed by Stanford. Future modules will address lifetime residential history, occupational history, and other factors such as hobbies that may involve exposures. Those modules could be used as surrogates, since urine and blood only indicate recent exposures.

- Dr. Vaught commented on GTEx, which is a collaboration of NHGRI, the Genome Research Institute, the National Institute of Mental Health (NIMH), and NCI. This project collects 35 to 40 separate tissues, including skin and muscle, from rapid autopsy patients or organ procurement donations. Brain tissue is also gathered when appropriate. Metastatic cancer is an exclusionary criterion. Tissues are viable for basic RNA integrity and RNA sequencing even if they are collected some time after death. They are also starting surgical collections. The surgical patients are often having amputations, and skin and muscle are major components of those collections. Data from this study will be available on dbGAP soon, and the tissues will eventually be available for sharing.

- Dr. Berry felt that before deciding which specimens should be collected, they should think about the analyses that may be conducted on them. The analyses will affect not only the specimens that will be collected, but also the way in which they are collected. Gross technical factors could determine what is feasible and what is not feasible. For example, if samples are collected when the donor has fasted for 12 hours, then good metabolomic studies can be conducted. If the donor has not been fasting, then those studies cannot be performed. Other considerations include the speed of the centrifuge; for instance, collection could be conducted in the home, but the person who does the collection may need a portable centrifuge. He suggested breaking the research questions into kinds of analyses, such as metabolomics, proteomics, genomics, genetics, epigenetics, gene expression, micro RNA, and others.

- Dr. Bidichandani said that in epigenetics, the chromatin immunoprecipitation (ChIP) process is preferred, and storage processes should be mindful of this.

- Dr. Brujin thought that if the list were to be extended, it should include human tissue, both fresh and from a short autopsy time frame, for immunoprecipitation. Dr. Bowser added "fixed and frozen."

- Dr. Horton understood that blood and urine samples provide a current picture of the patient. He asked what tissue can show in terms of exposure.

- Dr. Kamel said that the information gleaned from tissue depends upon the exposure. For example, lifetime lead exposure can be learned from bones and teeth. The timeframe is different depending on the particular bone. These samples can be collected non-invasively. Another example is organic chlorine pesticides or related compounds, which can be determined from fat tissue. Taking a fat biopsy can be invasive, and blood samples are not strong. There are few examples of environmental toxicants that are persistent enough in the body to be measured for long-term exposure.

- Dr. Weisskopf said that a large sample of blood serum and plasma is needed for organochlorines. Cadmium in urine is another example of a reasonable measurement from a sample. He said that controls are critical for looking at ALS incidence from an epidemiological perspective. Another consideration could be to think of the tissue as potentially predictive of survival of ALS. Then, variation among the cases could be examined. With that approach, blood, urine, hair, and nails could be relevant for environmental contaminants. The collection methods for those samples are very precise and specific.

- Dr. Bradley commented that organic chlorine pesticides have been demonstrated to be higher in Parkinson's Disease patients than in controls. He noted that brain banking is a difficult field, and suggested a different model for the development of funding for brain tissue banking for the National ALS Repository. Fifty years ago, the autopsy rate was about 40%, and now it is less than 10%. This change is due to a number of reasons, including public resistance, religious resistance, and physician resistance because imaging can yield information, so physicians may not feel that autopsies are necessary. However, since the advent of imaging, published papers have indicated that 25% of autopsies reveal information which, if it had been known in life, would have played a role in the disease or condition management. He suggested that an investigator who is interested in collecting tissue can be extremely effective. He shared his experience in studying metal levels, which included collecting autopsies. He and his colleagues developed a system in which patients were admitted to the hospital for their terminal care. The time from death to autopsy was approximately 75 minutes, and rapid autopsies are important for RNA preservation. Many ALS patients desire to donate their bodies after death, and the majority of them do not have that service provided to them because of challenges in linkage. Therefore, a network of centers is needed to serve as collecting centers. He suggested creating this network of funded neurologists who will serve as the basis for collecting patients centrally for brain banking.

- Dr. Bowser agreed, noting that post-mortem brain and spinal cord collection depends on a dedicated service of experienced and willing providers. A neuropathologist is needed in addition to the neurologists who can recruit patients. Rather than a centralized process where samples are sent in typical post-mortem times of 24 to 48 hours, he advocated for utilizing multiple sites across the country that can collect and process samples immediately. A central or virtual repository could be created, but a system would need to be created to

determine which samples could be kept in-house and which would be sent to the centralized repository.

- Dr. Kaye asked how a representative sample of brains could be collected in this schema and how to reach people who do not have access to neurologists. Dr. Bradley and Dr. Bowser indicated that those persons would not be reached by the system.

- Regarding biomarkers, Mr. Tison asked whether researchers may desire to use longitudinal samples to correlate with ALSFRS progression rate from the 6-month repeated survey module.

- Dr. Bradley and Dr. Bowser indicated that a number of researchers were interested in the correlation not only from a longitudinal perspective, but also post-mortem.

- Dr. Kasarskis suggested organizing their approach by specimen and by the types of research questions that can be answered by a given specimen. Then the plan can flow according to the viability of the specimen, whether it is adaptable to future technologies or future genetic identifications, and other considerations. Post-mortem tissue is important for confirmation. Questions of what is inexhaustible, the ease of sample collection, side effects, morbidity, cost, post-mortem interval, et cetera, can be answered for each type of specimen.

- Dr. Gwinn said that the draft protocol begins that analysis, based on the available literature. They know what can be measured by certain biofluids, and they know types of markers that can be correlated with different kinds of exposures, but specialized and tissue-specific elements have not yet been incorporated into the protocol. It also does not take into account specific factors such as the potential need for an onsite centrifuge, or special tubes for RNA collection or collection of samples for metal analysis.

- Dr. Kasarskis commended Dr. Gwinn for the draft protocol and suggested that the expert panel could begin to fill in the gaps.

- Dr. Brujin clarified that they could standardize a set of questions for each sample type.

- Dr. Brooks asked whether the registry is for use by the agency for environmental disease correlation, or whether the samples will be available for the scientific community to request for hypothesis-driven research.

- Dr. Horton answered that ATSDR looks to the expert panel for advice regarding how the bioregistry can be useful to the field. Samples that are collected will be available to the general scientific community for research. The bioregistry could operate through a “check box” in the National ALS Registry so that upon joining the registry, participants can indicate their willingness to donate samples and the kind of samples they are willing to donate.

- Dr. Horn asked whether the purpose of the collection is to link samples to the National ALS Registry to make them available to researchers, or whether the purpose is to create a bioregistry that does not exist and that represents the best resource for researchers. If samples already exist, why should this bioregistry be created?

- Dr. Brujin said that the National ALS Registry brings people with ALS and information to a central place. Other collections are important, but the registry represents an opportunity to involve ALS patients in studies. Further, a partnership for accomplishing the bioregistry will be valuable. It is important to connect to other efforts.

- Dr. Bradley added that the registry has the advantage of being a national database with non-selected patients. The endeavor will have funding, and epidemiological data will be available for patients in the bioregistry.

- Dr. Kamel wondered whether it would be valuable to use some of that funding to collect samples from control patients, even if it meant that fewer samples and fewer types of samples could be collected for ALS patients, to achieve comparability. Studies can be conducted with only ALS samples, but more studies are possible with controls. Part of the problem with controls is that people who do not have a disease tend not to be interested in donating samples, particularly if they are invasive. One strategy for this challenge is to enroll a spouse or friend of the case. There are limitations to this approach, even when the control cases are eager to participate. An extension of this strategy is to ask the person close to the ALS patient to recruit someone to participate as a control rather than to use a co-worker, spouse, or family member who may have had the same environmental exposures as the ALS patients.

- Dr. Bowser said that the RFA stipulates that people in the bioregistry must be in the National ALS Registry. Therefore, the feasibility study will not likely include a control population. The pilot study should consider additional important characteristics of samples that might be acquired that are less represented in other biorepositories. The epidemiological information that is collected with the samples is critical, so the specimens that are collected could be determined based on their relevance to epidemiologically-focused studies. Whole blood would be useful, for instance, and post-mortem teeth collection could assess lifetime exposure to metals. That work would make a major impact on the field.

- Ms. Ritsick noted that the deliberations of the panel can affect the scope of the contract and the number of samples to be collected.

- Dr. Kasarskis recalled that the Coriell repository required a convenience, non-ALS sample blood tube. Dr. Corriveau said that the NINDS Repository welcomes convenience controls, but does not require them. Dr. Gubitz added that the repository includes neurological control samples.

- Dr. Kasarskis recalled a comparable page of clinical data elements. He envisioned that an ALS patient in the National ALS Registry could recruit a non-ALS control, which would require broadening the scope of the registry to include a parallel arm.

- Dr. Kamel noted that there are often problems associated with collecting samples from people who already have a disease because it is not known how their condition has impacted the sample. Data such as history of exposure can be correlated with the sample and compared to NHANES data to determine whether samples that have been collected after diagnosis have been altered in ways that make them less useful because they are not prospective.

- Dr. Horton said that they would evaluate what NHANES is collecting and whether those biological specimens might be useful as a control. Mr. Kingon added the possibility of adding specimens to NHANES in the future.

- Dr. Weisskopf pointed out that there will be always be concerns about how the NHANES samples were collected and processed.

- Dr. Traino commented that normal tissue can potentially be collected from organ and tissue donors. While they are focused on the tissue, they should not forget the people who are providing the tissue and what they can provide. Her group is conducting surveys with family members of deceased ALS patients who donated tissue.

- Dr. Horton asked about gaps in the field, such as specimens that are not being collected and should be collected and researched.

- Dr. Brooks responded that the gut microbiome in ALS compared to other diseases is not being collected.

- Dr. Weisskopf noted that if biological samples will be collected from controls, and the controls are not necessarily random, then it will be important also to utilize the modules that are used with the ALS patients.

- Dr. Bouzyk added the gap of CLIA, and the pros and cons of running a national biorepository to CLIA standards. There are pros and cons to the approach, as CLIA does bring confidence to biomarkers. Additionally, it would be interesting and helpful to learn about global best practices as a reference point.

- Dr. Kaye asked whether it is important to immortalize the cell line.

- Dr. Corriveau responded that it is important not to immortalize a lymphoblastoid cell line. In modern genetics, there are frequent needs to use DNA directly from tissue or whole blood that has not been changed. If there is a need for cells, it may be worth considering other options, such as skin biopsies for fibroblasts.

- Dr. Bouzyk concurred with the importance of being able to go to the source. He agreed with extending the table of specimens to be collected and with thinking holistically about what is being collected. For instance, if blood and blood products are being collected, the rationale for collecting saliva as well is not strong.

- Dr. Kaye asked whether it is worth collecting saliva if blood cannot be collected.

- Dr. Bouzyk said that it is worth it, and a prioritized approach could be part of the protocol.

- Dr. Berry reminded the group that at times, it is easier to get a skin biopsy than a blood sample.

- Dr. Weisskopf suggested that the pilot could try to collect all of the samples to determine which ones tend to work.

- Dr. Corriveau said that the DNA yield from blood is higher than from saliva. Dr. Vaught added that bacterial DNA can be captured by the mouthwash protocol. Dr. Horn noted that processing saliva samples for DNA is usually more expensive.

- Dr. Vaught commented that their pilot studies led them to use PAXgene® Tissue to preserve and ship tissue to the analysis laboratory. The samples last for several days and up to a week at ambient temperature. The RNA quality and integrity is very good.

- Dr. Gallagher said that if saliva collection will be offered to people who are uncomfortable with donating blood, the choice may have to be presented to participants upfront. This choice could potentially bias the collection toward saliva, which is clearly a suboptimal specimen. If blood is collected for serum, then the clot can be recovered as an additional source of DNA.

- Dr. Kasarskis commented that skin sample collection is a physician procedure.

- Dr. Horn noted that there are models for collecting tissue outside the point of care. For instance, the Susan G. Komen Foundation maintains a normal tissue breast bank. They conduct core biopsies of breasts of normal women at community outreach events.

- Dr. Corriveau pointed out that if there are problems with collecting blood, the problems could mushroom into more serious concerns for the project as a whole. Serum and plasma are the most important samples to collect for biomarkers, epigenetics, genetics, and other critical areas.

- Dr. Bouzyk believed that blood is the gold standard for collection. Some studies utilize an electronic consent form that first asks potential participants whether they are prepared to give blood. If they reply “no,” then the next question asks whether they are willing to donate saliva.

- Dr. Corriveau said that in biomarker work, it is important to find people as early as possible after diagnosis and collecting samples of blood for serum and plasma every 4 months.

- Dr. Weisskopf agreed that if the consent form presents the possibility of giving either blood or saliva, then there will be bias against donating blood. It could be argued that blood and saliva are both needed, for different reasons.

- Dr. Kaye envisioned presenting a list of tissues to be collected so that participants can contribute the biospecimens that they are interested in providing.

- Dr. Gwinn asked whether there is a reason to collect saliva if a blood sample is available.

- Dr. Corriveau said that if a particular visit does not include blood collection, and a back-up is needed to ensure that samples are being collected from the same person, then saliva could serve as that confirmation.

- Dr. Berry said that there are medium-sized banks of fibroblasts, skin biopsies, and immortalized cell lines. It may be beneficial to reach out to some of these banks and learn about their work, as the work is very expensive, and they should consider resource utilization.

- Dr. Brujin explained that skin fibroblasts are used to generate the embryonic stem cells, which can then be differentiated to make motor neurons and a variety of cell types. They should determine how many of these lines they want. NINDS and other groups are investing in supporting and generating the Coriell induced pluripotent stem (iPS) cell line and differentiating the cells. There is a question of what will be gathered in addition and whether environmental data is needed. The iPS lines are not necessarily valuable for determining exposures, but for developing phenotypes. The environmental and clinical data may not be helpful in this instance.

- Dr. Kasarskis said that fibroblasts can be collected and banked without the intention to create iPS cell lines. That project may never be within the scope of the bioregistry. Collecting fibroblasts could set the stage for future research.

- Dr. Gwinn said that one of the potential uses of the biorepository is to validate measurements that have been made or to look at a larger distribution of values in an unselected population. She asked about particular measures that this might be suitable for. For example, some genotypes have been associated with both familial and sporadic ALS cases. It might be beneficial to have a national estimate of the frequency distribution of those mutations.

- Dr. Brooks returned to the issue of the purpose of the biorepository. The field needs peripheral blood, mononuclear cell protein, and RNA pellet from as many National ALS Registry participants as possible. With that sample and the addition of a skin biopsy before and after gastroscopy may provide a core of samples that would be desired by the field.

- Dr. Bowser said that for validation purposes, CSF or blood would be needed.

- Dr. Boylan said that the biomarker literature includes cross-sectional studies of moderate size. Collecting samples from more than 1 time point in the same patient can be challenging because they are expensive and time-consuming. If this bioregistry built in multiple time points for longitudinal foundations, it would represent a great contribution to the field.

- Dr. Kaye asked about ideal time periods for specimens to be collected.

- Dr. Boylan said that ultimately, the intervals should be clinically relevant, perhaps 3 – 4 months apart. The collection points should follow the patient through the course of the disease, and that interval varies from patient to patient. The ideal biomarker study would enroll patients early in their disease and follow them on a 3- to 6-month basis until death.

- Dr. Horton said that currently registry participants take the ALSFRS module every 6 months.

- Dr. Bowser said if only 2 samples could be collected, then a change in the FRS could trigger collection of the second set of samples. That approach would ensure that samples would be collected at different disease stages. If only a small number of samples will be collected, he hoped to follow the disease as far along as possible. A clinical FRS assessment could identify a unit of change to indicate significant progress of the disease. If only 2 samples are taken, and they are taken at intervals in which the disease has not progressed significantly, then they samples will be less useful than if they were collected at different disease stages.

- Dr. Corriveau reminded them of the importance of best practices. For instance, no more than 2 hours should elapse between the vein puncture to centrifuge and allocation to appropriate tubes, preferably in liquid nitrogen. The results of the work will reflect the quality of the samples that are collected.

- Dr. Bradley said that the feasibility of repeated collections of CSF is a major issue. Increasing numbers of pharmaceutical industry studies in ALS require CSF collection. It might be possible to get permission to obtain residual specimens from that work.

- Dr. Sejvar reinforced the importance of specimen handling and stringent SOPs, as well as a means for documenting the conditions under which the specimens were collected, which can reflect on the results that are obtained.

- Dr. Horton said that the sample size for the feasibility study was suggested to be 150. He wondered about the most appropriate use of the sample.

- Ms. Ritsick clarified that the numbers could change, and multiple samples could be taken.

- Dr. Brooks said that some of his gene expression studies need a sample size of 8 to 15 for a gene that is approximately 30% overexpressed. For a protein, the sample size is 25 to 30.

- Dr. Bowser asked that if 4 samples are collected from 1 person over time, whether that work is 4 samples, or 1 sample. Dr. Kaye replied that the budget allows for 150 people providing multiple specimens. The panel's recommendations can change the deliverable.

- Dr. Brooks said that some data are available from NEALS and from Dr. Vaught. He further noted that some samples will not be able to be used.

- Dr. Corriveau said that it is possible to decide whether to collect fibroblasts from a particular individual based on what is learned in the first few months of the project.

- Dr. Berry suggested that they define how much tissue would be collected, versus how much biofluid.

- Dr. Bowser added that if brain tissue is to be collected, then only the patients who die within the contract frame will be able to have their tissue collected. Ms. Ritsick responded that arrangements have been made for ATSDR to continue collection beyond the contract period.

- Dr. Bouzyk reminded them of the importance of "future-proofing" the biorepository for downstream technologies. If they will collect fresh frozen tissue, will they collect Formalin Fixed Paraffin Embedded (FFPE) tissue as well? Many molecular-based studies are being conducted on FFPE tissue, and some of the latest technologies are on the cusp of using FFPE tissue.

- Dr. Corriveau asked to whom the kits would be shipped. Dr. Kaye replied that they would be shipped to the person collecting the specimen, not to the donor.

With no further business posed, Mr. Kingon adjourned the meeting for the day.

Day Two
March 27, 2012

Overview of Day 1 Discussion

Wendy Kaye, PhD
Senior Epidemiologist
McKing Consulting Corporation
National ALS Registry Program
Agency for Toxic Substances & Disease Registry

Dr. Kaye reviewed the previous day's discussion. She reminded the panel that participants in the proposed biorepository must be participants in the ATDSR National ALS Registry. The biorepository must be a representative sample of the registry, so participation cannot be limited to geography, level of care, or other issues. They are creating a pilot project to test the feasibility of a biorepository, not to conduct analyses or studies.

If the biorepository goes forward, then it might be built into the National ALS Registry so that participants can indicate willingness to donate specimens when they join the registry. Another consideration regards how to harmonize the proposed bioregistry with other, existing bioregistries. The goal of the proposed biorepository is to link specimens to the National ALS Registry. Partnerships are valuable, and it is important to connect with others. One of the advantages of this biorepository is that it will include national, non-selected patients. It is funded, and it is supported by a wealth of epidemiological data on the participants.

Discussion was rich regarding research questions that might need to be answered and that might guide the specimens that will be collected. The list of topics included:

- ❑ Metabolomics
- ❑ Proteomics
- ❑ Genetics
- ❑ Epigenetics
- ❑ Gene expression
- ❑ Environmental issues
- ❑ Micro RNA
- ❑ Issues of survival
- ❑ Human tissue, fresh and frozen

Discussion also focused on the sample size and the need for a control. The size of 300 was suggested. There was discussion regarding whether other data should be collected, and it was suggested that the ALSFRS should be collected every 6 months. Environmental data should be collected via questionnaires, as specimens mainly assess only recent exposures.

Regarding specimens, some panel members felt that it is important to collect as many samples as possible. Best practices were discussed, and it was noted that medically viable samples may not be suitable for research purposes. The need for a control group was emphasized, and the possibility of using NHANES as a control group was posited. Longitudinal specimens and recruiting possible controls were discussed. The panel also noted that immortalizing cell lines

would not be the best use of resources, and it would be preferable to have fibroblastic cells. DNA should come from the source rather than from immortalized cell lines. Blood is the preferred sample. There was discussion about PAXgene® tissue collection and about whether an IRB would require saliva as an option for people who do not want to give blood.

In addition to blood, other specimen suggestions included:

- ❑ Hair
- ❑ Nails
- ❑ Teeth
- ❑ Bones
- ❑ Skin
- ❑ Muscle
- ❑ Residual specimens from drug company research

Regarding brain banking, the discussion raised the issue of collecting other specimens post-mortem as well, such as teeth and bones. If control specimens are collected, organ donor patients could be a possibility. The panel also discussed gaps, including the gut microbiome and whether tests should be conducted using CLIA standards.

The specimens desired by the panel included:

- ❑ RNA
- ❑ Skin biopsies
- ❑ CSF
- ❑ Blood
- ❑ Longitudinal collection of some specimens every 3 to 6 months, perhaps linked to performance on the ALSFRS

Other comments included information that would need to be collected so that researchers could develop their own controls and would know how specimens were collected and processed. Additionally, there were comments regarding how specimens will be shared. The topic of specimen-sharing will be discussed at a later time.

Dr. Kaye reviewed the revised draft table of specimen types and their relative merits and disadvantages. The completed table is provided as Attachment 1 to this document.

Discussion Points

General Comments

- Dr. Kaye stated that the information provided by the panel will be used to develop the final protocol for the feasibility study.

- Dr. Gubitz commented that the goal of the specimen should be defined. For instance, teeth, hair, and nails are collected for exposure data. Since the National ALS Registry includes a great deal of epidemiological data, it would be helpful to collect biosamples to reflect that data.

- Dr. Kasarskis also hoped to identify the research function of each of the specimens. He recalled discussion about specimen types that could have broad potential for future use.

- Dr. Brujin added that the longevity of the specimen was also important, as some specimens retain their viability for a longer time than others.

Blood

- Dr. Kaye directed the group's attention to the column about blood.
- Dr. Kasarskis agreed that the time frame for collecting a blood sample can vary according to the patient. In the best case, it should take 10 minutes to capture blood; however, acquiring blood from an advanced ALS patient can be more challenging and take upwards of 15 to 20 minutes, and the process may not be successful. He suggested a time range of 10 to 25 minutes. Blood can also be collected longitudinally.
- Dr. Horn suggested dividing the category of blood into subtypes. There had been discussion of PBMCs as well as collecting serum for biomarkers. The different derivates will affect the collection methods.
- Dr. Gubitz suggested DNA and biomarkers as two goals for blood specimens. Dr. Bowser added that blood can sometimes be used for environmental exposures.
- Dr. Weisskopf noted that some analyses require a larger volume of a sample than others. Environmental analyses of blood, for instance, may require more samples. Metals require 1 or 2 milliliters of blood. Organochlorines or other organics may require even more.
- Dr. Kasarskis noted that some recent clinical drug trials drew 8 tubes of blood from a patient, so precedent has been set for drawing relatively large samples.
- Dr. Weisskopf agreed and noted that for the purposes of "future proofing," as techniques improve, less sample is required. Dr. Vaught noted that aliquots should be saved for future uses.
- Dr. Bouzyk asked for clarification regarding "candidate biomarkers." He said that a wealth of biomarkers is available from blood and blood products, and more will likely be used in the future.
- Mr. Hixon commented that handling of the blood specimen depends on the type of blood that is being collected. Dr. Bowser agreed that handling and processing can vary widely according to the specimen and its potential use.

CSF

- Dr. Kaye turned the group's attention to CSF and asked about the timeframe for its collection.
- Dr. Kasarskis suggested a collection timeframe for CSF of a minimum of 30 minutes, noting that 2 people are required for the collection. If a patient is in respiratory distress or discomfort, then the process can take longer. Dr. Thurman noted that it is not practical to collect CSF in the patient's home.

- Dr. Kaye added that CSF could be collected longitudinally and clarified that the goal of collecting CSF is biomarkers with the panel. She asked about future use of CSF.

- Dr. Brujin and Dr. Bowser said that the samples are viable for up to 4 years if they are stored properly.

Urine

- Dr. Kaye turned to urine collection, which takes about 5 minutes. It is easy to collect, and multiple samples are possible. The specimens can be used for environmental studies.

- Dr. Brooks said that urine has a long history in ALS studies. Dr. Bowser added that urine can be used for metabolic studies.

- Dr. Sowell suggested that urine is not very useful for environmental studies for ALS, because it can only yield information about recent exposures, which are not likely to have had an impact on the disease.

Saliva

- The panel agreed that the collection time for saliva is 5 minutes, and it can be collected longitudinally and easily. Saliva can be assessed for DNA and some biomarker work.

Skin and Muscle

- Dr. Kasarskis said that the goal of collecting skin and muscle is to get fibroblasts and cultures, which will take several weeks of work by a laboratory. Dr. Gubitz agreed, adding that processing requires dedicated effort.

- The panel agreed that potential for DNA and RNA is high, and collection can take 10 to 15 minutes. The time for collection for a needle biopsy was 15 minutes. There is no need to collect these samples longitudinally. The goal of muscle specimen collection is biomarkers.

- Dr. Kasarskis said that it would be possible to get repeated skin biopsies if needed, but if the RNA and DNA cells are being stored, there should not be a need for longitudinal collection.

- Dr. Bowser asked whether they were considering biopsy or postmortem samples. Dr. Kasarskis recalled that the original comment was as biopsy, but he was not sure if a needle punch or open biopsy was planned.

- Dr. Bouzyk felt that muscle was as easy as skin to collect.

- Dr. Bowser said that muscle stores well. Dr. Kaye asked whether muscle is likely to be useful in the future.

- Dr. Brujin felt that muscle would be useful in the future, as muscle is highly relevant to ALS. As more is learned about the disease, it is likely that muscle will become even more relevant.

- Dr. Bowser commented on his experience returning to muscle biopsies, which had been valuable.

- Dr. Kasarskis asked whether the end product of a punch muscle biopsy was growing myocytes or myoblasts in cultures. Someone could differentiate iPS cells into motor neurons and examine them with the target muscle in culture as a potential synapse formation.

- Dr. Bowser and Dr. Brujin commented that muscle would not be used for that function, and that iPS cells were used for differentiation. Dr. Brooks said that in muscle disease, it is used for biochemistry and for making sections.

Teeth and Bone

- The panel agreed that DNA and RNA could be collected from teeth, but it is not likely to be worth it. The time of collection is not relevant, as teeth are collected post-mortem.

- Dr. Weisskopf noted that if bones are collected post-mortem, then it is possible to conduct more extensive analysis on them and to learn about exposures other than lead. In theory, any bone can be useful; however, harder, cortical bone is ideal.

- Dr. Kasarskis noted that pathologists may take a sample from the vertebral body. That sample is easy to collect, as the vertebral body has to be removed to reach the spinal cord.

- Dr. Thurman wondered whether teeth and bone should be separated. While teeth are helpful in examining heavy metal exposure in children, for instance, teeth may not be as useful for analyzing adult exposures.

- Dr. Weisskopf said that teeth are still good samples for adult populations, as exposure can be measured below the enamel.

- Dr. Thurman suggested that they qualify the environmental exposures. Teeth and bone are good for assessing exposure to heavy metals. Dr. Weisskopf added that organics are a potential future exposure that can be assessed by teeth and bone.

- The panel agreed that teeth and bone are easy to collect and have good potential for future use.

Hair and Nails

- Dr. Kaye asked about the future use of hair and nails, which are easy to collect and store.
- Dr. Thurman said that hair and nails are good for determining intermediate exposures going back a few months, perhaps a year. He was not certain whether there was value to be gained regarding remote exposures.
- Dr. Weisskopf said that if the issue is incidence of ALS, then hair and nails are not likely to be useful. If the issue is survival, then they could be useful.
- Dr. Weisskopf said that the time of collection for hair could vary if patients do not have enough hair.
- The panel agreed that hair could be collected longitudinally. No biomarkers to date are associated with hair.

Brain tissue

- The panel agreed that the potential for DNA and RNA is high with brain tissue, and the potential for future use is high. The goal of the collection the specimen is to gain insight into the pathophysiology of the disease.
- Dr. Brady said that the actual procedure for collection is approximately 1 to 1.5 hours, but the time of collection may not be relevant, as the samples are collected postmortem.
- Dr. Bowser said that the candidate biomarkers should be a "3" because the analysis includes not only candidates, but also which cells are making them and at what times, if they are found in other biofluids. Dr. Weisskopf added that brain tissue can be useful for environmental exposure assessment.
- Dr. Kasarskis confirmed that spinal cord will be collected as well as brain.
- Dr. Bowser asked whether the VA collects muscle, and Dr. Brady indicated that they do not. Dr. Bowser said that his team will collect deltoid and quadriceps samples from the same side.
- Dr. Brady said that the practicality of handling and processing brain tissue depends on how it is collected. At some point, they should discuss types of storage, such as centralized versus decentralized. He disagreed with some previous points that were made regarding quality of tissue and centralized processing. The experience at the VA Brain Bank includes plenty of research-quality tissue with high RNA Integrity Number (RIN) values. They have considered post-mortem intervals (PMIs), which are important, but are not the primary driver of tissue quality. There are some extended PMIs in some of their collections; factors such as the tissue pH are more tightly related to RIN values. Tissue pH has been more related to agonal state than to PMI. The agonal state is an issue in ALS patients regardless of PMI. If a decentralized collection process is desired, then that process may contribute to the "status quo of haves and have-nots in the field." Having a centralized brain collection allows all researchers to have access to tissue. It is difficult for people in the field to get tissue, and it

is important to think about the kind of biorepository they want to build and the ability to get tissue to researchers who need it. A centralized bank to which researchers can apply will "level the playing field" for people doing ALS research.

General Discussion

- Dr. Kaye reminded the panel that although the table refers to CSF collection from a bioregistry participant, CSF can also be collected post-mortem.
- Dr. Kasarskis suggested adding olfactory nerve biopsy. Dr. Berry said that adding that sample would depend on how broad the list should be. A procedure room is needed for that collection. Its potential is good, however.
- Dr. Horn recalled that the bioregistry will be national. Some of the elements they had described were specialized, and she wondered how to prioritize the top specimens with that in mind.
- Dr. Gwinn replied that given the project parameters, they should balance what is feasible and what is desirable.
- Dr. Kaye reviewed information from the table, noting that that collection could take place either in the field or in a doctor's office, and the collection would have to occur in any part of the US. To be representative, collection from living patients cannot be limited to the ALS Centers. Collection could take place in a family doctor's office and not necessarily in a specialist's office. Regarding postmortem samples, a network of dieners will conduct that collection.
- Dr. Weisskopf suggested that the table combine teeth, bone, and brain into a category of "autopsy."

Panel members voted on their top 4 specimens to collect for the proposed biorepository. Dr. Kaye reviewed the results of the voting:

Specimen	Number of Votes
Skin	12
Muscle	7
Blood	29
CSF	22
Urine	3
Saliva	1
Hair/Nails	2
Postmortem	26

Blood Collection

- Dr. Kaye addressed a question regarding how many longitudinal specimens could be collected per person. Given the constraints of the approval process and the length of the contract, it could be possible to collect 2, perhaps 3, specimens from every participant at 6-month intervals. She asked the group to discuss amounts of samples that would be ideal to collect.

- Mr. Tison commented that ALSFRS is already collected every 6 months. It is misidentified as "quality of life." Dr. Kaye agreed, noting that blood specimens could be collected every 6 months, timed with the administration of the ALSFRS.

- Dr. Kaye asked whether collecting 40 milliliters of blood would be acceptable.

- Dr. Horn asked whether anything about this population would limit the amount of blood that could be drawn. The panel indicated that there was not.

- Dr. Bowser suggested thinking in terms of vials collected rather than total volume. The tubes used to collect for whole blood are different from the tubes used to collect for plasma.

- Dr. Bouzyk agreed that 40-45 ml is the typical amount that is drawn in studies. Dr. Gallagher said that the NHANES study for adults collects up to 100 ml. Mr. Hixon said that clinical trials conducted through his group collects 120 ml.

- Dr. Sowell said that if longitudinal samples will be collected, then a larger amount should be collected initially, with lesser amounts collected subsequently, as some elements change over time and some do not.

- Dr. Kaye referred to Table 8 in the protocol, which lists 3 tubes at 23 ml total as a minimum.

- Mr. Tison commented that blood draws are more difficult and less productive as ALS progresses. Dr. Kaye agreed and noted that the study will include people in all stages of the disease.

- Dr. Berry said that his group has permission to collect up to 120 ml. When that volume is collected at baseline, smaller collections are taken later. The amounts are based on need, and a practical concern is the difficulty of blood draws later in the disease. He suggested setting an upper limit, but building flexibility into the plan to account for disease condition.

- Dr. Gubitz expressed concern that patients might be deterred from participating in the study if they are expected to donate a great deal of blood.

- Dr. Corriveau suggested that at the first visit, 1 tube of 5-6 ml should be drawn for DNA. As the list includes biomarkers, they should discuss whether both serum and plasma are desired, or whether one of them is good enough. This approach may be preferable to collecting as much blood as possible.

- Dr. Kasarskis said that advanced ALS patients may have trouble with venous access. Sometimes the only accessible vein is on the back of the hand, and 2 people may be needed for the collection. There is pain and discomfort associated with finding the vein, and the process of the draw itself can be slow.

- Mr. Tison added that dehydration is common as the disease progresses.

- Dr. Horn said that in determining the amount of specimen, they should also consider yield and how many experiments are downstream. They should think about who will use the resource and how they will use it, even for the pilot study.

- Dr. Gubitz pointed out that the next generation of sequencing methodologies does not use DNA from lymphoblastoid cell lines. Dr. Bidichandani agreed that they are abnormal cells. Fibroblasts can be made from skin.

- Dr. Bouzyk agreed that they should delve into some details in order to be selective about the types of tubes that are used. If the first tube is used for DNA, the DNA could be extracted from it, or it could be spun for buffy coat (for future DNA isolation) and plasma -if the tube is a purple top EDTA tube.

- Dr. Berry said that it is difficult to set a minimum amount for blood, because they may not get any, or very little. The priorities are important, but from a practical perspective, blood is drawn in a certain order based on the tubes and the additives in the tubes. It is difficult to know *a priori* how much blood will be collected. For instance, Ethylenediaminetetraacetic acid (EDTA tube) needs to be drawn before a heparin tube. If both are desired, but the heparin is more important, the EDTA still needs to be drawn first.

- Dr. Kaye said that the pilot test will illuminate these issues. They can evaluate whether all donors are able to fulfill all of the desired samples. She said that the protocol includes tubes in order: EDTA, clot activator, and acid citrate dextrose. She asked whether this list was acceptable and whether tubes should be added or removed.

- Dr. Berry suggested adding at least one PAXgene® tube for RNA. Dr. Gallagher noted that the acid citrate dextrose tube could then be eliminated.

- Mr. Hixon added that the PAXgene® tube is a 10 ml tube, but it includes 3 ml of preservative, so the total draw is 7 ml.

- Dr. Berry commented that the NEALS Biobank receives many requests for plasma. They collect plasma in EDTA, as sodium heparin tubes have been less versatile. Dr. Bowser agreed that if multiple tubes will be collected, then ETDA is the preferable tube type.

- Dr. Kaye confirmed that if a fourth tube is taken in the minimum protocol, it should be a second EDTA tube. That addition brings them to 34 total ml.

- Dr. Kamel suggested collecting at least one tube to measure metals. Whole blood is needed, and it should probably be from the first tube to avoid contamination. If some of the other tubes do not work out, then whole blood can be used for DNA. One or two ml is a

sufficient amount to collect for assessment of metal exposure. If a study considers the effects of toxicants on survival, then the measurement could be useful.

- Dr. Sowell felt that metals should not be collected in the first tube. Dr. Weisskopf commented that different tubes could be used for certain organics that are longer-term markers than metals.

- Dr. Sowell said that if multiple tubes will be collected, then a butterfly will probably be used. Blood left over in the butterfly can be collected, and bloodspot papers can be stored. Finger stick collections could be used as well for small volumes.

- Dr. Weisskopf said that 2-3 ml may just yield 1 run of trace metals.

- Dr. Bouzyk said that if PAXgene® is used for RNA downstream, they might consider a cost analysis of Tempus™ tubes as an alternative.

- Dr. Gallagher noted that the residual clot from tube 2 could be stored as a backup in case the DNA extraction fails.

Postmortem Specimens

- Dr. Kaye turned to the postmortem specimens. After blood, they received the most votes, indicating that the panel agreed that the biorepository should include postmortem specimens. Brain and spinal cord samples were suggested in order to align with the VA Biorepository. She asked about additional postmortem samples that may be needed in addition to brain and spinal cord, CSF, teeth, bones, skin, and muscle.

- Dr. Kasarskis observed that the samples will not be collected in the same way. The vertebral body bone is easy to collect. Psoas muscle is available as part of access to reperitoneal space. He was not sure whether pathologists would embrace the collection of teeth, and there could be pushback from a cosmetic standpoint.

- Dr. Weisskopf said that molars can be collected to address cosmetic concerns. Another issue is that teeth may need to be extracted by someone with expertise in extraction.

- Dr. Bowser asked whether collecting either bone or teeth would preclude the necessity for the other. Dr. Weisskopf said that one does not necessarily preclude the other. Bone may not be necessary if teeth are available, but bone may be easier to collect. One tooth is sufficient, and canines are preferable, but molars are acceptable.

- Dr. Brady turned to the issue of practicality, as teeth are outside the normal autopsy. Collecting a spinal cord can be challenging, and adding teeth to the protocol may increase the fail rate.

- Dr. Bradley agreed, noting that adding teeth to the protocol may discourage dieners in their network. He asked what can be done with teeth that cannot be done with bone and whether teeth are essential.

- Dr. Weisskopf said that teeth are preferable for assessing adult exposure, as bone "turns over" more.

- Dr. Bowser did not think that skin would be needed postmortem if it has already been collected. He asked Dr. Brady about the possibility of asking the dieners in the network about collecting teeth as part of the feasibility study. Dr. Brady said they could ask the dieners about their willingness to conduct teeth collection.

- Dr. Kamel understood that collecting teeth could be too much to ask of the dieners, but metals, especially lead, turn over in bone and do not in teeth. Any lead in a tooth started being there in childhood.

- Dr. Kasarskis noted that postmortem fat can be acquired. Dr. Thurman said that fat could be useful for longer-term organic exposures.

- Dr. Brady said that the standard VA pathology departments would probably be comfortable with these collections. Regarding the diener networks, there were challenges with convincing them to collect the spinal cord. Some will be willing to collect teeth as well, and some will not.

- Dr. Bowser felt that the area was valuable to explore, as a great benefit of this biorepository is its connection to the National ALS Registry and its epidemiological modules. They should enhance the ability to use the modules by collecting samples that address those questions. Even though the work is challenging and different from what people are accustomed to, it has the potential to make a significant impact on the field.

- Dr. Kasarskis asked fibroblasts can still be grown from skin is the PMI is 6 hours. Dr. Bradley said that nails continue to grow for 2-3 days, so there is viability.

- Dr. Kasarskis asked about the purpose of collecting muscle. Dr. Bowers answered that muscle addresses the pathobiology of the disease.

- Dr. Kasarskis added that skin can be collected from the omentum around the intestines.

Saliva, Urine, and Hair, and Nails

- Dr. Kaye reviewed the three specimens with the fewest votes: saliva, urine, and hair and nails. The only reason to collect saliva is if blood cannot be collected. Since this project is a pilot, saliva may not be necessary as part of the feasibility study.

- Mr. Hixon noted that these specimens can be self-collected.

- Dr. Thurman asked whether a buccal scraping would be collected with saliva, and Dr. Kaye replied that it would not. Saliva has higher yield than buccal scrapings.

- Dr. Horn said that saliva could possibly be relevant for longitudinal work and if blood was not collected as part of subsequent collections.

- Dr. Kaye asked about saliva's utility beyond DNA. Dr. Bowser said that saliva is being used as a biomarker in some contexts.

- Dr. Boylan suggested that saliva is "better than nothing" if a person is not able to contribute other biospecimens. Dr. Gubitz agreed.

- Regarding urine, Dr. Faye pointed out that it is easy to collect and store. Dr. Bouzyk suggested that its ease of collection is an argument to include it in the pilot study.

- Dr. Cross said that many samples and sample types in her biorepository are never used. They still have to be catalogued and stored. Rather than collecting a sample because it is easy to collect, it should be collected for a use.

- Dr. Bouzyk added that most biorepositories struggle with publicizing the availability of their resources to the investigator community. He has extracted DNA from urine samples that are more than 20 years old. For the purposes of the pilot study, the more the better. Further, ALS is a rare disorder with a small cohort.

- Dr. Cross said that they are collecting urine now, and 1 study will be using it.

- Dr. Kaye turned to hair and nails. Dr. Weisskopf noted that they are easy to store.

- Dr. Horton asked about information on exposure that can be gleaned from hair and nails. Dr. Weisskopf replied that exposure information for several months and up to 1 year would probably be available.

- Dr. Kasarskis said that saliva is a solid "Plan B" if blood is not available.

- Dr. Kaye said that the question was whether they would collect saliva from all participants to ensure that a biospecimen was collected from everyone, and collect blood from as many as possible. She asked whether saliva should be collected even when blood is collected.

- Dr. Bowser agreed that saliva should be "Plan B." Dr. Bouzyk said that an electronic form can first ask about willingness to donate blood and then suggest saliva if the participant does not want to donate blood.

- Dr. Kaye then turned to CSF, skin, and muscle. The panel agreed that CSF is very useful. She questioned whether it is practical to collect CSF on a large representative sample of people.

- Dr. Berry said that CSF has to be collected in an office by someone who is able and willing to do a lumbar puncture. This process asks more of participants, and many may not be willing to provide it.

- Dr. Kasarskis reminded them that there is morbidity associated with CSF. Headaches are common. Dr. Berry said that there are ways to collect CSF in ways that minimize adverse events, and management plans should be in place for adverse events.

- Dr. Bowser said that it might be important for the pilot study to determine the feasibility of collecting CSF.

- Dr. Bradley suggested that this collection could be linked with pharmaceutical trials.

- CSF is an important specimen, and it is not impossible to collect in a home setting.

- Mr. Tison said that he would volunteer again for a great cause.

- The panel discussed skin and muscle and agreed that skin can be collected in a home setting and does not necessarily require an office visit.

- Dr. Corriveau said that the primary purpose of the skin is fibroblasts. He wondered about sporadic fibroblasts and whether a mutation is involved. Given the expense and the investment already in the field, he was not sure that skin should be a priority specimen.

- Dr. Brujin agreed and reminded the group of the unique modules that accompany the samples. Fibroblasts may not be needed for IPS lines, but they could be needed for another application.

- Dr. Bidichandani said that that the point of collecting skin is for fibroblasts, but the point of collecting fibroblasts is not only to make iPS cells. Depending on the genes that are found to be mutated in individuals, it is possible to conduct several biochemical assays and cell-based assays. They can also be a back-up for DNA, RNA, and proteins.

- Dr. Corriveau said that if the cell biology is desired, then perhaps they should consider cryopreserved lymphocytes that do not obligate the immediate production of a cell line.

- Dr. Bidichandani said that epigenetic changes have been found reproducibly in fibroblasts, but not in lymphoblasts, in some of the repeat expansions diseases. Fibroblasts provide a tractable model to investigate a number of assays because they are available in huge numbers. He would place fibroblasts high on his list, because a priori it is not known what kinds of investigations will be done.

- Dr. Bowser agreed and noted that skin is the only immortalized specimen they are collecting. As they consider future uses for the bioregistry, there is potential value in those cell lines, despite challenges with storage and other issues.

- Dr. Kasarskis's colleagues are using fibroblasts from ALS to study gene regulation and enzyme levels. It is not possible to do this work with postmortem tissues. The flexibility of studying viable cells as fibroblasts is beneficial.

- Dr. Bradley asked whether skin can be cryopreserved without first culturing the fibroblasts. The panel was not certain. Dr. Horn indicated that the answer is "no" with keratinocytes.

- Dr. Berry said that if fibroblasts are put into a repository, then the consent form should address whether they can become iPS cells. If they can be, then the protocol must stipulate how they can be shared and used. Intellectual property considerations are important as well. If fibroblasts can be made, but not iPS cells, then that decision has implications as well.

- Dr. Kasarskis noted that the VA consent form covers those points, and patients can opt in or out of all of those categories. The donors are informed that the cells are not going to be used for therapeutic purposes.

Modules

- Dr. Kaye asked the panel whether additional data should be collected by the National ALS Registry above and beyond the current modules and the new modules that will be added on additional exposures, occupation, head trauma, electrocution, caffeine use, and pregnancy history. There is some concern that registry participants have not been completing the modules. If people participate in the biorepository, then they will be directed back to the surveys and will receive assistance in answering them, if necessary.

- Dr. Kasarskis asked about the military module. Dr. Kaye answered that the military module includes questions about the branch of service and service in a wartime theater.

- Dr. Kasarskis observed that the module is fairly global and wondered whether it could be developed more to learn more about types of exposures and environments. He asked whether ATSDR had consulted with epidemiologists in the military. Dr. Kaye answered that the modules have a 5-minute time limit.

- Dr. Horton said that there would be concerns associated with modifying an existing survey. Any new questions would need to be presented in a new module.

- Dr. Kaye added that the modules collect lifetime residential exposure, not occupational. The surveys ask about usual occupation and current occupation.

- Dr. Bradley emphasized that it is important to understand residential lifetime exposure. Dr. Kaye said that the new module will ask about lifetime residential history. It includes the type of water and whether the area was urban or rural.

- Dr. Weisskopf asked about "usual" and "current" employment. It might be possible instead to ask specifically about a participant's military occupation. This information is important. He suggested adding history of traumatic brain injury (TBI) or blast injuries.

- Dr. Kaye said that there are separate questionnaires for TBI and electrocution. The old modules are demographics, smoking and alcohol use, occupation, military service, physical activity, family history, and the ALSFRS on a 6-month basis. The new modules include lifetime residential history, exposure to pesticides, exposure to hobbies, TBI and electrocution, caffeine use, female reproductive history, a clinical module, and open-ended questions on concerns about ALS and insurance.

- Dr. Horton said that ATSDR actively solicits feedback and encouraged the panel to forward additional ideas to them.

- Dr. Gubitz said that the modules are comprehensive and cover relevant information without making the process too burdensome for patients. Although familial mutations are rare, the family history module does not capture elements of them. She suggested that one of the new models could provide a way for patients to indicate whether they have a familial mutation.

- Dr. Kaye said that the clinical module asks whether a patient has undergone genetic testing. Dr. Horton said that any question should be answered easily by the patient.

- Dr. Boylan said that the question was kept general because new genes are found all the time. Dr. Gubitz felt that a general question would suffice. Many ALS patients are well-educated and know a lot about their disease. They may want to share this information.

- Dr. McCarty asked about consent to access medical records. Dr. Kaye answered that all of the information is self-reported. Access to medical records is usually time-limited by the IRB.

- Dr. McCarty asked about medication use. Dr. Kaye said that Stanford advised against asking about medication use. The clinical module asks about RILUTEK®.

- Mr. Tison suggested adding more gradation to the physical activity module, as many ALS patients are serious athletes. Current questions only separate those who engage in little or no physical activity from those who engage in more physical activity. Regarding genetics, he suggested asking about second-degree relatives with ALS.

- Dr. Horton said that the issue of physical activity and sports has been raised. The clinical linking mechanism will be able to identify participants who are physically active, and there may be a way to query the system so that a researcher could identify a subset of individuals and conduct a study based on those who said they were physically active.

- Dr. Berry said that when the biospecimens are collected, they should ask questions about the time of day; when the patient last ate, drank, or consumed caffeine; and other germane questions. Dr. Kaye said that those questions would be separate from the epidemiological questions.

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- Dr. Brujin encouraged them to use the materials that were developed as part of the NINDS common data elements work.

Size of the Pilot Project

- Dr. Kaye asked about the size of the pilot project. What is a reasonable number for a feasibility study? Should they test the idea of doing longitudinal specimens? What about control specimens?

- Dr. Brooks felt that the feasibility pilot should include at least 2 consecutive samples from the same person.

- Dr. Kaye confirmed that the samples would be blood. It is not likely that the CDC IRB will approve two collections of CSF. Since they are conducting a feasibility study, should they plan for a large enough sample size to randomize people into groups of 1 sample or 2 samples?

- Dr. Brujin said that 2 should be collected wherever possible in order to have a comparison.

- Dr. Horton said that part of the study was to determine what can be collected from individuals, and how easily. Dr. Weisskopf agreed that part of the point of the study was to determine whether participants were willing to give a second sample. Dr. Boylan agreed, noting that part of testing feasibility is to offer everyone the same options.

- Dr. Kasarskis asked who would administer the consent forms. Dr. Kaye answered that the process is not finalized, but the person collecting the specimen will probably administer the consent form. Participants who indicate interest in participating will receive the form in the mail. The forms will be reviewed by trained interviewers via telephone. Then the signed consent will be obtained at the time of collection.

- Dr. Kasarskis expressed concern that the depth of the information in the consent form would be within the scope of knowledge of the person administering consent over the telephone so that the IRB will be assured that the patient understands the project completely.

- Dr. Kaye indicated that the types of cells were not included in the consent initially, so the process may need to be revisited. Participants will probably not go to physicians' offices for specimen collection.

- Dr. Kasarskis said that skin samples are operative procedures for most medical centers. In order to get a comprehensive set of samples, face-to-face with a physician may be needed. Dr. Kaye said that skin may not be possible to include.

- Dr. Horn asked about training for people who get consent and collect the samples. Their model includes dedicated coordinators that are trained in consenting procedures. Dr. Kaye said that vendors will be utilized, and there will be training packages. The logistics will be addressed separately.

- Mr. Tison said that for timing to get postmortems near term, lower ALSFRS scores may need to be targeted. It also may be necessary to ask about invasive ventilation preference and target those opposed.

- Dr. Kaye said that they will engage in targeting for postmortem specimens. They will have the opportunity to participate in all sample types. The study will target those with lower scores on the ALSFRS or who have been in the registry for a longer period of time. She asked about absolute numbers of living persons to include in the study.

- Dr. Horn suggested consulting with a statistician. Dr. Brujin suggested that they ensure that the sample is diverse and to get representation in difficult areas.

- Dr. Kasarskis was concerned that the first pass might include the ALS patients who are the most willing to participate and may therefore not be longitudinally representative.

- Dr. Kaye said that all patients will be in the National ALS Registry and have indicated their willingness to be contacted for opportunities. A sample of those people will be contacted. If they want to make comparisons, then they will need a large enough sample.

- Dr. Williamson noted that there will still be a bias within the registry, as the participants will have had to indicate their willingness to be contacted for studies. Dr. Kaye confirmed that everyone in the registry has indicated willingness to be contacted.

- Dr. Brujin suggested that they have as large a sample as possible. Dr. Boylan stressed that the sample size would probably be driven by what they can afford.

- Dr. Horton said that if they increase the sample size, they will have to justify the reasoning for a larger size than 150. Dr. Kaye and Mr. Kingon pointed out that 150 is a fairly small number.

- Dr. Thurman said that it is important to have sufficient numbers of people who live in remote areas far from a referral medical center to determine whether they can be reached. The sample may need to be weighted. Input from a statistician will be needed, and he suggested that a number higher than 150 may be needed.

- Dr. Berry suggested approaching the calculation in a manner similar to a tolerability study in a randomized controlled trial.

- Dr. Bouzyk pointed out that large cohorts are needed in case control association studies. If the work is hypothesis-driven or focuses on a familial component, then a few hundred or fewer participants may be needed. It might be worthwhile to draw blood from first- and second-degree relatives and build up pedigrees of the ALS patients themselves.

- Mr. Tison asked Dr. Berry whether it would make sense to target a size match with the NEALS Biomarker Research Study for longitudinal samples. Dr. Berry said that it might make sense to choose a number that is larger than a biorepository that exists. NEALS has 200 patients with early ALS and 50 additional participants with pure upper or lower motor neuron signs. They also include disease controls.

- Dr. Kaye confirmed that there was agreement among the panel that a sample size of approximately 300 seemed appropriate.

- Dr. Cross asked whether related individuals in the registry are linked. It may be important in the long-term to capture families. Dr. Kaye replied that the registry does not ask that question, and it would be challenging to do, as a person must be living to be in the registry.

- Dr. Brooks asked what the agency would consider a successful study.

- Dr. Horton replied that the goal of the RFA was to determine whether it was feasible to create a biorepository. They will consult with statisticians to determine a quantitative benchmark.

- Dr. Brooks said that they have a capturable sample, as opposed to a convenience sample. He proposed that a success would be 2000 patients, as was achieved by Coriell.

- Dr. Gubitz said that Coriell focused on blood only. Dr. Kaye said that 2000 is not needed for a feasibility study. Dr. Horton agreed that when the biorepository goes live, they want as many participants as possible.

- Dr. Kaye said that the pilot study needs enough specimens to determine feasibility for long-term viability. They also would like to have enough specimens so that they can also be used for research activities if the biorepository does not go forward.

- Dr. Brujin said that if they set a goal of 300, but only capture 200, they should consider the outcome that will convince CDC to pursue the project.

- Dr. Kaye said that feasibility includes not only the number of samples, but also how many samples were collected per person, the logistics, the costs, and more.

- Mr. Tison suggested collecting the Social Security Numbers of affected relatives to link siblings with familial ALS. Close relatives could easily acquire that information.

- Dr. Boylan understood that one of the underlying interests in the study was geographic and demographic representations of the population, which are important statistical considerations.

- Dr. Kaye said that other considerations include urban versus rural, race, and gender. Those who self-registered with the Registry may not be as diverse as the population as a whole.

- Dr. Bowser indicated that NEALS collected 200 samples in 2 years. This pilot study could aim to collect at least 300 samples from a geographically dispersed area. The study may not reach 300 samples, but could reach a goal of geographic distribution of samples or level of racial or ethnic diversity. The feasibility could still be a success.

- Dr. Williamson said that statistics will not drive this feasibility study. Little baseline information is available, and part of the purpose of the study is to obtain baseline data in order to design studies.

Attachment 1: Final Table Following Discussion

Characteristic	Blood	CSF	Urine	Saliva	Skin	Muscle	Teeth Bone	Hair Nail	Brain Spine
Proximity to CNS	++	+++	+	+	+	+	+	+	+++
Less Invasive	++	+	+++	+++	++	+	+	+++	+
Practicality	+++	+	+++	++	++	+	++	+++	+
Handling/processing	++	+	++	+++	+	+	+++	+++	+
Potential for DNA/RNA	+++	+	+	++	+++	+++	+	+	+++
Time for Collection	10-25	30-45	5	5	10-15	15		5	
Longitudinal	+++	++	+++	+++	NA	NA		+++	
Ease of Collection	++	+	+++	+++	++	++		+++	
Candidate biomarkers	++	+++	+	+	++	+	+	+	+++
Goal of Specimen	DNA Biom	Biom	Env Meta	DNA Biom	DNA IPS	Biom bioche	Env IPS?	Env	Path Env
Future use	+++	++	+	+	+++	+++	+++	+	+++

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