Appendix S

National ALS Biorepository

Version 1 – Amendment 6

Table of Contents

Introduction

No single study or database can provide answers to the most pressing questions about ALS, such as: How many people have ALS? What are the underlying causes? How can understanding these causes lead to prevention and treatment? What biomarkers are useful for predicting disease progression and treatment response? Answering such questions requires the integration of epidemiologic, clinical, and basic research findings to place individual measurements in context, suggest hypotheses, and provide a basis for inference. This biorepository will enhance the Registry's utility for research on ALS. It will differ from existing biorepositories because it is population-based and nationally comprehensive, rather than defined by geographic area, exposure, or clinical characteristics. The National ALS Biorepository will increase the number of biological samples available for research and will make them available to ALS researchers regardless of institutional affiliation. Persons with ALS have expressed interest in providing biological specimens through the National ALS Registry.

Background

ALS Epidemiology and Genetics

ALS occurs worldwide but because of its rarity, variation and trends in ALS incidence are difficult to measure. The only consistently identified risk factors for ALS are increasing age and male sex. Although tobacco smoking has been proposed as an additional "established risk factor" (Armon 2009), findings among studies are inconsistent and the effect on risk (if any) is small (Alonso 2010). Epidemiologic studies have examined many other potential risk factors for ALS, including occupation, trauma, infections, exposure to metals and other chemicals, and electric shock (**Table 1**). Potential biomarkers have been proposed for some of these exposures or their pathologic sequelae; however, so far few epidemiologic studies have incorporated biomarkers.

Like other degenerative neurologic diseases, including Alzheimer's and Parkinson's diseases, ALS is thought to develop as a result of complex interactions among multiple genetic and environmental risk factors (Ahmed 2011; Siddique 2011). The clinical heterogeneity of ALS suggests that different underlying mechanisms and pathways could be involved.

Familial ALS. Approximately 10% of people diagnosed with ALS have an affected first-degree relative (Fang 2009; Siddique 2011). Fang et al. conducted a retrospective cohort study of familial ALS by analyzing the Swedish Multi-Generation Register from 1961 to 2009. They found that the risk of ALS was increased about 10-fold in siblings and children of affected persons; risk was further increased if the proband was diagnosed at a younger age. Clustering of ALS within families suggests that genetic factors contribute to susceptibility.

Beginning in the 1990's, genetic studies of families with multiple affected members implicated mutations in several genes that typically confer risk in an autosomal dominant manner, although autosomal recessive inheritance has been observed in some families (OMIM, 2012). Variations in superoxide dismutase 1 (*SOD1*) were first associated with familial ALS in 1993; since then, more than 130 *SOD1* mutations have been implicated, accounting for approximately 10–20% of multiply affected ALS families (Millecamps 2010).

A recent French study of 162 distinct families with ALS examined the frequencies of mutations in five previously associated genes; results are summarized in **Table 2** (Millecamps 2010). A mutation in at least one of these five genes was found in only 36 (22%) of the 162 families; of the 31 distinct mutations identified, 7 (23%) had not been previously reported. The finding of largely private mutations in multiple genes attests to the heterogeneity of genetic susceptibility to ALS.

Recently, two studies implicated expansion of a GGGGCC hexanucleotide repeat in C9ORF72, a non-coding region of chromosome 9, in families of European ancestry affected by both ALS and frontotemporal dementia (FTD) (DeJesus-Hernandez 2011; Renton 2011). DeJesus-Hernandez et al. found this repeat expansion in 22.5% of familial ALS seen in three clinics (Mayo Clinic, University of British Columbia, and University of California—San Francisco). Renton et al. found the same expansion in nearly half of all familial ALS in Finland and in one-third of 268 familial ALS probands of European ancestry.

The ALS Online Database (ALSoD, http://alsod.iop.kcl.ac.uk), an open source data repository for sharing ALS genotype and phenotype data, was developed by a consortium within the World Federation of Neurology (Lill 2011). Consortium members submit their own data and extract data from scientific publications; users of the database can also upload their own data. The focus is on familial ALS, although data from non-familial cases are also accepted. As of January 2012, the database included more than 450 records for people with familial ALS; mutations in 17 genes were represented, including the five studied by Millecamps.

Discoveries of additional mutations in families affected by ALS continue to offer clues to underlying disease processes. For example, Wu et al. in 2012 used exome sequencing in families with ALS to discover associated mutations within the profilin 1 (*PFN1*) gene.

Sporadic ALS. New technologies and information stemming from the Human Genome Project have spurred genetic association studies of "sporadic" ALS in persons without a family history (**Figure 1**). These population case-control studies are the focus of the ALSGene database (www.alsgene.org), which as of January 2012 included more than 250 polymorphisms in more than 90 genes, including some previously identified in family studies (Lill 2011).

In genetic association studies of sporadic ALS, observed odds ratios are typically small (between 1.1 and 2.0), even for variants of genes implicated in familial ALS. Such findings are typical for genetic association studies of multifactorial chronic diseases, including atherosclerosis and cancer. No genetic association with sporadic ALS has been found to have effects as large and consistent as the association of *APOE* genotype with Alzheimer disease (meta-analysis odds ratio [OR] 3.68, 95% CI 3.30–4.11, www.alzgene.org, January 2012) and even strong genetic associations serve only as the starting point for further investigations of pathogenesis (Storandt 2012). Since 2005, genome-wide association studies (GWAS) have been a powerful tool for identifying new candidate genes in complex diseases. The results for GWAS of ALS have offered only a few leads and replication of the promising association with *DPP6* has been inconsistent (Chio 2009).

ALS Biomarkers

Advances in molecular biology and neuroimaging technologies are providing an increasingly detailed picture of neuropathology in ALS. Biomarkers of contributory exposures, disturbed physiologic processes, and tissue damage may be useful in discovering underlying physiologic mechanisms. Several proposed mechanisms and categories of potential biomarkers are summarized in **Table 3**. Many of these have been identified in basic research using neural tissue obtained postmortem.

A vital goal of pathophysiologic research is to identify biomarkers that can be used clinically, to diagnose ALS earlier and reduce diagnostic delay, predict disease progression, stratify patient populations for clinical trials, and assess response to treatment (Bowser, 2011, Otto 2012). Desirable attributes of clinical biomarkers include their presence in accessible specimens (e.g., blood, urine); detectability early in disease; high sensitivity and specificity; and high predictive value for relevant clinical outcomes. The successful development of such biomarkers requires integrating findings from epidemiologic, clinical, and basic science research.

A recently published article, "Roadmap and standard operating procedures for biobanking and discovery of neurochemical markers in ALS," followed from a 2010 workshop sponsored by the World Federation of Neurology (Otto 2012). This report systematically describes key considerations in the design and conduct of research on ALS biomarkers and the need for international collaboration and standardization. The authors point out that "biomarker studies are of limited or no use without reliable clinical characterization of patients and propose a "desirable minimum clinical dataset," which is included as **Table 4** of this protocol.

Bowser et al. (2011) recently reviewed candidate protein-based, neurophysiological, and neuroimaging biomarkers in ALS. Although all three types of biomarkers are potentially measurable in clinical settings, only protein-based biomarkers will be available from samples collected for the National ALS Biorepository. Candidate protein-based biomarkers include various molecules with a role in innate immunity (e.g., interleukins, chemokines), complement factors, and markers of neural tissue damage. Quantitative methods for measuring candidate protein biomarkers include antibody-based methods (quantitative ELISA, multiplex antibody capture) and various combinations of protein purification procedures (gel electrophoresis, liquid chromatography) with mass spectrometry.

The discovery and validation of biomarkers is an area of ongoing, active research. As of January 2012, the NIH clinical trials database (http://clinicaltrials.gov/) included at least 18 studies of ALS that included collection of biomarkers, of which four were actively recruiting. For example, enrollment began in November 2011 for a new longitudinal study of ALS biomarkers (NCT01495390) at Massachusetts General Hospital.

ALS Biorepositories

Understanding the complex pathobiology of ALS, which appears to involve multiple complex, interdependent processes over time, requires the capacity to detect factors with small effects on overall risk. Thus, there is a strong incentive for ALS clinical researchers to collaborate in establishing

standardized diagnostic criteria (such as the El Escorial criteria, first published in 1994) and frameworks for sharing clinical data and biological research specimens.

Many ALS clinical registries have been established at academic and other research centers; typically, these registries consist of data and specimens collected from patients at the time of diagnosis and treatment or during the course of clinical trials. The data and specimens may be shared in ad hoc research collaborations. ARISLA, an Italian foundation for research on ALS, maintains a list of European and U.S. biobanks and repositories (with brief descriptions and links) at http://www.alscience.it/?view=52#v52.

Tissue banks containing postmortem specimens of brain, spinal cord, and muscle tissue are also important resources for ALS research. The International Brain Banking Network (IBBN) (http://www.intbbn.org/) facilitates communication among brain banks and conducts annual workshops in association with the American Association of Neuropathologists. The IBBN website includes a directory of brain banks in the U.S. and other countries (http://www.intbbn.org/registry-of-brain-banks.aspx); most of these banks are at universities and many have an Alzheimer research focus.

Several independent organizations offer specimen retrieval services that will query registries and tissue banks for specimens with specific characteristics and request them for use by outside researchers. For example, the National Disease Research Interchange (http://www.ndriresource.org) is a not-for-profit corporation funded mostly by NIH that locates biological research materials and maintains a specimen bank for the NIH Office of Rare Diseases Research (ORDR, http://biospecimens.ordr.info.nih.gov/). SpecimenCentral.com (http://specimencentral.com/) is a commercial broker, connecting researchers with biobanks holding the types of biological samples they are seeking.

Prospective collaborations in the US and elsewhere have developed clinical research resources for ALS, including biospecimen collections. The principal examples are summarized briefly below and in **Table 5**.

New England ALS (NEALS) Consortium. The NEALS Consortium is a network for ALS clinical research that was formed in 1994 and has grown to more than 90 members. NEALS provides infrastructure for multi-center clinical trials and other clinical research studies. The Massachusetts General Hospital (MGH) serves as the Coordinating Center for many of these studies and houses an associated biofluid repository containing a variety of blood, urine, plasma, cerebrospinal fluid (CSF), and extracted DNA samples. Biorepository samples are linked to clinical data and stored under controlled conditions, providing potentially larger sample sizes for research than are available from single studies. The NEALS Sample Sharing Committee oversees sample sharing policies and reviews data and sample requests. A scalable "virtual biobank" platform has been developed to allow investigators in ALS clinical research centers to share and search for samples while retaining control of their own specimens and data (Sherman 2011).

National Institute of Neurologic Diseases and Stroke (NINDS), NIH. In 2004, NINDS added ALS to the conditions included in a DNA bank and cell line repository established at the Coriell Institute to support large-scale research on the genetics of neurologic diseases. This effort was a public-private collaboration of NINDS with the ALS Association (ALSA), the Muscular Dystrophy Association (MDA), and academic

researchers from 62 centers across the United States, including many members of the ALS Research Group (http://www.alsrg.org) (Gwinn 2007). The repository is a national research resource of biological samples linked to individual phenotypic datasets. For all included subjects, NINDS Clinical Data Elements (CDE) must be complete; these include demographic, clinical, and medical and family history data (Gwinn 2007, Appendix S1B). With the exception of smoking (optional), data on potential environmental exposures are not collected. The repository includes specimens from approximately 2000 people with ALS, including blood, immortalized lymphocytes, and fibroblasts. Some samples from unaffected and affected blood relatives of subjects, spouses, and healthy individuals (including population and convenience controls) are also included. The repository is not actively adding specimens, although researchers may apply to submit their own collections. More information is available at http://ccr.coriell.org/Sections/Collections/NINDS/Motor.aspx?PgId=192&coll=ND.

Medical Research Council (MRC) London Brain Bank for Neurodegenerative Diseases. The MRC London Brain Bank was established in 1989 in the Department of Neuropathology, Institute of Psychiatry, King's College London, UK, to make clinically and pathologically well-characterized brain and spinal cord tissue available to researchers worldwide (http://www.iop.kcl.ac.uk/departments/?locator=380). The MRC London Brain Bank focuses on neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, frontotemporal dementia, and motor neuron disease (including ALS). Currently, specimens are available from 189 persons with motor neuron disease and the bank maintains an ongoing program to "facilitate brain donation through an ethically approved program of informed consent for cohort studies and ad-hoc donations."

Several epidemiologic studies of ALS have used population-based registries to identify potential participants, who are contacted to request biological specimens (Chio 2009). Very few population-based ALS registries have been designed to collect specimens prospectively. In the US, the only large population-based ALS registry with an associated biorepository is the National Registry of Veterans with ALS. This biorepository includes blood (or buccal cell) specimens collected for DNA analysis, as well as a brain bank; because it offers the most relevant model for development of the National ALS Biorepository, it is described in additional detail here.

National Registry of Veterans with ALS. The registry was established by the Veterans Administration (VA) after increased incidence of ALS was reported in veterans deployed to the Persian Gulf in 1990–91 (Kasarskis 2004). The objectives of the registry were to identify living US military veterans with ALS, follow their health status, collect data for epidemiologic research, and inform veterans about clinical trials for which they could be eligible (Allen 2008). Veterans with ALS were identified from VA medical databases and via nationwide solicitation; a brief telephone interview screened potential participants for eligibility. During the enrollment period (April 2003–September 2007) more than 2,000 participants aged 23–93 years were enrolled, spanning combat eras from World War II to the 1990–91Gulf War. Registrants were asked to provide contact information for all providers of healthcare since their onset of ALS symptoms. Their medical records were retrieved and abstracted by trained personnel, who recorded results of physical and electromyographic examinations, pulmonary function tests, and laboratory and imaging studies, as well as medical history (including surgery and physical trauma) and family history of ALS. An integral component of the registry is the DNA bank, which is a collaboration of

the ALS Registry (Epidemiologic Research and Information Center, Durham VAMC), the VA DNA Coordinating Center (Palo Alto VAMC), and the Genetic Tissue Core Laboratory at the Massachusetts Veterans Epidemiology Research and Information Center (Boston VAMC). All registry participants were asked to contribute a specimen, although it was not required. DNA specimens were collected in participants' homes by trained nurses associated with a nationwide home health agency. Nurses were supplied with blood collection kits, as well as mouthwash for use in collecting buccal cells when no blood specimen could be obtained. Of the more than 2,000 veterans enrolled in the registry, 76% provided written consent for participation in the DNA bank; 8% refused and the remainder were excluded for other reasons. More than 1,000 specimens were obtained, of which 85% were blood and 15% were buccal cells only.

A brain bank component was added to the registry in 2006. The VAB Brain Bank is coordinated at the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC) at VA Boston Healthcare System (VABHS). Tissue is analyzed, processed, and stored at the Southern Arizona Core Tissue Laboratory (SACTL) at the Southern Arizona VA Healthcare System (SAVAHCS) in Tucson, AZ. Although enrollment in the registry ended in 2007, regular telephone follow-up of enrolled Veterans has been continued by the VAB. This follow-up includes semi-annual administration of the ALS Functional Rating Scale. Postmortem collection of brain tissue continues for previously enrolled participants. Additionally, the VAB continues to enroll Veterans with ALS via referrals from the Neurology Service at VA Boston, and other VAs. A Scientific Review Committee provides expert review for all requests to use registry data and samples. Multiple studies have already been conducted on a range of topics, including genetics, environmental exposures, disease progression, psychosocial issues, and a brain-computer interface for patients (Allen 2008). The National Registry of Veterans with ALS is also the source of cases for the GENEVA Study, a case-control study of genes, environmental exposures, and gene-environment interactions in ALS (Schmidt 2008; Schmidt 2010). The VAB has begun to release tissue and associated data to investigators.

Best Practices for Biorepositories

International Society for Biological and Environmental Repositories (ISBER). ISBER publishes best practices for the collection, storage, retrieval and distribution of biological materials for research. The second and most recent edition was published in 2012 and is available online at https://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_Best_Practices_3rd_Edi.pdf (ISBER 2012). Guidelines in this document address all aspects of biorepository management, including administration and records management, facilities and equipment, safety, quality assurance, specimen tracking and management, and ethical and legal issues, as well as best practices for human biospecimen collection, transport, and storage. A key point notes the value of pilot or feasibility studies for identifying problems in specimen collection and handling before beginning a larger study.

National Cancer Institute (NCI). NCI has invested in extensive biorepository programs to support basic, epidemiologic, and clinical research on cancer. Recognizing the increased value and changing uses of biospecimens in clinical research, NCI commissioned the National Biospecimen Network Blueprint (2003), which recommends ways to enhance the collection of biospecimens and the management of biorepositories. The NCI Clinical Trials Cooperative Group Program, which includes more than 3,000

institutions and more than 14,000 investigators, also maintains the network of Cooperative Group Banks (http://cgb.cancer.gov/). Banked biospecimens include formalin-fixed, paraffin-embedded tumor and normal tissue, fresh frozen tumor and normal tissue, blood, serum, plasma, urine, genomic DNA, and bone marrow. A directory of participating Cooperative Group Banks is available online at http://cgb.cancer.gov/contacts/index.html.

In 2006, NCI's Office of Biorepositories and Biospecimen Research (OBBR) established the Biospecimen Research Network to assess the effects of pre-analytical factors (e.g., specimen types, collection methods, transport, processing, and storage) on the outcomes of genomic and proteomic studies in cancer research. OBBR has issued Best Practices for Biospecimen Resources that address scientific and technical considerations, as well as ethical and legal issues. Last updated in 2011, these Best Practices are available online at http://biospecimens.cancer.gov/bestpractices.

Public Population Project in Genomics (P3G). P3G is an international non-profit organization that promotes collaboration among researchers in population-based genomic research. The P3G Observatory (http://www.p3gobservatory.org/) is an online repository of information and tools for harmonization among individual biobanks. The Observatory includes a directory of relevant guidelines and best practices at http://www.p3gobservatory.org/repository/sampleCollection.htm. In addition to the ISBER and NCI guidelines already mentioned, these include Best Practice Guidelines for Biological Resource Centers published by the Organization for Economic Co-operation and Development (OECD) and other national and international research organizations.

Anticipating Research Uses

Anticipating future uses of biorepository samples is challenging, especially because technology continues to expand the realm of possible analyses. Although versatile specimens—such as whole blood, serum, and plasma—are likely to be most useful in the long run, collecting specimens for future use always requires trade-offs: for example, between collecting a larger variety and volume of specimens vs. cost of collection and storage, or between asking participants to consent to unspecified future analyses vs. their willingness to participate.

Choosing the types and quantities of specimens to be collected for a biorepository depends on multiple considerations. A recently published biobanking "roadmap" for ALS biomarker research compared the advantages and disadvantages of several types of specimens, which are summarized in **Table 7** (Otto 2012). In addition to blood and cerebrospinal fluid (CSF), which have been studied most often, the roadmap considered urine, saliva, skin, and muscle tissue. Urine and saliva offer practical advantages relative to blood: collection procedures are less invasive and analysis is simpler (although also more limited) because of their less complex molecular composition. Urine has potential added value as a specimen for measuring levels of drugs and other environmental chemicals and their metabolites. Saliva provides an alternative source of DNA when blood collection is not possible. CSF, skin, and muscle tissues can only be collected in a clinical setting. Nail and hair clippings can provide information about past environmental exposures (such as those in **Table 1**), mostly limited to near- and intermediate-term exposures (Goullé 2009).

Whole blood remains the preferred specimen type for biobanks intended to serve as long-term research resources. Its advantages include:

- DNA yield (quality and quantity);
- versatility (e.g., for analysis of RNA and other biomolecules, as well as markers of environmental exposures);
- straightforward creation of aliquots for sample sharing;
- long-term stability in storage; and
- potential for creating cell lines.

Whole blood specimens must be collected with the intended analyses in mind to prevent interference by anticoagulants. For example, collecting blood in anticoagulant-containing tubes causes the release of cytokines, resulting in spuriously elevated concentrations. Whole blood specimens collected on filter paper (Guthrie cards) share many of these advantages with conventional blood specimens; however, their small volume limits the numbers and types of possible analyses and they can't be used to establish cell lines.

Pilot Study Results

Through a contract ATSDR conducted a study to pilot methods for collecting and banking biological specimens from participants in ATSDR's National ALS Registry. The Pilot Study included two specimen collection components: biological specimens from living participants (in-home) and postmortem specimens. The in-home component enrolled 330 participants, from whom specimens were collected on two occasions, approximately six months apart. The postmortem component enrolled 30 participants, who could also participate in the in-home study. In-home collection included blood, urine, hair, and nails. The postmortem collection included the collection of brain, spinal cord, CSF, muscle, bone, and skin specimens for the creation of cell lines.

Persons participating in the in-home collection were asked to provide consent over the phone. Those participating in the postmortem collection were visited in their homes and asked to provide consent in person. When skin specimens were added to the tissue types collected postmortem, a consent addendum was created and approved by the IRB. Participants who provided consent to participate before the addition of skin specimens were contacted about participating in this additional specimen type and asked to provide consent over the phone. For all parts of the consent process that took place over the phone, the participant returned a signed copy of the consent form to McKing before any study procedures took place. Participants were provided a copy of the signed consent form for their records.

Three hundred and thirty-nine individuals provided consent to take part in the Pilot Study, 202 (59.5%) male and 137 (40.5%) female. Of the 339 biospecimen participants, 221 (64.8%) were recruited and 118 (35.2%) were volunteers. Nine of the 339 participants were unable to schedule an appointment, resulting in 330 participants completing at least one specimen collection. Thirty individuals provided consent to take part in the postmortem part of the study. Of the 30 postmortem participants, 5 (16.7) were recruited and 25 (83.3) were volunteers. All 30 postmortem participants provided consent for the overall donation of brain, spinal cord, bone, muscle, and CSF. In addition, 27 of the 30 the postmortem participants provided consent for skin sample collection. Three participants did not provide consent for

skin sample collection (one died before skin sample collection was added to the protocol, one refused, and one died before the signed skin consent form was received).

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

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

Three hundred and twenty-one participants provided at least one blood specimen and 15 provided a saliva sample. Nine participants were unable to provide any blood; however, eight provided hair samples, nine provided nail samples, nine provided a urine sample, and four provided a saliva sample. Most (78%) participants were able to provide specimens at both collection appointments.

Eighteen postmortem participants have donated specimens as of November 12, 2015. The length of time in the study for these participants from date of consent to date of death ranged from 1-24 months and the length of time in the study for participants that are still living, ranged from 20-27 months. The age at death for the deceased participants ranged from 43-76 years of age. Two participants are deceased but withdrew from the postmortem part of the study and did not provide postmortem donations. However, these participants did take part in the biospecimen part of the study.

Creating a geographically-diverse biorepository had unique challenges. Recruitment was slower than expected, finding reliable phlebotomists across the country was difficult, and there were unexpected issues related to shipping specimens including higher than average temperatures and mechanical failure. In addition, after the first specimens were collected there was a larger than expected number of individuals that were too sick or deceased and could not participate in the second specimen collection, decreasing the number of paired specimens. However, we were able to recruit the target sample size and process the varied specimen types. In March 2015, the Expert Panel extensively discussed the results of the pilot study and endorsed adding specimen collection as a component of the National ALS Registry. The Expert panel felt that the specific specimens collected should remain flexible so that there could be changes as research priorities and technology changed. In addition, they felt that because of the logistics and costs specimens should be collected at only one point in time. The discussion on the types of samples that should be collected as well as some changes to the logistics have been incorporated into the current proposal.

Approach

We will collect specimens from ALS patients enrolled in the National ALS Registry. The specimens to be collected will be determined on a yearly basis depending on the scientific demand. Currently, McKing is

accessing demand by reviewing the literature and talking with key ALS researchers. In subsequent years, we will evaluate the actual researcher requests. For example, although hair and nails are easy to collect there is still a cost to the laboratory for inventory control and storage. Therefore, we may not collect any additional hair or nail samples until the ones from the pilot study have been used up. Specimen types that could be included for in-home collection are blood, urine, hair, and nails. The amount of blood collected will not exceed 50 ml. Types of blood specimens could include blood for serum and DNA, blood for plasma, metals free collection of blood, blood for RNA extraction, and blood to create cell lines. For the postmortem collection, we will collect the brain, spinal cord, CSF, bone, muscle, and skin.

Population. Eligible participants for either component must have enrolled in the congressionally mandated National ALS Registry. After an individual has joined the Registry he/she will have the opportunity to ask for additional information about the biorepository and provide needed contact information (**Appendix A**). Additional specific eligibility requirements are outlined for each component below.

In-home component. Specimens will primarily be collected in participants' homes by trained phlebotomists. This was the process used by the biorepository pilot study and VA ALS Registry specimen collection. This approach is more convenient for participants and less likely to discourage those whose mobility is severely restricted. Minimizing limitations on when or where specimen collection can take place improves participation.

During the pandemic, th. A detailed restart safety plan can be found in Appendix B-6b.e Registry paused Biorepository specimen collections to protect the health of the Biorepository participants and members of their household

It is possible that ATSDR will want to collect blood specimens that need immediate processing and special handling. For example, some analytic techniques require plasma samples that have been centrifuged within 15 minutes of collection and then frozen. If this is the case, we will arrange for some participants to come to central locations for these specialized collections.

The phlebotomist will use a commercially available kit to collect the specimens and will be responsible for sending collected specimens to the laboratory according to the shipping protocol. Practical issues preclude the collection of specimen types that require a medical procedure or immediate processing (e.g., skin or muscle biopsy, lumbar puncture for CSF). **Table 8** indicates minimum specimen collection requirements, as well as potential additional specimens that could be self-collected and shipped by the participant or caregiver.

In-home saliva only component. DNA is one of the most used specimen types as more and more genes associated with ALS are identified. In order to increase the number and diversity of DNA specimens available for analysis, we will collect only saliva from an additional group of ALS patients enrolled in the Registry. We will sent a salvia collection kit to the participant's home with instructions for collection along with a pre-paid shipping label to return it to the lab.

Postmortem component. Research on the pathobiology of ALS has naturally emphasized analysis of brain, spinal cord, and CSF, the tissues most proximal to the disease process. The Boston University Alzheimer's Disease Center Brain Bank has developed procedures based on ISBER best practices (ISBER 2012) to recover these tissues postmortem. Their procedures will be used for the postmortem component of the biorepository with additions to include collection of muscle and bone tissue specimens.

Sample size. The number of participants will vary from year to year depending on funding. The National ALS Biorepository would like to collect a full set of biological specimens from 250 – 325 participants per year. Based on the blood failure rate in the pilot study, we anticipate that about 10% of the participants will be unable to give blood and will be offered the opportunity to provide a saliva sample. In addition, we will collect saliva only on 350 participants per year. Those who provide saliva only will be a combination of those who were unable to provide a blood specimen and those selected for saliva only collection. Therefore in a year with 325 participants in the full biological specimen component and 350 saliva only collections, we are expecting 675 participants. We would like to obtain postmortem tissue from 40 participants, i.e., approximately 10 per year.

Training staff. All biorepository staff will be trained on biorepository procedures and data security by the Senior Scientist. All biorepository staff will be required to provide documentation of training in HIPAA or take a certified course in HIPAA regulations that pertains to research. All biorepository staff will also take approved Human Subjects Protection training.

Outreach. Staff from the National ALS Biorepository will attend ALS related events (e.g., ALS Walks and ALS Association meetings) to answer questions about the project and handout IRB approved materials such as the factsheet and introductory letter. These activities will increase the biorepository visibility and better establish the connection with the National ALS Registry.

In-home Biorepository Component

Selecting prospective participants

Eligibility. To be eligible to donate specimens, prospective participants must have enrolled in the National ALS Registry and requested additional information about the biorepository

Identifying prospective participants. To achieve the target sample size of 675 participants per year, we estimate that it will be necessary to contact approximately 325-350 persons enrolled in the National ALS Registry for the full collection and 350-400 for saliva only collection. After providing consent to take part in the Registry, a potential participant will be asked if he/she would like to more information about the Biorepository (Appendix A). On a monthly basis, ATSDR will provide a list of those in the National ALS Registry who have expressed an interest in the biorepository. This list will include the date of birth, sex, current address, telephone number, and date of diagnosis for each registrant. Each month individuals will be selected to receive an invitation to participate in the biorepository based on geographic area. If a

person with ALS calls and wants to provide specimens for the biorepository, he/she will be provided information on how to join the Registry and indicate interest in the biorepository.

Selection full collection and saliva. The number of participants to be recruited each month will be determined by the number for the entire year. Each month ATSDR will provide McKing with a listing of Registry participants interested in the biorepository. To better address the congressional mandate to examine genetic and environmental risk factors that may cluster by geographic area, we will select a convenience sample from those who are interested in the biorepository proportional to state population and with at least one person from each state. We will recruit from the harder to fill states, e.g., Wyoming, Rhode Island, first and then distribute the cases throughout the other states. Because recruitment tends to get individuals from the same town to enroll during the same time period, selection in states where we are recruiting multiple participants will be distributed across the states in any given month. Based on our experience with the pilot study, this will give a good distribution of those living in urban and rural areas in addition to good state representation. At the time of selection, potential participants will be assigned to full collection or saliva only and receive the appropriate recruitment materials and consent form.

Demographic composition of biorepository population. A spreadsheet will be developed to track recruitment which includes age, sex, city, and state. We will attempt to assure the participants are diverse as possible within those participating in the National ALS Registry. After each round of participant selection, biorepository staff will examine the distribution of enrolled participants by geographic area, age, and sex, so that subsequent recruitment can target enrollment of the underrepresented groups. We will determine the number of individuals that should be recruited per state based on state population.

Enrolling participants

Recruitment. A sample of persons indicating an interest in the biorepository (full collection or saliva only) will be mailed a packet of information that includes an introductory letter (**Appendix B-1a(1) or B-1a(2)**), factsheet (**Appendix B-2a(1) or B-2a(2)**), and consent form (**Appendix B-3a or B-3b**). Approximately one week (5-10 days) after the packet is mailed, McKing biorepository staff will contact the potential participant to determine if he/she has received the packet, answer any questions, and if interested in participating, go over the consent form. The consent form will be signed, by the participant or witness, at the time of the phone call and returned in a self-addressed stamped envelope. The witness is just attesting that the consent form was explained to the participant and he/she agreed. Only the participant can provide consent. If a participant decides after reviewing the consent form that he/she does not want to participate, the individual will be removed from future contact lists for the biorepository. We will attempt to reach the person by phone up to 3 times.

Scheduling specimen collection. McKing biorepository staff will answer all questions about the biorepository and maintain all direct contact with participants except for collecting the specimens. Each prospective participant who verbally confirms his/her willingness to participate will be offered a range of dates and times to schedule a visit by a phlebotomist to collect the specimens (**Appendix B-1b**). We anticipate that most participants will choose to have specimens collected at their place of residence; however, acceptable alternative locations will be found for those who prefer for specimen collection to

occur elsewhere. Biorepository staff will assign a unique identification number (Biorepository ID) to each participant at the time his/her first appointment for specimen collection is scheduled.

Within one week after scheduling an appointment for specimen collection, biorepository staff will send the participant a confirmation letter with the appointment location, date, and time (**Appendix B-1c**). The letter will include and instructions for how to open the specimen collection kit to remove the urine collection portion (**Appendix B-2b**). If the participant is only providing a saliva sample, a kit will be mailed within one week of receiving the signed consent form.

No appointment will be scheduled for the saliva only collection.

Preparing for specimen collection

Alternative collection arrangements

Because of the pandemic we are unable to send phlebotomists to participant's houses. Therefore, we would like to allow home health care workers and physicians to draw the bloods. This would only be done at regularly scheduled doctor's visits or with home health care workers already employed by the participant. This will ensure no additional potential exposures to corona virus. McKing Biorepository staff will confirm qualifications to draw blood such as a nursing license or Certification of Phlebotomy Training (CPT).

In addition, while the Biorepository usually collects blood or saliva, there are some circumstances where we might want to collect both such as, but not limited to, the participant provided saliva when we were unable to collect blood, e.g., during the pandemic, or the participant had ALS related genetic findings where having blood would increase the usefulness of samples for research.

All other aspects related to recruitment, obtaining informed consent, kit shipment and specimen processing will remain the same.

Arranging the home visit. McKing has hired phlebotomy companies to provide phlebotomy services. They have the capability to provide nationwide specimen collection by hiring experienced local, licensed phlebotomists to collect specimens from participants who live in various regions of the U.S. McKing will provide information packets about the biorepository and collection requirements that will be provided to the contract phlebotomist. The phlebotomist will be provided via a secure method the name of the participant, date, time, address, phone number and location for specimen collection, at least one day prior to the scheduled in-home visit. The specimen collection kit, which includes detailed instructions for collecting the specimens, will be sent to the participant's home.

The phlebotomist will contact the participant via telephone introduce herself and confirm the appointment for the next day. If there are any changes at this time, the phlebotomist contacts McKing to notify them of any changes. If the participant states on this call that he/she has decided against participating in the biorepository, the appointment will be cancelled and the participant will be removed from the biorepository contact list. Otherwise, the participant will be reminded about the procedures

that will take place and given basic instructions related to eating and drinking before the specimen collection.

If the participant does not feel well enough on the day of specimen collection but still wants to participate in the biorepository, biorepository staff will reschedule the appointment (**Appendix B-1d**). The participant may call the McKing biorepository coordinator or notify the phlebotomist upon arrival at the participant's home. Appointments will be rescheduled up to two times.

Specimen collection kits. Kits will be created that contain the necessary instructions and equipment for collecting each type of specimen (blood, urine, hair, nail clippings, saliva), as well as appropriate shipping containers with instructions for transporting specimens to the laboratory for processing. McKing will distribute and track kits mailed to the participants.

All materials shipped to and received back from the phlebotomist will be tagged with a barcode. The barcode will contain information about the type of sample and the Biorepository ID for each participant, which does not contain personally identifying information. A list of the barcodes and a data collection form will be included with each specimen shipment.

Collecting specimens

Detailed information on specimen collection and processing can be found in **Appendix B-4**.

Urine. Specimen collection will be a random or "spot" urine, because a 24-hour urine or timed urine is not feasible. Participants will receive instructions on how to open the specimen collection kit and remove the urine collection container (**Appendix B-2b**) before the visit from the phlebotomist with instructions on how to obtain the specimen (**Appendix B-2c**). Participants will be encouraged to collect the specimen in the morning prior to the appointment to obtain the first morning void.

Blood. Blood will be collected using a 21-gauge x ¾" multi-sample butterfly needle blood collection set, with 12" tubing and safety-lock for the needle. Specimens will be collected in a predefined order to minimize cross contamination of specimens.

Hair. Hair will be collected from the nape area of the head directly into the shipping container using scissors contained in the collection kit. Hair will not be collected from those who are bald or whose hair is too short.

Fingernails. Fingernails will be collected from all ten (10) fingers using a stainless steel nail clipper. Fingernail clippings will be collected directly into the shipping container. Fingernails will be collected from as many nails as possible. Fingernails will not be collected if they are too short. Toenails will not be collected because of possible circulation issues in the feet of ALS patients making it riskier than collecting fingernails.

Specimen processing form. Information necessary to process and interpret the results of the specimen analysis will be collected by the phlebotomist and shipped with the specimens (**Appendix B-2e**). The phlebotomist will have to ask the participant for some of the information required.

Saliva. Saliva will be collected using a self-collection kit from Oragene. The participant will be asked to spit into a small funnel mailed to his/her home. Instructions for providing the saliva specimen will be mailed with the kit. No additional information is collected with the saliva.

After specimens are collected

Shipping specimens to the laboratory. The phlebotomist will be responsible for shipping the specimens to the laboratory according to instructions included in the kit. The shipping containers provided in the kit are sufficient to maintain the contents at the correct temperature for at least 24 hours beyond anticipated time of arrival. To monitor temperature during transit, we will use multi-use temperature data loggers. These can provide continuous temperature data during transit. These are easy to use and allow the sites to activate them at time of shipment with the push of the button. The phlebotomist will take the package to the nearest FedEx manned drop-off and can call for shipment pick-up in remote areas if approved by McKing. The laboratory will notify biorepository staff via email when the shipment has been received.

Saliva will be shipped back to the laboratory by the participant. A self-addressed FedEx label will be included with collection kit along with directions on how to drop the box in a FedEx box or call for a FedEx pick-up.

Laboratory processing. The laboratory will aliquot, process, and store all specimens according to their standard operating procedures. Once inventoried, samples will be immediately placed into permanent locations and stored at the appropriate temperature for the sample type. The storage unit(s) will be monitored 24/7 by an electronic monitoring system in accordance with pre-approved temperature specifications.

Unsuccessful collection. If the blood draw fails during an in-home collection visit, the phlebotomist will notify biorepository staff. Biorepository staff will contact the participant and ask if he/she would like to provide a saliva specimen **(Appendix B-1f)**. If yes, biorepository staff will send a self-collection saliva kit to the participant's home **(Appendix B-1e and Appendix B-2d)**.

If specimens become unusable after collection, biorepository staff will contact the participant, explain what happened, and ask if he/she are willing to provide a saliva specimen **(Appendix B-1f)**. Once the participant agrees to provide a saliva specimen, biorepository staff will send a saliva self-collection kit and guidelines directly to the participant's home **(Appendix B-1g and Appendix B-2d).** Biorepository staff will not ask participants to donate blood or urine again; however, if a participant offers to repeat the donation, biorepository staff will schedule a follow-up appointment for a phlebotomist to revisit the participant's home at least 8 weeks after the first blood draw. Following completion of the biospecimen collection, a thank you letter will be mailed to each to thank them for taking part in the biorepository

(Appendix B-1i). A repeat blood draw will only be considered for those where the blood was unusable due to shipping or laboratory error. Repeat blood draws will not be considered for failed blood draws.

Participant follow-up. Biorepository staff will send a thank-you note when specimens have been received (A**ppendix B-1i**).

Event collection Biorepository Component

To increase participation in areas that are under-represented in the Biorepository, we will work with our partners, e.g., the ALS Association, to conduct specimen collections at events such support groups and symposiums. Once a person expresses interest in providing a sample, the procedures will be the same as those for in-home collections for saliva or blood except that the sample(s) will be collected immediately after obtaining consent rather than mailing the kit to the participant's home. The collections will be in a private area with dividers separating the areas. Partners will announce the event using the email in **Appendix B-5**.

Postmortem Biorepository Component

Selecting prospective participants

Eligibility. Once the person has been contacted and has expressed interested in participating in the postmortem collection, the potential participant must be deemed medically eligible based on information from his/her treating neurologist.

Identifying prospective participants. To achieve the target sample size of 40 participants, we estimate that it will be necessary to contact approximately 100.

Demographic composition of biorepository population. The size is too small to recruit a sample that is representative by geographic area, race or ethnicity.

Enrolling participants

Introducing the biorepository. Prospective participants can elect to participate in both components of the biorepository (i.e., in-home biospecimen or postmortem), in only one component, or in neither. Enrollment and specimen collection for all who choose to participate in the in-home component will proceed as outlined in the previous section (**In-home Component**). Recruitment for the **Postmortem Component** is described in the next section. For those who elect to participate in both components of the biorepository, the recruitment call will cover both portions. However, calls to obtain consent will be done separately.

Recruitment. A sample of persons indicating an interest in the biorepository will be mailed a packet of information that includes an introductory letter (**Appendix C-1a**), factsheet (**Appendix C-2a**), HIPAA authorization form (**Appendix C-2b**) and consent forms (**Appendix C-3** and **Appendix C-3a**). Approximately one week (5-10 days) after the packet is mailed, McKing biorepository staff will contact the potential participant to determine if he/she has received the packet, answer any questions, and if

interested in participating, go over the process. Biorepository staff will ask if the person is interested in participating that he/she sign and return the HIPAA authorization form allowing us to speak to his/her treating neurologist in the self-addressed stamped envelope. Once the HIPAA authorization form is received, we will contact their treating neurologist to confirm eligibility.

Screening prospective participants. McKing biorepository staff will answer all questions about the biorepository and maintain all direct contact with participants except for collecting the specimens. Biorepository staff will consult with the prospective participant's treating neurologist to ensure that they meet the following criteria: 1) has an expected lifespan of 12-24 months from last date of contact with the treating neurologist; 2) has not been diagnosed with dementia; 3) has not been diagnosed with other neurological diseases; 4) has no plans to go onto a respirator as part of their ALS treatment plan; and 5) has no other medical or family issues that the treating neurologist believes makes the prospective participant a poor candidate for brain donation (**Appendix C-2c**). Following consultation with the treating neurologist, the biorepository staff will contact the prospective participant to notify them that they meet or do not meet the eligibility criteria for participation (**Appendix C-1e and Appendix C-1f**).

Informed consent. Within two weeks of confirming a prospective participant's eligibility to participate in the Postmortem Component, biorepository staff will contact the prospective participant and arrange a time for a call with them and their family member (**Appendix C-1f**). One week prior to the meeting, a letter to confirm the appointment will be mailed to the participant (**Appendix C-1g**). At this meeting, biorepository staff will explain the purpose of the biorepository, the process by which the postmortem tissues will be harvested, answer all questions that the prospective participant or family member may have, and review the consent form in detail. If the prospective participant is ready to consent to the biorepository at this meeting, biorepository staff will ask the prospective participant to sign the consent form. The Family Authorization Form (**Appendix C-2d**) for postmortem donation will be signed by the family member at the same time. The signed consent form and Family Authorization form will be returned to McKing in a self-addressed stamped envelope.

If the prospective participant is not ready to consent to the biorepository during this call, biorepository staff will follow up with them within two weeks following the call to determine if they are interested in participating (**Appendix C-1j**). If at any time during this process the prospective participant informs biorepository staff that he/she does not want to participate, his/her name will be removed from the biorepository contact list and they will not be contacted again. If after follow-up the prospective participant is not ready to consent, he/she will be considered a passive refusal and will not be contacted again. However, if a prospective participant later contacts staff to say that he/she is ready to consent; he/she may be included if the sample size has not been reached.

McKing will assign a Biorepository ID to each participant at the time his/her consent form has been signed. At this time a letter will be mailed to the participant to thank them for agreeing to participate in the biorepository (**Appendix C-1k**).

Preparing for specimen collection

Planning for Tissue Recovery. Specimen collection will be coordinated by the National Disease Research Interchange (NDRI), a non-profit, federally funded organization dedicated to retrieval of human tissues and organs for research, prepared, preserved, and shipped according to specific scientific protocols. After an individual is consented, he/she will be given the name of an NDRI staff member that will contact them to answer any questions and help facilitate the donation. McKing biorepository staff and NDRI will develop an individualized tissue recovery plan for each participant within 60 days from their consent to participate**.** The tissue collection plan used by McKing will include: 1) family primary point of contact, including name and contact information; 2) information on funeral plans, including name and contact information for funeral home; 3) name and contact information for transportation of the participant's body to and from harvest site; 4) name and contact information for the diener who will harvest the tissues; 5) tissue collection site and contact information; and 6) name and contact information for backup diener in case the primary diener is unavailable at the time of the participant's death. Once the tissue collection plan is complete, biorepository staff will notify the participant. The family will be provided with a toll-free, 24/7 phone number to call upon death of the participant. McKing will complete a donation plan that will be shared with the participant to make sure his/her family knows what will be done so donation goes smoothly (**Appendix C-1l**). NDRI is responsible for ensuring all applicable requirements (including consent if required) of the jurisdiction are met before harvesting tissues.

Tracking participants. Biorepository staff will maintain contact with and track all enrolled participants in order to monitor the progression of their disease (**Appendix C-1m**). Monitoring the participant's condition will allow biorepository staff to be aware when death is imminent and better prepared to implement the tissue recovery plan. Once every three months, biorepository staff will contact the participant or his/her family via phone to check in. As the participant's disease progresses and they appear closer to death, biorepository staff will increase their contacts with the participant or their family to once a month. Biorepository staff will document key points of these conversations and file them in the participant's file.

Family Authorization. At least one family member at the request of the participant will be included in discussions with the participant so that he/she can participate in the decision of the person with ALS to participate in the postmortem specimen collection. The family member will be kept informed of and included in any subsequent discussions with the person with ALS and will be present when the person consents. At the time of death, the family member will contact biorepository staff to arrange transit of the remains for specimen collection. If for any reason the family member decides not to proceed with the donation, the collection will be cancelled.

Specimen collection kits. Individual postmortem tissue collection kits will be assembled by NDRI and distributed to sites to assist in the proper collection and transport of tissue. The kits will include pre-labeled collection supplies such as pre-filled formalin containers, collection cups, and collection forms. Each kit will include an insulated shipper; all required shipping supplies/labels to meet IATA regulations; a pre-paid return FedEx air bill; notification form and packing/shipping instructions. All specimens will be labeled with the Biorepository ID number with an extension for the type of specimen collected.

Therefore, Biorepository ID numbers will be the same if a participant agrees to be in both the in-home collection and the postmortem portions of the study.

Collecting specimens

The body will be transported to a site designated for specimen collection. Specimen collection will be coordinated by NDRI. Detailed information on specimen collection can be found in **Appendix C-4**. The goal of the protocol is to complete all collections and return the body to the funeral home within two days. However, we will allow three days if obstacles are encountered. We will calculate the time from death notification to receipt of the body for each participant.

Brain, spinal cord, and CSF. A contracted diener will obtain the brain, spinal cord, and CSF specimens using standard procedures, which usually take two to three hours. The diener will pack the specimens on ice and a courier will deliver them to the nearest airport for shipping to the Boston University Alzheimer's Disease Center Brain Bank.

Muscle and bone. The diener will obtain muscle and bone specimens during the brain and spinal cord recovery process. Muscle and bone specimens will be deposited in separate formalin-filled vials and a courier will deliver them to for shipping to Fisher BioServices.

Skin. The diener will obtain a skin specimen during the brain and spinal cord recovery process. The skin specimen will be deposited into a separate vial and shipped to Zen-Bio Incorporated for creation of a cell line.

After specimens are collected

Specimens will be tracked from shipment of the specimen collection kit to receipt of specimens. Once we are notified that the specimens have been collected, a letter will be sent to the family of the participant to recognize the donation and to thank them for their support (**Appendix C-1n**).

Shipping specimens to the laboratory. Brain, spinal cord, and CSF will be shipped to the Boston University Alzheimer's Disease Center Brain Bank. Muscle and bone specimens will be shipped to Fisher BioServices and skin specimen shipped to Zen-Bio.

Laboratory processing. Brain, spinal cord, and CSF specimens will be processed at the Boston University Alzheimer's Disease Center Brain Bank according to procedures detailed in **Appendix C-5** and **Appendix C-6**. Muscle and bone specimens will be storage-ready and will not require additional processing. Once inventoried, samples will be immediately placed into permanent locations and stored at the appropriate temperature for the sample type. The storage unit(s) will be monitored 24/7 by an electronic monitoring system in accordance with pre-approved temperature specifications. The skin specimen will go to ZenBio for cell-line creation.

Unsuccessful recovery. Timing of postmortem tissue collection is critically important. The tissues must be harvested within 48 hours of death and then processed for storage within 24 hours of harvesting. If tissue recovery fails due to family member refusing participation after death or timing problems during

the process, these issues will be documented. If the collection fails due to any issue other than family withdrawal, the family will be notified. No follow-up action with the participant's family member will occur if the family member refuses participation.

Human Subjects Considerations

In-home Component

Biorepository staff will schedule telephone calls with each person interested in participating in the biorepository to go over the consent form (**Appendix B-3**) and answer questions. At the end of the call, the biorepository staff will ask those interested in participating to sign and date the consent form. All participants will be asked to provide consent themselves, however some persons with ALS have disease progression limiting their physical abilities. Because disease progression varies by individual, it is impossible to predict who, or the number of individuals that might be unable to sign. For individuals unable to sign because of physical disabilities, we are requesting a waiver of documentation. This research is no more than minimal risk and does not include any procedures that would require documentation of consent outside of a research setting. In these cases, we will require that there be a witness to the consenting process and consent of the person. The witness will be requested to sign the consent form. Because this is only a witness and not a legally authorized representative, we would permit the witness to be a family member, caregiver, or friend. The participant will send the signed consent form to McKing in a self-addressed stamped envelope. A copy of the signed consent form will be sent to the participant with his/her thank you letter in the event he/she has any questions. We are requesting permission to not include information addressing GINA on the consent form. Unlike many conditions, it is unlikely that individuals will be unaware that an individual or family is affected by ALS. This information is just as likely, if not more so, to cause discrimination than information about a gene that is only associated with ALS. In addition, it is likely to cause confusion and concern that is unnecessary.

Postmortem Component

Consent to determine eligibility will be obtained through the use of a script and written information sheet and will be documented via the signed HIPAA authorization form. After expressing interest in the postmortem component, the potential participant will be mailed a HIPAA authorization form (**Appendix C-2b**) to allow biorepository personnel to speak with his/her treating neurologist to determine eligibility (**Appendix C-2c**). After determining that a person is eligible, biorepository staff will contact the person to schedule a conference call with the potential participant and the family member most likely to be in charge of his/her final arrangements. The biorepository staff will go over the consent form (**Appendix C-3**) and answer any questions. All participants will be asked to provide consent themselves. However some persons with ALS have disease progression limiting their physical abilities, therefore we are requesting a waiver of documentation for those who are unable to sign. In these cases, we will require that there be a witness to the consenting process and consent of the person. The witness will be requested to sign the consent form. Because this is only a witness and not a legally authorized representative, we would permit the witness to be a family member, caregiver, or friend . In addition, we will ask the family member to sign the Family Authorization Form (**Appendix C-2d**). This form attests

to the family member's understanding of his/her family member's wishes regarding postmortem donation and his/her agreement to abide by his/her wishes. Although this form is nonbinding and we would not collect specimens against a family member's wishes, it involves the family member in the process and makes it more likely he/she will honor the family member's wishes. A copy of the signed consent form and HIPAA authorization form will be mailed to the participant. We are requesting permission to not include information addressing GINA on the consent form. Unlike many conditions, it is unlikely that individuals will be unaware that an individual or family is affected by ALS. This information is just as likely, if not more so, to cause discrimination than information about a gene that is only associated with ALS. In addition, it is likely to cause confusion and concern that is unnecessary.

Distribution of Specimens

The goal of the National ALS Biorepository is to have specimens available for ALS research. ATSDR will develop an application and review process for researchers to request specimens from the Biorepository. Once the request is approved, Biorepository staff will work with the research to send the approved specimens. Specimens will have the Biorepository ID number on the vials so that Biorepository staff can address questions about specific specimens, however at no time will researcher outside of ATSDR have access to the key. Any epidemiologic data that accompanies the specimens will be coded in such a way as to make inadvertent identification unlikely. For example, exact age will not be provided but will be recoded to age group, e.g., 55 will become 50-59. The Biorepository ID numbers will be added to the National ALS Registry to facilitate the linking of the biospecimens and the epidemiological data.

Specimen Analysis Conducted by the National ALS Registry

Some specimen analyses will be done by the National ALS Registry including those that contribute to one of the purposes outlined in the legislation, i.e., examine appropriate factors, such as environmental and occupational, that might be associated with the disease. Analytic results such as levels of metals in blood will become part of the Registry and available to other researchers. This increases the usefulness of the Registry and Biorepository and decreases duplication of effort.

Metals Analysis

Given that familial ALS occurs in 5%–10% of cases, it has been hypothesized that exposure to environmental factors may interact with genetic susceptibility in the development of ALS. Familial ALS can be caused by mutations in several genes, including C9orf72, SOD1, TDP43, and FUS genes, and these genes also contribute to the development of sporadic ALS (Ajourd-Driss 2015). Proposed environmental risk factors for ALS include exposure to cyanobacteria, various metals (lead, methyl mercury, selenium, zinc, copper and cadmium), pesticides, cigarette smoking, electromagnetic fields (EMF), and electrical shock (Trojsi 2013). The National ALS Registry conducted a pilot biorepository with voluntary participation from registrants. The Registry is unique because it includes persons with ALS (PALS) in the general population while most other human studies have been conducted on occupational cohorts or clinic patients. So far, limited studies have measured environmental chemicals in ALS biospecimens. We will start our exploratory analyses of environmental chemicals for an association with ALS. We will first focus on metals and metalloids, including mercury, cadmium, lead, manganese, arsenic, chromium, selenium, copper, and possibly others (that are tested in conjunction with them, such as beryllium and

uranium measured in the lead assay). To examine the hypothesis that exposure to metals is associated with the development of ALS, we will use biospecimens that were collected as part of the ALS biorepository. We anticipate the sample size will be between 300 and 600, depending on the registrants' response. The NCEH Division of Laboratory Sciences will conduct the bioassays using each aliquot tube labeled only with the participant's coded ID number. No personally identifiable information will be shared. Because the pilot biorepository did not include a control group, we will utilize the NHANES data to obtain estimates of the demographic distribution of metal levels for the US Population and test for:

• Null hypothesis: ALS registrants' distribution of metal levels is the same as a similar group of the US Population

• Alternative hypothesis: ALS registrants have higher metal levels than a similar group of the US Population

We will fit multivariate Cox proportional hazards models to determine whether survival time is associated with metal levels. The dependent variable will be interval from registration to death. The date of death and multiple causes of death will be obtained from the National Death Index. We will model the metal levels as continuous variables after making proper transformations for each metal. All models will include the following covariates: sex, age at diagnosis, race/ethnicity, and smoking status. We will also examine the available exposure information by linking the ALS registrants' survey data.

Genomic Analysis

Finally, the DNA samples of registrants will be genotyped using the NeuroX version 2 SNP chip (from Illumina) at Dr. Bryan Traynor's laboratory at the NIH. This SNP chip consists of a standard component with additional custom content. The Illumina HumanOnniCore forms the backbone of this array, assaying about 300,000 common SNPs across the human genome. The custom content focuses on neurodegeneration, and assays about 100k SNPs. These custom markers target specific regions of the genome implicated in neurological diseases and mutations in genes that cause ALS, Parkinson's disease, Alzheimer's disease, multiple system atrophy and other neurological diseases (Nalls 2015). Dr. Bryan Traynor has an IRB exemption to ascertain individuals with a clinical diagnosis of a movement disorder, their affected and unaffected family members, and unrelated, healthy individuals (to provide control samples); to characterize their phenotypes; and to identify and further characterize genetic contributions to etiology by collecting blood samples, saliva samples, and/or skin biopsies on these individuals for DNA and induced Pluripotent stem cell line preparation. He will be receiving deidentified DNA samples from the ALS biorepository for his testing. Genetic test results on ALS registrants will be transmitted to ATSDR to link with the ALS registrant, their survey data and other testing that is done by the biorepository. We will examine the potential relationships between the metals shown to be associated with ALS and mutations in known neurodegenerative genes (C9orf72, SOD1, TDP43, and FUS genes and others). We will assemble appropriate control groups for future metals and genetic test comparisons.

Beta-N-methylamino-L-alanine (BMAA) Analysis

Matched brain, spinal cord, and cerebral spinal fluid (CSF) material from 25 registrants will be analyzed to detect and quantify BMAA. Members of the Emergency Response Branch (ERB) and Tobacco and

Volatiles Branch (TVB) of the Division of Laboratory Sciences (DLS), National Center for Environmental Health (NCEH) will support this investigation via the development and validation of methods to detect and quantify BMAA in human tissues. Biorepository samples will be delivered to the Incident Response Laboratory (IRL), where IRL analysts will ensure samples are cataloged, blinded and disseminated to analysts. Sample preparation of brain tissue, spinal cord and CSF will extract the small molecules from the matrices and coupled liquid chromatography (LC) with mass spectrometry (MS) analysis will provide the concentration data. Brain and spinal cord tissue samples will be prepared using bead pulverization, followed by an organic extraction; CSF will have the proteins removed by precipitation. All organics will be hydrolyzed with strong acid and processed by solid-phase extraction (SPE). The LC/MS analysis of BMAA and its isomers will be done by a method previously published by TVB that utilizes an ion pairing technique for baseline separations of constitutional isomers; the LC/MS analysis will include isomer compounds of BMAA prevalent in algae to ensure there are no false positives. Analysts from TVB will upload the results into STARLIMS and the IRL will provide EHSB with an interpretation key. We will assemble appropriate control samples for future BMAA comparisons in persons with ALS and people with no ALS diagnosis.

Results of Research

Results of research will not be returned to participants for several reasons. It is unclear when specific samples will be used for research and could be many years in the future when the person with ALS is no longer alive. The National ALS Registry and the National ALS Biorepository do not collect information on next-of-kin. In addition, the Registry and the Biorepository many not actually have the results of research conducted by approved researchers outside of CDC/ATSDR. Therefore, it is not logistically possible to provide the results of research to participants or their families. ATSDR will post aggregate results of research on their website for the ALS community.

References

Ahmed A, Wicklund MP. Amyotrophic lateral sclerosis: what role does environment play? Neurol Clin. 2011 Aug;29(3):689-711.

Ajroud-Driss S, Siddique T. Sporadic and hereditary amyotrophic lateral sclerosis (ALS). Biochim Biophys Acta. 2015. 1852(4):679-684.

Allen KD, Kasarskis EJ, Bedlack RS, Rozear MP, Morgenlander JC, Sabet A, Sams L, Lindquist JH, Harrelson M L, Coffman C J, Oddone EZ. The National Registry of Veterans with amyotrophic lateral sclerosis The National Registry of Veterans with amyotrophic lateral sclerosis. Neuroepidemiology 2008 30(3):180-90.

Alonso A, Logroscino G, Hernán MA. Smoking and the risk of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2010 Nov;81(11):1249-52.

Armon C. Smoking may be considered an established risk factor for sporadic ALS. Neurology. 2009 Nov 17;73(20):1693-8.

Bowser R, Turner MR, Shefner J. Biomarkers in amyotrophic lateral sclerosis: opportunities and limitations. Nat Rev Neurol. 2011 Oct 11;7(11):631-8.

Chiò A, Schymick JC, Restagno G, et al. A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis. Hum Mol Genet. 2009 Apr 15;18(8):1524-32.

DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011 Oct 20;72(2):245-56.

Goullé JP, Saussereau E, Mahieu L, et al. Application of inductively coupled plasma mass spectrometry multielement analysis in fingernail and toenail as a biomarker of metal exposure. J Anal Toxicol. 2009 Mar;33(2):92-8.

Gwinn K, Corriveau RA, Mitsumoto H, Bednarz K, Brown RH Jr, Cudkowicz M, Gordon PH, Hardy J, Kasarskis EJ, Kaufmann P, Miller R, Sorenson E, Tandan R, Traynor BJ, Nash J, Sherman A, Mailman MD, Ostell J, Bruijn L, Cwik V, Rich SS, Singleton A, Refolo L,. Andrews J, Zhang R, Conwit R, Keller MA; ALS Research Group. Amyotrophic lateral sclerosis: an emerging era of collaborative gene discovery. PLoS One. 2007 Dec 5;2(12):e1254

Fang F, Kamel F, Lichtenstein P, et al. Familial aggregation of amyotrophic lateral sclerosis. Ann Neurol. 2009 Jul;66(1):94-9.

International Society for Biological and Environmental Repositories (ISBER). Best practices for repositories: collection, storage, retrieval, and distribution of biological materials for research. 3rd ed. Available at: https://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_Best_Practices_3rd_Edi.pdf

Kasarskis EJ, Dominic K, Oddone E. The National Registry of Veterans with Amyotrophic Lateral Sclerosis: Department of Veterans Affairs Cooperative Studies Program (SCSP) #500a. Amyotroph Lateral Scler Other Motor Neuron Disord 2004 5(Suppl1):129-32.

Lill CM, Abel O, Bertram L, Al-Chalabi A. Keeping up with genetic discoveries in amyotrophic lateral sclerosis: the ALSoD and ALSGene databases. Amyotroph Lateral Scler. 2011 Jul;12(4):238-49.

Millecamps S, Da Barroca S, Cazeneuve C, Salachas F, Pradat PF, Danel-Brunaud V, Vandenberghe N, Lacomblez L, Le Forestier N, Bruneteau G, Camu W, Brice A, Meininger V, LeGuern E. Questioning on the role of D amino acid oxidase in familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):E107

Nalls MA., Jose B, Dena GH, Margaux FK,, Elisa M, Alan ER et al. NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. Neurobiol Aging. 2015. 36(3):1605.e7-12.

National Biospecimen Network Blueprint, Andrew Friede, Ruth Grossman, Rachel Hunt, Rose Maria Li, and Susan Stern, eds. Constella Group, Inc., Durham, NC, 2003. Available at: http://biospecimens.cancer.gov/global/pdfs/FINAL_NBN_Blueprint.pdf

Otto M, Bowser R, Turner M, et al. Roadmap and standard operating procedures for biobanking and discovery of neurochemical markers in ALS. Amyotroph Lateral Scler. 2012 Jan;13(1):1-10.

Public Population Project in Genomics (P3G) Observatory. Repository of information and tools. Sample collection and processing. Available at: http://www.p3gobservatory.org/repository/sampleCollection.htm

Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011 Oct 20;72(2):257-68.

Schmidt S, Allen KD, Loiacono VT, et al. Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis: the GENEVA study. Rationale, study design and demographic characteristics. Neuroepidemiology. 2008;30(3):191-204.

Schmidt S, Kwee LC, Allen KD, Oddone EZ. Association of ALS with head injury, cigarette smoking and APOE genotypes. J Neurol Sci. 2010 Apr 15;291(1-2):22-9.

Sherman A, Bowser R, Grasso D, et al. Proposed BioRepository platform solution for the ALS research community. Amyotroph Lateral Scler. 2011 Jan;12(1):11-6.

Siddique T, Ajroud-Driss G. Familial amyotrophic lateral sclerosis, a historical perspective. Acta Myol 2011 Oct;30(2):117-20.

Storandt M, Head D, Fagan AM, Holtzman DM, Morris JC. Toward a multifactorial model of Alzheimer disease. Neurobiol Aging. 2012 Jan 17.

Trojsi F, Monsurrò MR, and TedeschG. Exposure to Environmental Toxicants and Pathogenesis of Amyotrophic Lateral Sclerosis: State of the Art and Research Perspectives. Int J Mol Sci. 2013. 14(8): 15286–15311.

Wu CH, Fallini C, Ticozzi N, et al. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature. 2012 Jul 15. doi: 10.1038/nature11280. [Epub ahead of print]

Table 1. Factors previously studied for association with ALS, with selected examples citing relevant biomarkers or exposures.

Factor/example	Review or study	Biomarker or history of exposure
Infectious agents		
EX: Enterovirus	Vandenberghe 2010. PMID 19900148	PCR for enterovirus RNA (CSF, fixed or frozen brain or spinal cord)
Cyanobacteria toxins	Cox 2009. PMID 19254284	HPLC/mass spectrometry analysis of protein-bound BMAA (archived brain or spinal cord)
Pesticides		
EX: Organophosphates	Johnson 2009. PMID 19632272	Paraoxonase enzyme activity, *PON1* genotype (blood)
Metals		
EX: Lead, mercury	Johnson 2009. PMID 19632272	Metals and organometallic compounds (brain, blood, CSF)
Drugs and other chemicals		
EX: Statins	Sorensen 2009. PMID 19930099	Medical history
Formaldehyde	Weisskopf 2009. PMC2765376	Occupational history
Injury		
EX: Head trauma	Schmidt 2011. PMC2840700	A-beta protein deposition (brain), interaction with *APOE* genotype (blood)
Electrical shock	Abhinav 2007. PMC2117843	History of lightning strike or other electrical injury
Occupation		
EX: Military service	Weisskopf 2005. PMID 15642900	Occupational history
Professional soccer	Chio 2009. PMID 19267274	Occupational history
Smoking	Alonso 2010. PMID 20639382	Smoking history
Diet	Morozova 2008. PMID 18300717	Dietary history

Table 2. Frequencies of mutations in five genes associated with familial ALS in a study of 162 families (Millecamps 2010).

Gene	Families (n)	Distinct mutations (n)	Novel mutations (n)
SOD1	20	18	3
TARDBP	7	6	2
FUS	7	5	2
ANG	1	1	0
VAPB	1	1	0

Table 3. Pathophysiologic processes with hypothesized roles in ALS, with selected examples citing approaches to measurement.

Pathophysiologic process	Review or study	Examples of measurement approaches
Gene expression and RNA metabolism		
EX: Gene expression	Mougeot 2011. PMC3219589	Microarray (peripheral blood lymphocytes)
EX: RNA processing	Lagier-Tourenne 2010. PMC3167692	Immunohistochemistry (brain and spinal cord)
EX: Endogenous retrovirus	Douville 2011. PMC3052883	PCR, sequencing, immunohistochemistry (brain)
Protein expression and metabolism		
EX: Protein expression	Kudo 2010. 20530642	Laser capture microdissection, tissue microarray (spinal cord)
Protein degradation	Ryberg 2010. PMID 20583124	Mass spectrometry-proteomics (cerebrospinal fluid)
Protein misfolding	Bosco 2011. PMC2967729	Immunoblot, immunohistochemistry (spinal cord)
Energy metabolism		
EX: Lipid metabolism	Dupuis 2011. PMID 21035400	Lipid levels (blood), mitochondrial function (muscle)
Oxidative stress	Lawton 2012. PMID 22117131	Mass spectrometry-metabolomics (plasma)
Inflammation		
EX: Cytokines	Fiala 2010. PMC2992053	ELISA (serum), microarray, immunohistochemistry (spinal cord)
Astrocyte toxicity	Haidet-Phillips 2011. PMID 21832997	Cell co-culture, mircroarray (spinal cord)

Table 4. Proposed desirable minimum clinical dataset for ALS biomarker studies (Otto 2012).

Category	Clinical information
Generic	Age
	Gender
	Ethnicity
	Drugs
	Past medical history
	Family history of neurodegenerative disorders (back two generations)
Phenotype	Diagnostic certainty (ALS by revised El Escorial criteria plus PLS or PMA), confirmed each time-point.
	Symptom onset (months/years)
	Date of diagnosis
	Site of onset (bulbar, upper/lower limb, respiratory)
	Regional phenotypes (flail arm, flail leg, progressive bulbar palsy, upper versus lower motor neuron-predominant ALS, cognitive involvement e.g. ALS-FTD)
Evaluation	ALSFRS-R
	Forced Vital Capacity
	Upper/lower motor neuron involvement
	Body Mass Index
	Cognitive assessment, e.g. Verbal Fluency Index
Interventions	Gastrostomy
	Non-invasive ventilation
	Cough-assist device
	Tracheostomy
	Date of death (with consideration of stratification of patients according to time of sampling in relation to overall disease course)

Copy of Table IV in Otto 2012.

Table 5. Biorepositories and brain banks with samples from people with ALS

Biorepository	Sponsor	Home	Sample types	Number with ALS	Access
Clinical biorepositories					
Northeast ALS Consortium (NEALS)	Consortium	Massachusetts General Hospital Boston, MA	serum, plasma, CSF, whole blood, extracted DNA, urine	5 clinical trials and 7 biomarker studies, each enrolling ~30-300 participants; ongoing open enrollment	applications reviewed by NEALS committee, with priority given to NEALS and other sites that contributed samples
NINDS Motor Neuron Disease Collection	National Institute for Neurologic Diseases and Stroke (NINDS, NIH)	Coriell Institute for Medical Research Camden, NJ (USA)	DNA, fibroblasts, immortalized lymphocytes	2021 persons	available on order with material transfer agreement and statement of research intent
Population-based biorepository					
National Registry of Veterans with ALS	Veterans Administration (VA)	Boston VA Medical Center Boston, MA	DNA (blood 85%, saliva 15%)	>1200 persons	collaborative research with Duke University
Brain banks					
VA Biorepository (VAB) Brain Bank	Veterans Administration (VA)	Southern Arizona VA Healthcare System Tucson, AZ	brain, spinal cord	not specified	not specified
MRC London Brain Bank for Neurodegenerative Diseases	Medical Research Council (MRC) London, UK	Institute of Psychiatry, King's College London, UK	fixed and frozen human brain tissue and spinal cord, sections on slides, frozen CSF, micro-dissected regions, extracted DNA/RNA	189 persons with motor neuron disease	available to researchers by application

Table 6. UK Biobank specimen collection protocol (adapted from Elliott 2008).

Collection priority	Sample preservative	Volume (ml)	Transport temp (°C)	Specifications
1	EDTA	9	4	Spray dried to give a final concentration of 1.8 mg/ml blood
2	Lithium heparin	8	4	*Plasma separation tube; spray dried to give a final concentration of 17 IU/ml.
3	Clot activator	8	4	*Serum separation tube; silica clot accelerator used in plastic collection tubes.
4	EDTA	9	4	
5	Acid citrate dextrose	6	18	Tube contains 1.0 ml of solution giving 2.2 mg/ml blood sodium citrate, 0.8 mg/ml citric acid, 2.45 mg/ml dextrose
6	EDTA	4	4	
				*indicates use of gel plug that forms a barrier to cellular material but allows serum or plasma to pass through
	Urine	9	4	

Table 7. Relative advantages of different specimen types for biomarker research (Otto 2012).

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

Adapted from Table V in Otto 2012. Note that skin specimens will not be collected for the National ALS Biorepository.

Table 8. Proposed minimum specimen collection protocol, with examples of potential analyses using each sample type.

Collection priority	Sample preservative	# tubes	ml / tube	Fractions	Examples
Blood					
1	K_2EDTA	1	10	White cells (buffy coat), red cells, plasma	DNA, proteins, red blood cell lipids
2	K_2EDTA	1	6	Whole blood	Lead, other metals
3	Plain, (no anticoagulant	1	10	Serum	Clinical biochemistries, metabolic products, other small molecules
4	CPT	1	8.0	Cells	Cell line creation
5	PAXgene RNA	1-2	5	RNA-stabilized whole blood	Intracellular RNA
Urine			9	--	Electrolytes, environmental chemicals, metabolic products
Nail clippings			--	--	Metals
Hair clippings			--	--	Metals
Saliva[1] (Oragene Collection Kit)			2	--	DNA

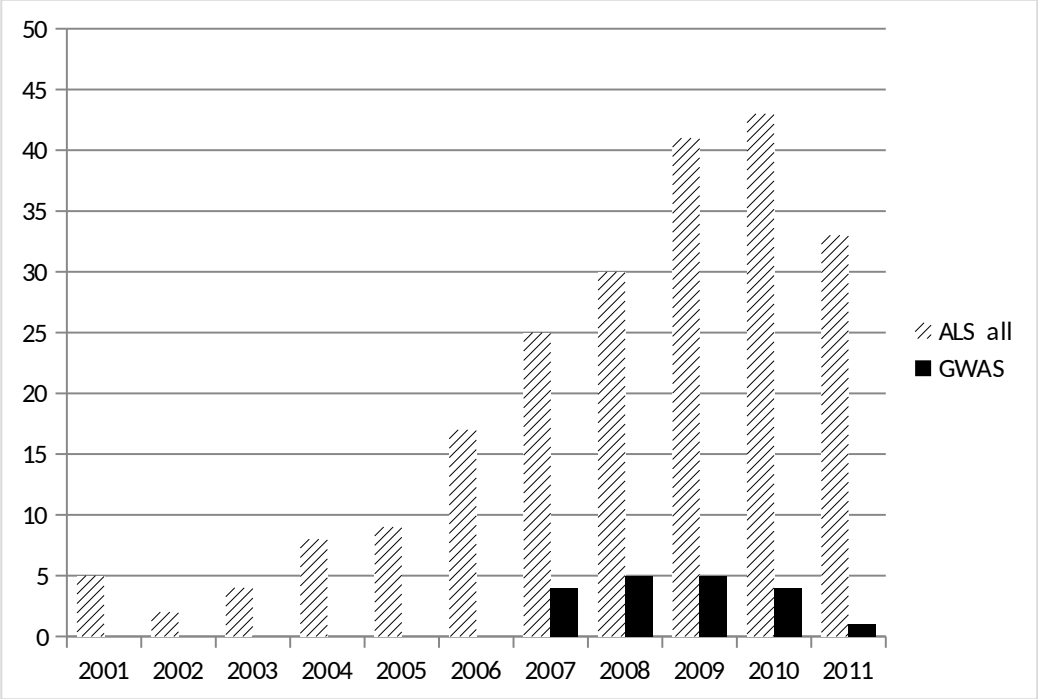
Sources: Eliott 2008, Holland 2003, Otto 2012

[1]Note: saliva samples will not be collected by technicians; self-collection of saliva will be used only when blood collection fails.

Drawing the 6.0-mL EDTA in second order allows the butterfly needle line to be flushed of potential heavy metal contamination beforehand. The PAXgene tubes are always collected last.

When blood is collected from a participant, the Vacutainers™ are inverted 10 times to mix the anti-coagulant/preservative with the whole blood (or to activate clotting in the red-top tubes with no anticoagulant). Using the butterfly collection device allows the phlebotomist to mix the previous tube thoroughly while the next tube is being collected.

Figure 1. Genetic association studies of sporadic ALS by year, 2001-2011 (as of January 2012).



GWAS = genome-wide association studies

Data from HuGE Navigator, www.hugenavigator.net.

Appendix A. Request for additional information

Research is an important part of the National ALS Registry. Specimens are needed for much of this research. The National ALS Biorepository is a new part of the National ALS Registry. If you are interested in learning more about the Biorepository, please check the "I Agree" box. A sample of interested PALS will get information packets.

☐ I Agree

If "I Agree", new screen

☐ I would like to learn more about donating biospecimens to the National ALS Biorepository.

☐ I would like to learn more about donating postmortem tissues to the National ALS Biorepository.

☐ I would like to learn more about donating both biospecimens and postmortem tissues to the National ALS Biorepository

Please provide your mailing address and phone number. This information will be used to make sure we ask PALS from all over the US to take part. We will also use this information to contact you if you are selected.

Mailing address: _____City:_____ State: _____ Zip code: _____

Phone number: ( ) ____-_____

Appendix B. In-home Biorepository Component
Appendix B-1. Communications
B-1a. – Email/letter to those expressing interest in biorepository

B-1a(1). – Email/letter (full collection)

This will be sent to participants who expressed an interest in participating only in the biospecimen portion of the biorepository. A copy of the fact sheet and consent form will be enclosed with this letter.

[Date]

Name
Address
City, State Zip

Dear [Participant Name]:

The Agency for Toxic Substances and Disease Registry (ATSDR) has launched the National ALS Biorepository (Biorepository). The Biorepository is a new part of the National ALS Registry. They have contracted with McKing Consulting (McKing) to manage the Biorepository. McKing would like to thank you for your interest in the Biorepository. The Biorepository will collect, store and distribute specimens from individuals with ALS. You are free to join or not. Your decision will have no impact on your eligibility to take part in the National ALS Registry or any future studies.

We are asking people who are enrolled in the National ALS Registry to donate biological specimens. This part of the Biorepository, may collect blood, urine, hair and nail specimens at your home. If you choose to take part in this part of the Biorepository, you will be asked to answer a few questions and to provide blood, urine, hair and nail specimens.

We are enclosing a fact sheet that explains the biospecimen part of the Biorepository. It also has answers to some frequently asked questions. We have enclosed a copy of the consent form for the Biorepository. Signing this form indicates that you agree to take part in the Biorepository. We will call you to review the consent form and answer any questions. If any part of this form is not clear to you, be sure to ask questions. Do not sign until you get answers to all of your questions. Once you have reviewed the consent form and agreed to participate in the biospecimen part of the Biorepository, we will set up an appointment for a phlebotomist to come to your home and collect specimens.

Your specimens will be saved and banked for future ALS research. We will store your specimens with some data about you, such as your age, race, and sex. We will not put your name on the specimen. We do not know how long we will keep these specimens. All information you provide will be kept private to the extent allowed by law. To protect your privacy your name will not appear with any of your information. A summary of the results of this specimen collection will be posted on the National ALS Registry website. Individuals will not be identified. Taking part in this Biorepository may help us learn

more about ALS. Although the Biorepository will not benefit you directly it may be of future benefit to others.

If you have any questions or want more information, please contact the Biorepository Coordinator, Laurie Wagner by email at lwagner@secure.mcking.com or by phone at 1-855-874-6912.

Thank you for supporting the National ALS Biorepository. We look forward to hearing from you soon.

Sincerely,

Wendy E. Kaye, PhD
Senior Scientist, National ALS Biorepository

Enclosures
Reading Level 9.9 (changing biorepository with study 7.9)

B-1a(2). – Email/letter (saliva only)

This will be sent to participants who expressed an interest in participating only in the biospecimen portion of the biorepository. A copy of the fact sheet and consent form will be enclosed with this letter.

[Date]

Name
Address
City, State Zip

Dear [Participant Name]:

The Agency for Toxic Substances and Disease Registry (ATSDR) has launched the National ALS Biorepository (Biorepository). The Biorepository is a new part of the National ALS Registry. They have contracted with McKing Consulting (McKing) to manage the Biorepository. McKing would like to thank you for your interest in the Biorepository. The Biorepository will collect, store and distribute specimens from individuals with ALS. You are free to join or not. Your decision will have no impact on your eligibility to take part in the National ALS Registry or any future studies.

We are asking people who are enrolled in the National ALS Registry to donate biological specimens. This part of the Biorepository, will collect a saliva specimen. If you choose to take part in this part of the Biorepository, you will be asked to provide a saliva specimen using a self-collection kit that will be mailed to your home.

We are enclosing a fact sheet that explains the saliva collection part of the Biorepository. It also has answers to some frequently asked questions. We have enclosed a copy of the consent form for the Biorepository. Signing this form indicates that you agree to take part in the Biorepository. We will call you to review the consent form and answer any questions. If any part of this form is not clear to you, be sure to ask questions. Do not sign until you get answers to all of your questions. Once you have reviewed the consent form and agreed to participate in the biospecimen part of the Biorepository, we will mail a kit to your home to provide a saliva specimen at your convenience.

Your specimens will be saved and banked for future ALS research. We will store your specimens with some data about you, such as your age, race, and sex. We will not put your name on the specimen. We do not know how long we will keep these specimens. All information you provide will be kept private to the extent allowed by law. To protect your privacy your name will not appear with any of your information. A summary of the results of this specimen collection will be posted on the National ALS Registry website. Individuals will not be identified. Taking part in this Biorepository may help us learn more about ALS. Although the Biorepository will not benefit you directly it may be of future benefit to others.

If you have any questions or want more information, please contact the Biorepository Coordinator, Laurie Wagner by email at lwagner@secure.mcking.com or by phone at 1-855-874-6912.

Thank you for supporting the National ALS Biorepository. We look forward to hearing from you soon.

Sincerely,

Wendy E. Kaye, PhD
Senior Scientist, National ALS Biorepository

Enclosures
Reading Level 10 (changing biorepository with study 8)

B-1b. – Phone script to schedule appointment, review instructions, and obtain consent

This is a phone script for making calls to individuals who received an information packet. The purpose of this call is to determine if the individual would like to participate and if so, consent the individual through a thorough review of the consent form. After the individual has provided consent, make an appointment for biospecimen collection.

INTRODUCTION:

- Ask for participant, introduce yourself and say that you are following up with potential participant

IF PARTICIPANT IS NOT AVAILABLE:

- Ask when would be a good time to contact (him/her)?

RECORD TIME FOR NEXT CONTACT:

Day/Date: _____

Time: _____

- If person asks on phone for your contact information leave your name and phone number only
- Thank them and end call

IF PARTICIPANT IS AVAILABLE:

- Introduce yourself and let the participant know that you are calling on behalf of the National ALS Biorepository
- Ask if he/she received the information packet
- Inform participant that you are calling to review the consent form
- Ask participant if they have had a chance to review the consent form and have a copy available at this time

a) If participant does not have a copy of the consent form available:

- Offer to send or email another copy and reschedule a time to call back (depending on their mode of contact.

RECORD TIME FOR NEXT CONTACT:

- Day/Date: _____
- Time: _____
- Remind participant to review consent form and to have a copy available for the next call
- Thank them and end call

b) If participant does have a copy of consent form but has not reviewed it:

- Allow participant time to review consent form while you wait on the phone
- Ask if participant wants to continue to participate in the biorepository

c) If participant does have a copy available and has reviewed consent form:

- Ask if participant wants to continue to participate in the biorepository

IF PARTICIPANT DOES NOT WANT TO CONTINUE TO PARTICIPATE IN THE BIOREPOSITORY:

- Remind participant of your name
- Thank them and end call

IF PARTICIPANT DOES WANT TO CONTINUE TO PARTICIPATE IN THE BIOREPOSITORY:

- Review each section of the consent form
- Ask participant if they have any questions at the end of each section
- Ask participant if they still want to participate in biorepository

If no,

- Remind participant of your name
- Thank them and end call

If yes,

- Ask participant or witness to sign the consent form
- Inform participant that the signed copy should be mailed back in the pre-stamped envelope sent with the information packet

CONFIRM PARTICIPANT CONTACT INFORMATION:

- Review the contact information that you have on file and make any corrections necessary; (name, address, phone, email etc.)
- Ask if the participant prefers US mail or email communication.

SCHEDULE APPONTMENT DATE AND TIME:

WHAT'S NEXT:

- Schedule appointment for in-home specimen collection
- Inform participant that a box containing the collection kit should arrive at their home
- Inform participant that the phlebotomist will call them 1 day before their appointment as a reminder
- Ask participant if there is anyone in the household that you can speak to about the biorepository if they are not available to come to the phone

If no,

- Make note in database not to speak with anyone else in the home about this biorepository

If yes,

- Document name and relationship to participant

IN CLOSING:

- Ask participant if they have any questions
- Remind participant of your name
- Thank them and end call

B-1c. – Letter/email appointment confirmation and instructions

This will be sent as an email or letter depending on preference of communication indicated by the participant when consent is obtained. This letter will be sent once the collection appointment is confirmed with a phlebotomist. The purpose of the letter is to confirm the appointment and provide instructions to the participant on preparatory activities.

[Date]

Name
Address
City, State, Zip Code
Dear [Participant Name]:

This letter is to confirm your appointment for a phlebotomist to come to your home and collect the following specimens:

- Blood
- Urine
- Hair clippings
- Finger nail clippings

Your specimen collection appointment is scheduled for
(Day/Date): _____ at (Time): _____.
Below are instructions to follow prior to your appointment:

- Do not cut your hair for at least 1 week before your appointment. [Delete if not collecting hair]
- Hair should be free of all gels, oils and hair creams or sprays prior to sample collection. The hair to be collected should be untreated (not permed, dyed or bleached). [Delete if not collecting hair]
- Do not cut your fingernails for at least 1 week before your appointment. Please remove nail polish for the day of your appointment. [Delete if not collecting fingernails]
- You will find a urine collection cup in the specimen kit sent to your house. Please provide your urine specimen the morning before your appointment.
- Drink plenty of water the days leading up to your blood draw and on the day of your blood draw.

If you have any questions feel free to contact me by phone at 1-855-874-6912 or by email at lwagner@secure.mcking.com. Thank you for your support of the National ALS Biorepository.

Sincerely,

Laurie Wagner, MPH
Coordinator, National ALS Biorepository

Enclosure

Reading Level 6.8

B-1c.1 – Letter/email appointment confirmation and instructions for just blood draw

This will be sent as an email or letter depending on preference of communication indicated by the participant when consent is obtained. This letter will be sent once the collection appointment is confirmed with a phlebotomist. The purpose of the letter is to confirm the appointment and provide instructions to the participant on preparatory activities.

[Date]

Name
Address
City, State Zip

Dear [Participant Name]:

This letter is to confirm your appointment for a phlebotomist to come to your home and collect the following specimens:

- Blood
- Urine
- Hair clippings
- Finger nail clippings

Your specimen collection appointment is scheduled for
(Day/Date): _____ at (Time): _____.
Below are instructions to follow prior to your appointment:

- Drink plenty of water the days leading up to your blood draw and on the day of your blood draw.

If you have any questions feel free to contact me by phone at 1-855-874-6912 or by email at lwagner@secure.mcking.com. Thank you for your support of the National ALS Biorepository.

Sincerely,

Laurie Wagner, MPH
Coordinator
National ALS Biorepository

Enclosure

B-1d. – Phone script to reschedule appointment

This is a phone script for individuals that need to reschedule their appointment for the biospecimen collection. The appointment can be rescheduled up to 2 times after which we will remove participant from active participant list and he/she can contact us when he/she is ready to have specimens collected.

INCOMING CALL INTRODUCTION:

- Answer phone, state your name and that you are with the National ALS Biorepository
- Ask potential participant how you can help them

OUTGOING CALL INTRODUCTION

- Ask for potential participant, introduce yourself and say that you are following up with potential participant

IF PARTICIPANT WANTS TO CANCEL THEIR APPOINTMENT:

- Look up participant name in database
- Verify date and time of appointment
- Cancel appointment
- Ask if participant would like to reschedule their appointment

IF PARTICIPANT DOES NOT WANT TO RESCHEDULE THEIR APPOINTMENT:

- If collection kit has already been shipped to participant, provide instructions on returning the kit
- Thank them and end call (remove name from future contact lists)

IF PARTICIPANT DOES WANT TO RESCHEDULE THEIR APPOINTMENT:

- Look up participant name in database
- Determine how many times they have canceled appointment (if more than 2 times then do not reschedule appointment, let participant call when they want specimens collected then reschedule)
- Schedule new appointment

Day/Date: _____

Time (am/pm):_____

CONFIRM PARTICIPANT CONTACT INFORMATION:

- Read participant the contact information that you have on file and make any necessary corrections; (name, address, phone, email)

WHAT'S NEXT:

- Confirm participant has specimen collection box.
- Ask them to place the box in a safe place because it will be used at the rescheduled appointment.

IN CLOSING:

- Ask if participant has any questions
- Remind participant of your name
- Thank them and end call

B-1e. – Email/letter blood draw failure

This will be sent as an email or letter depending on preference of communication indicated by the participant when consent is obtained. This letter will be sent to the participant if the blood draw failed during the in-home collection. The letter offers the participant the opportunity to complete an alternative form of DNA collection through a self-collection saliva kit.

[Date]

Dear [Participant Name]:

Thank you for agreeing to take part in the National ALS Biorepository. We are sorry we were not able to take your blood during our visit. We would like to know if you would like to donate a saliva specimen instead. We have enclosed a kit and directions. If you would like to provide a saliva specimen, please follow the enclosed directions. Please return the saliva specimen using the pre-paid FedEx air bill and original shipping box.

If you have any questions, please contact Laurie Wagner by phone at 1-855-874-6912 or by email at lwagner@secure.mcking.com. Thank you for your support of the National ALS Biorepository.

Sincerely,

Laurie Wagner, MPH
Coordinator, National ALS Biorepository

Reading level 10.0 (replacing biorepository with study 8.9)

B-1f. – Phone script for failed blood draw or unusable blood

This is a phone script for participants whose blood draws were unusable because the specimen was damaged during transit or processing or the participants was unable to give blood. The purpose of the call is to inform the participant the specimen is unusable as well as offer an alternative collection of DNA through a self-collected saliva kit.

INTRODUCTION:

- Ask for participant, introduce yourself and say that you are following up with participant

IF PARTICIPANT IS NOT AVAILABLE:

- Ask when would be a good time to contact (him/her)?
- RECORD TIME FOR NEXT CONTACT.
  Day/Date: _____
  Time: _____
  - If person asks for your contact information leave your name and phone number only
  - Thank them and end call

IF PARTICIPANT IS AVAILABLE:

- Introduce yourself and let the PALS know that you are calling on behalf of the National ALS Biorepository
  - Inform them that we are unable to use their blood because of a lab/shipping error OR that we were unable to obtain blood
  - Describe option for failed blood draw (saliva only)
  - Ask if they would like to provide a saliva sample
    - For those whose blood was unusable because of shipping or laboratory error, a second blood draw will be considered if the person offers. Please consult with the Biorepository Director or Coordinator.

IF PARTICIPANT DOES NOT WANT TO PROVIDE A SALIVA SAMPLE:

- Give participant the biorepository phone number
- Thank them and end call

IF PARTICIPANT DOES WANT TO PROVIDE A SALIVA SAMPLE:

- Thank them for agreeing to provide saliva sample
- Let them know that you will mail a saliva self-collection kit

IF PARTICIPANT VOLUNTEERS TO HAVE BLOOD SAMPLE DRAWN AGAIN (DO NOT ASK THEM) SCHEDULE A NEW APPOINTMENT:

Day/Date: _____
Time: _____

CONFIRM PARTICIPANT CONTACT INFORMATION:

- Review the contact information that you have on file and make any corrections necessary; (name, address, phone, email)

WHAT'S NEXT:

Saliva sample:

- Let participant know that the saliva kit should arrive within the next week with instructions.

Blood sample:

- Let participant know that only blood will be drawn and no other specimens collected.
- Confirm appointment, blood draw must be at least 8 weeks since the first draw.

IN CLOSING:

- Ask if participant has any questions
- Remind participant of your name
- Thank them and end call

B-1g. – Email/letter for saliva collection

This will be sent as an email or letter depending on preference of communication indicated by the participant when consent was obtained. This letter will be sent if the blood sample was damaged during transit or processing and becomes unusable. The letter offers the participant the opportunity to complete an alternative form of DNA collection through a self-collection saliva kit.

[Date]

Dear [Participant Name]:

Thank you for taking part in the National ALS Biorepository. We are sorry to inform you that we are unable to use your blood because of a lab/shipping error. We would like to know if you would like to donate a saliva specimen instead. We have enclosed a saliva specimen collection kit and directions. If you would like to provide a saliva specimen, please follow the enclosed directions

If you have any questions, please contact Laurie Wagner by phone at 1-855-874-6912 or by email at lwagner@secure.mcking.com. Thank you for your support of the National ALS Biorepository.

Sincerely,

Laurie Wagner, MPH
Coordinator, National ALS Biorepository

Reading level 10.6 (without study name 9.4)

B-1h. – Phone script follow-up saliva collection

This is a phone script for following up on saliva kits that have not been returned. The purpose of this call is to confirm the saliva kit has arrived at the participant's home, remind them to complete the collection and return it to the laboratory.

INTRODUCTION:

- Ask for participant, introduce yourself and say that you are following up with potential participant

IF PARTICIPANT IS NOT AVAILABLE:

- Ask when would be a good time to contact (him/her)?

RECORD TIME FOR NEXT CONTACT:

Day/Date: _____

Time: _____

- If person asks for your contact information leave your name and phone number only
- Thank them and end call

IF PARTICIPANT IS AVAILABLE:

- Introduce yourself and let the participant know that you are calling on behalf of the National ALS Biorepository
- Ask if they received the saliva collection kit

IF PARTICIPANT DID NOT RECEIVE THE SALIVA SAMPLE COLLECTION KIT:

- Let participant know that you will mail another saliva collection kit

CONFIRM PARTICIPANT CONTACT INFORMATION:

- Review the contact information that you have on file and make any corrections necessary; (name, address, phone, email)

WHAT'S NEXT:

- Let participant know that the saliva kit should arrive within 1 week with instructions and to call if they have any questions once it arrives
- Ask participant to mail kit back within 1 week after they provide sample

IF PARTICIPANT DID RECEIVE THE SALIVA SAMPLE COLLECTION KIT:

- Ask if the participant is interested in providing a saliva specimen
- Ask if participant has any questions or need any assistance
- Ask participant when they plan to mail the kit back

IN CLOSING:

- Ask if participant has any questions
- Remind participant of your name

- Thank them and end call

B-1i. – Email/letter thank you for participating in the biorepository

This will be sent as an email or letter depending on preference of communication indicated by the participant when consent was obtained. This letter will be sent following the completion of the biospecimen collection to thank the participant for participation in the biorepository.

[Date]

Dear [Participant Name]:

Thank you for taking part in the National ALS Biorepository. This Biorepository is being done to increase the number of specimens available for ALS research. A summary of the results of this specimen collection will be posted on the National ALS Registry website. Participants and other interested persons will be able to view the report. We will post a list of studies that used specimens from the Biorepository.

Collecting and storing biological specimens can lead to research. This research can improve clinical care for ALS patients. Many studies into the causes, progression, and prevention of ALS need specimens. Your specimens will be available to ALS Researchers. Data from the National ALS Registry risk factor surveys can help approved researchers using your specimens. If you have not already done so, please consider logging into your Registry account and taking the surveys. Your generous donation will help ALS research in the future.

If you have any questions, please contact Laurie Wagner, the Biorepository Coordinator, by phone at 1-855-874-6912. You can reach her by email at lwagner@secure.mcking.com. Once again, we thank you for your support of the National ALS Biorepository.

Sincerely,

Wendy E. Kaye, PhD
Senior Scientist, National ALS Biorepository

Reading level 11.2 (changing biorepository to study 7

Appendix B-2. Other participant materials

B-2a.—Fact Sheets

B-2a(1) – Fact Sheet Full collection

This will serve as a FAQ document. It will be included in the packet of information mailed to selected registry enrollees that have indicated interest in participating in the biorepository. It will also be emailed or mailed to any enrollees that ask for more information about the biorepository.

National Amyotrophic Lateral Sclerosis (ALS) Registry Biorepository Fact Sheet: All Biospecimens

What is the National ALS Biorepository about?	The National ALS Biorepository (Biorepository) was created by the Agency for Toxic Substances and Disease Registry (ATSDR) to collect, store, and share samples from participants in the National ALS Registry.
What is a Biorepository?	A biorepository is a facility that collects and stores samples of biological material. This could include blood, urine, tissue, cells, DNA, and proteins. Some medical information may also be stored along with a written consent form. These samples will be used for future research.
Who is managing the Biorepository?	This Biorepository is funded by ATSDR. ATSDR is a federal public health agency located in Atlanta, Georgia. McKing Consulting Corporation (McKing) has been awarded a contract to collect and distribute specimens for the Biorepository.
Why is this Biorepository important?	The specimens in this biorepository will complement the Registry's epidemiologic data. It will also add to the total numbers of biological specimens available for research on ALS. The National ALS Biorepository will differ from others already in existence because it will include ALS patients from the entire country. In addition, it will not be limited to those with specific exposures or clinical findings.
Who can take part in the Biorepository?	Persons with ALS enrolled in the National ALS Registry.
What information about me will be collected?	You will be asked to sign a consent form and answer a few brief questions. Then the phlebotomist will draw blood, clip hair and nail specimens, and you will provide a urine specimen. The samples will be stored for future research on ALS.
Where will the specimens be taken?	We will contact you to set up a time for a trained professional to come to your home and draw your blood and collect the other specimens.
Is there any risk to me?	Very little. We will try to make you as comfortable as possible but taking your blood may hurt a little. You will feel a slight "pinch "when the needle is put in. You may feel some discomfort or see a small bruise where the blood was drawn. There is no risk for the other

	specimens being collected.
Is there any benefit to me?	There is no direct benefit to you. The Biorepository will collect, store and process biospecimen samples. However, with further research, your samples may help us to better understand ALS in the future.
Will the information I tell you be kept private?	Yes. Just like when you talk to your doctor, everything you tell us will be kept private to the extent allowed by law. Any information with your name on it will be kept in a locked area. Only authorized employees will be able to look at this information. Results will be posted on the National ALS Registry website. Individuals will not be identified.
What will happen after I provide specimens?	Results of the Biorepository will be summarized and posted on the National ALS Registry website for all participants and other interested persons to view. Individuals will not be identified. ATSDR will keep your specimens for future ALS research.
Is there anything I need to do to prepare for the day I have the specimens taken?	Yes. • Do not cut your hair for at least 1 week before your appointment. • Hair should be free of all gels, oils and hair creams or sprays prior to sample collection. The hair to be collected should be untreated (not permed, dyed or bleached). • Do not cut your fingernails for at least 1 week before your appointment.
Is there anything I need to do on the day I have the specimens taken?	Yes. • Drink plenty of water on the day of your blood draw. • You may want to provide your urine specimen collection the morning before your appointment to have specimens taken. • Please remove nail polish for the day of your appointment to have specimens taken.
How long will it take?	Each specimen collection home visit should take about 30 minutes.
Is there any cost to me for the specimen collection?	No. There is no charge for the specimens being collected.
Do I have to participate in the Biorepository?	No. Taking part in the Biorepository is completely voluntary. You can refuse to answer any question for any reason. You can also refuse to provide a blood sample. You may choose to leave the Biorepository at any time even after signing the consent form. There is no penalty for leaving the Biorepository. Your decision will have no impact on your medical care, other services provided by your neurologist, or your participation in the National ALS Registry.

For more information about the Biorepository, contact **Laurie Wagner, MPH, Biorepository Coordinator**
Toll-free: 1-855-874-6912
Email: lwagner@secure.mcking.com
Website: wwwn.cdc.gov/als/alsBioRegistry.aspx

Reading level 8.1

B-2a(2).—Fact Sheet without Hair and Nails

This will serve as a FAQ document. It will be included in the packet of information mailed to selected registry enrollees that have indicated interest in participating in the biorepository. It will also be emailed or mailed to any enrollees that ask for more information about the biorepository.

National Amyotrophic Lateral Sclerosis (ALS) Registry Biorepository Fact Sheet: Biospecimens

	participants in the National ALS Registry.
What is the National ALS Biorepository about?	
What is a Biorepository?	A biorepository is a facility that collects and stores samples of biological material. This could include blood, urine, tissue, cells, DNA, and proteins. Some medical information may also be stored along with a written consent form. These samples will be used for future research.
Who is managing the Biorepository?	This Biorepository is funded by ATSDR. ATSDR is a federal public health agency located in Atlanta, Georgia. McKing Consulting Corporation (McKing) has been awarded a contract to collect and distribute specimens for the Biorepository.
Why is this Biorepository important?	The specimens in this biorepository will complement the Registry's epidemiologic data. It will also add to the total numbers of biological specimens available for research on ALS. The National ALS Biorepository will differ from others already in existence because it will include ALS patients from the entire country. In addition, it will not be limited to those with specific exposures or clinical findings.
Who can take part in the Biorepository?	Persons with ALS enrolled in the National ALS Registry.
What information about me will be collected?	You will be asked to sign a consent form and answer a few brief questions. Then the phlebotomist will draw blood and you will provide a urine specimen. The samples will be stored for future research on ALS.
Where will the specimens be taken?	We will contact you to set up a time for a trained professional to come to your home and draw your blood and collect the other specimens.
Is there an alternative collection process?	Yes. If you have a scheduled doctor's visits or a home health care worker already coming to your home, we can coordinate with them to collect your specimens.
Is there any risk to me?	Very little. We will try to make you as comfortable as possible but taking your blood may hurt a little. You will feel a slight "pinch "when the needle is put in. You may feel some discomfort or see a small bruise where the blood was drawn. There is no risk for the other specimens being collected.
Is there any benefit	There is no direct benefit to you. The Biorepository will collect, store and process

to me?	biospecimen samples. However, with further research, your samples may help us to better understand ALS in the future.
Will the information I tell you be kept private?	Yes. Just like when you talk to your doctor, everything you tell us will be kept private to the extent allowed by law. Any information with your name on it will be kept in a locked area. Only authorized employees will be able to look at this information. Results will be posted on the National ALS Registry website. Individuals will not be identified.
What will happen after I provide specimens?	Results of the Biorepository will be summarized and posted on the National ALS Registry website for all participants and other interested persons to view. Individuals will not be identified. ATSDR will keep your specimens for future ALS research.
Is there anything I need to do on the day I have the specimens taken?	Yes. • Drink plenty of water on the day of your blood draw. • You may want to provide your urine specimen collection the morning before your appointment to have specimens taken.
How long will it take?	Each specimen collection home visit should take about 30 minutes.
Is there any cost to me for the specimen collection?	No. There is no charge for the specimens being collected.
Do I have to participate in the Biorepository?	No. Taking part in the Biorepository is completely voluntary. You can refuse to answer any question for any reason. You can also refuse to provide a blood sample. You may choose to leave the Biorepository at any time even after signing the consent form. There is no penalty for leaving the Biorepository. Your decision will have no impact on your medical care, other services provided by your neurologist, or your participation in the National ALS Registry.

For more information about the Biorepository, contact **Laurie Wagner, MPH, Biorepository Coordinator** **Toll-free:** 1-855-874-6912 **Email:** lwagner@secure.mcking.com **Website:** wwwn.cdc.gov/als/alsBioRegistry.aspx	Reading level 8.6

B-2a(3).—Fact Sheet Saliva only

This will serve as a FAQ document. It will be included in the packet of information mailed to selected registry enrollees that have indicated interest in participating in the biorepository. It will also be emailed or mailed to any enrollees that ask for more information about the biorepository.

National Amyotrophic Lateral Sclerosis (ALS) Registry Biorepository Fact Sheet: Saliva

	Substances and Disease Registry (ATSDR) to collect, store, and share samples from participants in the National ALS Registry.
What is the National ALS Biorepository about?	
What is a Biorepository?	A biorepository is a facility that collects and stores samples of biological material. This could include blood, urine, tissue, cells, DNA, and proteins. Some medical information may also be stored along with a written consent form. These samples will be used for future research.
Who is managing the Biorepository?	This Biorepository is funded by ATSDR. ATSDR is a federal public health agency located in Atlanta, Georgia. McKing Consulting Corporation (McKing) has been awarded a contract to collect and distribute specimens for the Biorepository.
Why is this Biorepository important?	The specimens in this biorepository will complement the Registry's epidemiologic data. It will also add to the total numbers of biological specimens available for research on ALS. The National ALS Biorepository will differ from others already in existence because it will include ALS patients from the entire country. In addition, it will not be limited to those with specific exposures or clinical findings.
Who can take part in the Biorepository?	Persons with ALS enrolled in the National ALS Registry.
What information about me will be collected?	You will be asked to sign a consent form. Your sample will be stored for future research on ALS.
Where will the specimens be taken?	You will provide a saliva specimen in your home at your convenience.
Is there any risk to me?	There is no risk when providing a saliva specimen.
Is there any benefit to me?	There is no direct benefit to you. The Biorepository will collect, store and process biospecimen samples. However, with further research, your samples may help us to better understand ALS in the future.
Will the information I tell you be kept private?	Yes. Just like when you talk to your doctor, everything you tell us will be kept private to the extent allowed by law. Any information with your name on it will be kept in a locked area. Only authorized employees will be able to look at this information. Results will be

	posted on the National ALS Registry website. Individuals will not be identified.
What will happen after I provide specimens?	Results of the Biorepository will be summarized and posted on the National ALS Registry website for all participants and other interested persons to view. Individuals will not be identified. ATSDR will keep your specimens for future ALS research.
Is there anything I need to do before donating specimens?	Do not eat, drink, smoke or chew gum for 30 minutes before giving your saliva sample.
How will I provide my specimen?	You will be asked to spit into a small funnel that will be mailed to your home. Instructions for providing the saliva specimen will be mailed with the kit.
How long will it take?	Giving a saliva specimen should take about 10 minutes.
Is there any cost to me for the specimen collection?	No. There is no charge for the specimens being collected.
Do I have to participate in the Biorepository?	No. Taking part in the Biorepository is completely voluntary. You can refuse to answer any question for any reason. You can also refuse to provide a saliva specimen. You may choose to leave the Biorepository at any time even after signing the consent form. There is no penalty for leaving the Biorepository. Your decision will have no impact on your medical care, other services provided by your neurologist, or your participation in the National ALS Registry.

For more information about the Biorepository, contact **Laurie Wagner, MPH, Biorepository Coordinator** **Toll-free:** 1-855-874-6912 **Email:** lwagner@secure.mcking.com **Website:** wwwn.cdc.gov/als/alsBioRegistry.aspx	

Reading level 9.1

B-2b. – Instructions for opening the specimen kit

These guidelines will provide instructions to participants receiving and opening the specimen collection kit from the laboratory. This will be sent with Email/Letter to interested PALS (B-1c.).

National ALS Biorepository
Guidelines for opening Collection Kit

Step 1: Open the outer shipping box.

Step 2: Take the lid off of the Styrofoam container.

Step 3: Remove the yellow bag that contains the Urine Collection Kit.

Step 4: Remove the two cooling gel packs.

Step 5: Once the Urine Collection Kit and cooling gel packs are removed, replace the Styrofoam lid and set aside the Collection Kit and outer shipping box. The Collection Kit will not be used again until the phlebotomist arrives, the day of your specimen collection appointment.

Step 6: Open the yellow bag when you are ready to provide your urine sample. It is best to get the sample when you first get up in the morning on the day of your appointment.

The Urine Collection Kit should contain the following:

1. Plastic collection container
2. Disposable gloves
3. Towelette

Step 7: Follow the directions included with the urine collection kit.

Step 8: Place the urine container back in the resealable yellow bag and place the bag and cooling gel packs in the refrigerator.

Step 9: Call a member of the National ALS Biorepository team at 1-855-874-6912 if you have questions.

Reading Level 8.7

B-2b.1 – Instructions for opening the specimen kit when not collecting urine

These guidelines will provide instructions to participants receiving and opening the specimen collection kit from the laboratory when urine is not being collected. This will be sent with Email/Letter to interested PALS (B-1c.).

National ALS Biorepository
Guidelines for opening Collection Kit

Step 1: Open the outer shipping box.

Step 2: Take the lid off of the Styrofoam container.

Step 3: Remove the two cooling gel packs.

Step 4: Once the cooling gel packs are removed, replace the Styrofoam lid and set aside the Collection Kit and outer shipping box (Do not put box in refrigerator). The Collection Kit and outer shipping box will not be used again until the phlebotomist arrives, the day of your specimen collection.

Step 5: Place cooling gel packs in the refrigerator (Do not put in freezer).

Step 6: Call a member of the National ALS Biorepository team at 1-855-874-6912 if you have questions.

Reading level 8.9

B-2c. – Instructions for participant urine collection

These guidelines provide instructions to the participant on completing the urine collection prior to the arrival of the phlebotomist. This document will be included in participant information packet as well as including in the urine collection package that will arrive with the collection kit from the laboratory.

OPEN THE URINE COLLECTION KIT:

- Open the urine collection cup package. DO NOT REMOVE the label from the top of the Urine Collection Cup. There is a needle under the label.
- Put the yellow-top tubes to the side for the phlebotomist to use
- Remove the sterile wipe

PREPARE FOR URINE COLLECTION:

- Wash hands thoroughly with soap and water
- Unscrew the blue cap
- Place the blue cap on the counter with "straw" facing upwards. Do not touch the inside of cap or the straw.

FEMALE COLLECTION INSTRUCTIONS:

- Separate the folds of skin around the urinary opening.
- Using the sterile wipe, clean the area using front-to-back strokes
- Discard the wipe
- Void a small amount of urine into the toilet
- As you continue to urinate, bring the collection cup into the midstream to collect the urine sample
- Do not touch the inside or lip of the cup
- Replace the blue cap onto the collection cup
- Place cup inside bag and place in the refrigerator
- Give to the phlebotomist when she arrives

MALE COLLECTION INSTRUCTIONS:

- Pull foreskin back (if necessary)
- Using the sterile wipe, clean the end of the penis by rubbing in a circular motion starting at the urethra opening and moving outwards
- Discard the wipe
- Void a small amount of urine into the toilet
- As you continue to urinate, bring the collection cup into the midstream to collect the urine sample
- Do not touch the inside or lip of the cup
- Replace the blue cap onto the collection cup
- Place cup inside bag and place in the refrigerator
- Give to the phlebotomist when she arrives

Reading Level 5.1

B-2d. – Instructions for saliva collection

These guidelines will provide instructions to participants completing the self-collected saliva collection. This will be included in the saliva collection kit package that is shipped to their home.

ORAgene®·DISCOVER
OGR-500

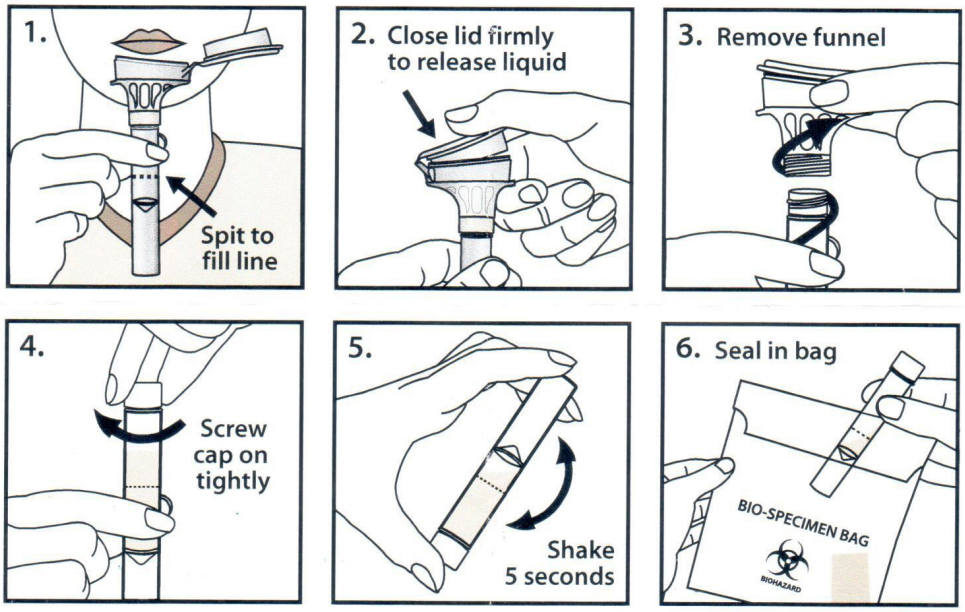
Oragene®·DISCOVER self-collection kit instructions

For complete instructions and additional languages, see inside kit.

Collection precautions: Do NOT eat, drink, smoke or chew gum for 30 minutes before giving your saliva sample.

Do NOT remove the plastic film from the funnel lid.

Procedure: Most people take between 2 and 5 minutes to deliver a saliva sample following steps 1 to 6.



This product is designed for the collection of human DNA from saliva samples.

Warnings and precautions: Wash with water if the stabilizing liquid comes in contact with eyes or skin. Do NOT ingest. See MSDS at www.dnagenotek.com.

Small cap, choking hazard.

Storage: Store at room temperature 15–30°C.

Oragene®·DISCOVER is for research use only, not for use in diagnostic procedures. Some DNA Genotek products may not be available in all geographic regions. ®Oragene is a registered trademark of DNA Genotek Inc.

U.S. Patent No. 7,482,116;
European Patent No. 1 513 952 and 1 956 969; Patent pending
Canadian Design Nos. 127470; 132896; 132897
U.S. D631,554 S and D640,795 S
Community Design Nos. 001095186-0001; -0002; -0003

Superior samples • Proven performance

PD-PR-00261 Issue 1/2012-05

DNA genotek
www.dnagenotek.com

National ALS Biorepository Consent Form (Biospecimens)

Why am I being asked?

You are being asked to take part in the biorepository because you are enrolled in the National ALS Registry and have told us you are interested in learning more about the biorepository. The biorepository is a new part of the National ALS Registry maintained by the Agency for Toxic Substances and Disease Registry (ATSDR). We ask that you read this form and ask any questions you may have before agreeing to be in the biorepository. McKing is the company that was awarded a contract to manage the biorepository. The biorepository Senior Scientist is Dr. Wendy E. Kaye.

Taking part in this biorepository is voluntary. You should feel free to ask the researchers any questions you may have. Your decision whether or not to take part will not affect your current or future relations with the National ALS Registry. If you decide to take part, you are free to withdraw at any time without affecting that relationship.

What is the purpose of this biorepository?

This biorepository is being created to increase the number of specimens available for ALS researchers. The specimens in this Biorepository will complement the National Registry's epidemiologic data.

Approximately 675 people will be asked to contribute specimens to the biorepository each year.

What are the procedures involved?

You will be asked to have specimens collected from you at your home. This should take about 30 minutes.

You are being asked to donate specimens. We will make an appointment for a phlebotomist to come to your home to collect specimens at a time convenient for you. We will ask you to donate the specimen types checked below.

- A urine specimen collection kit with directions will be sent to your home before your appointment. You will be asked to provide a urine specimen (9 ml) before the end of your appointment. The best time to give a urine specimen is when you first get up in the morning on the day of your appointment.
- A blood sample will be drawn from your arm with a needle by a trained phlebotomist. There will be 5 tubes of blood taken (about 40 ml or 2.5 tablespoons).
- Fingernail clippings will be collected by the phlebotomist from all ten (10) fingers using a stainless steel nail clipper.
- Hair clippings will be collected by the phlebotomist on the day of your appointment. The hair specimen collection will be about 120 strands. These will be clipped from the back of your head, near your neck, and under your other hair.
- Saliva samples (2 ml) will be taken only if you are unable to give blood or if there is a problem with the blood. A saliva sample kit with directions will be mailed to your home and you will be asked to collect the saliva sample yourself.

Additional Instructions:

- Drink plenty of water on the day of your blood draw.
- You will be sent a cup to collect a urine specimen. We ask that you collect this the morning before your appointment.
- Do not cut your hair for at least 1 week before your appointment, if we are collecting hair.
    - Hair should be free of all gels, oils and hair creams or sprays prior to sample collection. The hair to be collected should be untreated (not permed, dyed or bleached).
- Do not cut your fingernails for at least 1 week before your appointment. Please remove nail polish for the day of your appointment, if we are collecting fingernails.

Tissue Banking

The purpose of this biorepository is to collect specimens for future research related to ALS. Your specimens will be stored with a number. No private information will be on the specimen container.

Researchers can ask to use specimens from the biorepository for research related to ALS. We do not know what types of research will be done. These studies will likely examine the DNA and RNA (genetic material) and other material from cells in the tissues. Researchers must provide a detailed plan of the study. This plan must be approved by ATSDR and the ethics committees at the researchers' institutions. Your specimens along with others will be provided to approved researchers. These specimens will not have your name on them. The specimens will include information about you such as your age or city where you lived. They may also request other data you gave the National ALS Registry. We will limit the amount of information about you to reduce the chances that you will be identified.

Results of research using these specimens will be available on the National ALS Registry website.

What are some of the risks and discomforts that may happen to people who are in this biorepository?

You will feel a slight "pinch" when the needle is put in to draw your blood. You may feel some pain or see a small bruise where the blood was drawn. There is little risk for the other specimens being collected.

There is a slight risk that someone could get access to the data we have stored about you. If information about you does leak out, ATSDR will not be able to guarantee that it will be protected.

There is a very small chance that genetic research in the future could give genetic information that could be used to identify you.

Are there benefits to taking part in this biorepository?

You will not directly benefit from taking part. It is hoped that knowledge gained from this research may benefit others with ALS in the future.

What other options are there?

You have the option to not to take part in this biorepository.

What about privacy and confidentiality?

If you take part in the biorepository, we will not have access to your private medical records. The people who will know that you take part in this biorepository are members of the biorepository team, individuals who may have access to your informed consent document due to their job function with the National ALS Biorepository, and people processing your specimens.

Information that identifies you and the consent form signed by you will be looked at by the ATSDR.

A possible risk of the biorepository is that people outside of the biorepository find out you are taking part in the biorepository or learn information about you and your health. Your specimens will be stored with a code. Your name will not be on the container.

When the results of the biorepository are published or discussed at meetings, no information will be included that would reveal your identity

Will my family be told biorepository results?

Your family will not be told of any new findings that come to light during the course of using your specimens. We will not tell you or your family any genetic testing results from research studies. ATSDR will post study results on the National ALS Registry website.

What are the costs for participating in this biorepository?

There is no cost for participating in this biorepository. You will not be charged for any lab tests.

Will I be reimbursed for any of my expenses or paid for my participation in this biorepository?

You will not be paid to take part in this biorepository.

Will my cells, tissues, blood, or other biological materials be used to develop commercial products?

It is possible that a commercial product may be developed from the tissue or blood samples collected as part of this biorepository. The nature of the research means that your sample is only one of many that will lead to this product and your sample will not have your name on it. You and your family will not profit financially from such a product. You will have no legal rights to any discovery or invention that either directly or indirectly results from the use of your specimens, individual information, or information from your samples.

Cells obtained from your body may be used to establish a cell line which may be shared in the future with other researchers and which may be of commercial value. A cell line is one which will grow indefinitely in the laboratory. Cell lines may be useful because of the characteristics of the cells and/or the products they may produce.

Can I withdraw or be removed from the biorepository?

Taking part in this biorepository is voluntary. If you choose not to take part or decide to withdraw your consent and stop taking part, this will not affect your relationship with National ALS Registry, or other benefits to which you are otherwise entitled.

You have the right to leave the biorepository at any time without penalty. If you withdraw from the biorepository, we will destroy any of your remaining samples. However, we will not be able to remove your samples that have already been used or shared with researchers.

Who should I contact if I have questions regarding the biorepository? Who should I contact if I wish to voice concerns or complaints? Who can I talk to about my rights or want to withdraw my samples?

If you have questions, concerns, or complaints, think the biorepository has hurt you, or if you want to withdraw your samples, you can talk to Wendy E. Kaye, Ph.D., Senior Scientist or Laurie Wagner, MPH, Biorepository Coordinator at 1-855-874-6912.

If you want to speak with someone who is not directly involved in this biorepository, or have questions about your rights, or wish to voice questions, concerns or complaints, you may contact the CDC Human Research Protection Office at 1-800-584-8814.

Remember

Taking part in this biorepository is voluntary. Your decision whether or not to take part will not affect your current or future relations with the National ALS Registry. If you decide to take part, you are free to withdraw at any time without affecting that relationship.

Subjects' Rights:

I have read the above information. I have discussed this biorepository with the person obtaining consent, been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this biorepository. I will be given a copy of this signed and dated consent form.

_____

Print Subject name

_____ _____

Signature of Subject Date

If participant is physically unable to sign the consent form, please complete the following

_____ _____

Witness Signature Date

I, _____, witnessed that _____ was explained the consent form and has agreed to take part in this biorepository. Due to the progression of the disease, the participant is physically unable to sign the consent form.

Reading level 9.5 (changing biorepository to study 8.0)

National ALS Biorepository Consent Form (Saliva)

Why am I being asked?

You are being asked to take part in the biorepository because you are enrolled in the National ALS Registry and have told us you are interested in learning more about the biorepository. The biorepository is a new part of the National ALS Registry maintained by the Agency for Toxic Substances and Disease Registry (ATSDR). We ask that you read this form and ask any questions you may have before agreeing to be in the biorepository. McKing is the company that was awarded a contract to manage the biorepository. The biorepository Senior Scientist is Dr. Wendy E. Kaye.

Taking part in this biorepository is voluntary. You should feel free to ask the researchers any questions you may have. Your decision whether or not to take part will not affect your current or future relations with the National ALS Registry. If you decide to take part, you are free to withdraw at any time without affecting that relationship.

What is the purpose of this biorepository?

This biorepository is being created to increase the number of specimens available for ALS researchers. The specimens in this Biorepository will complement the National Registry's epidemiologic data.

Approximately 675 people will be asked to contribute specimens to the biorepository each year.

What are the procedures involved?

You will be asked to collect saliva from which DNA can be extracted. We will send a special kit to your home. This should take about 10 minutes.

Tissue Banking

The purpose of this biorepository is to collect specimens for future research related to ALS. Your specimens will be stored with a number. No private information will be on the specimen container.

Researchers can ask to use specimens from the biorepository for research related to ALS. We do not know what types of research will be done. These studies will likely examine the DNA and RNA (genetic material) and other material from cells in the tissues. Researchers must provide a detailed plan of the study. This plan must be approved by ATSDR and the ethics committees at the researchers' institutions. Your specimens along with others will be provided to approved researchers. These specimens will not have your name on them. The specimens will include information about you such as your age or city where you lived. They may also request other data you gave the National ALS Registry. We will limit the amount of information about you to reduce the chances that you will be identified.

Results of research using these specimens will be available on the National ALS Registry website.

What are some of the risks and discomforts that may happen to people who are in this biorepository?

There is little risk for collecting saliva.

There is a slight risk that someone could get access to the data we have stored about you. If information about you does leak out, ATSDR will not be able to guarantee that it will be protected.

There is a very small chance that genetic research in the future could give genetic information that could be used to identify you.

Are there benefits to taking part in this biorepository?
You will not directly benefit from taking part. It is hoped that knowledge gained from this research may benefit others with ALS in the future.

What other options are there?
You have the option to not to take part in this biorepository.

What about privacy and confidentiality?
If you take part in the biorepository, we will not have access to your private medical records.
The people who will know that you take part in this biorepository are members of the biorepository team, individuals who may have access to your informed consent document due to their job function with the National ALS Biorepository, and people processing your specimens.

Information that identifies you and the consent form signed by you will be looked at by the ATSDR.

A possible risk of the biorepository is that people outside of the biorepository find out you are taking part in the biorepository or learn information about you and your health. Your specimens will be stored with a code. Your name will not be on the container.

When the results of the biorepository are published or discussed at meetings, no information will be included that would reveal your identity

Will my family be told biorepository results?
Your family will not be told of any new findings that come to light during the course of using your specimens. We will not tell you or your family any genetic testing results from research studies. ATSDR will post study results on the National ALS Registry website.

What are the costs for participating in this biorepository?
There is no cost for participating in this biorepository. You will not be charged for any lab tests.

Will I be reimbursed for any of my expenses or paid for my participation in this biorepository?
You will not be paid to take part in this biorepository.

Will my cells, tissues, blood, or other biological materials be used to develop commercial products?
It is possible that a commercial product may be developed from your saliva sample collected as part of this biorepository. The nature of the research means that your sample is only one of many that will lead to this product and your sample will not have your name on it. You and your family will not profit financially from such a product. You will have no legal rights to any discovery or invention that either directly or indirectly results from the use of your specimens, individual information, or information from your samples.

Can I withdraw or be removed from the biorepository?

Taking part in this biorepository is voluntary. If you choose not to take part or decide to withdraw your consent and stop taking part, this will not affect your relationship with National ALS Registry, or other benefits to which you are otherwise entitled.

You have the right to leave the biorepository at any time without penalty. If you withdraw from the biorepository, we will destroy any of your remaining samples. However, we will not be able to remove your samples that have already been used or shared with researchers.

Who should I contact if I have questions regarding the biorepository? Who should I contact if I wish to voice concerns or complaints? Who can I talk to about my rights or want to withdraw my samples?

If you have questions, concerns, or complaints, think the biorepository has hurt you, or if you want to withdraw your samples, you can talk to Wendy E. Kaye, Ph.D., Senior Scientist or Laurie Wagner, MPH, Biorepository Coordinator at 1-855-874-6912.

If you want to speak with someone who is not directly involved in this biorepository, or have questions about your rights, or wish to voice questions, concerns or complaints, you may contact the CDC Human Research Protection Office at 1-800-584-8814.

Remember

Taking part in this biorepository is voluntary. Your decision whether or not to take part will not affect your current or future relations with the National ALS Registry. If you decide to take part, you are free to withdraw at any time without affecting that relationship.

Subjects' Rights:

I have read the above information. I have discussed this biorepository with the person obtaining consent, been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this biorepository. I will be given a copy of this signed and dated consent form.

_____

Print Subject name

_____ _____

Signature of Subject Date

If participant is physically unable to sign the consent form, please complete the following

_____ _____

Witness Signature Date

I, _____, witnessed that _____ was explained the consent form and has agreed to take part in this biorepository. Due to the progression of the disease, the participant is physically unable to sign the consent form.

Appendix B-4. In-home specimen collection procedures

B-4a. Urine

Urine collection will be a spot random collection on the day of the appointment for the blood draw. The urine collection kit will be mailed to the participant prior to the visit with instructions on how to collect the specimen. Participants will be encouraged to collect the specimen in the morning and provide it to the phlebotomist when he/she comes for the home visit or it may be collected at the time of the visit.

Upon arrival, the phlebotomist will ask the participant if he/she has already obtained the urine specimen. If not, the specimen will be collected at the end of the visit.

Participants will be provided a prescreened (for heavy metals and phthalates) high-density polypropylene 150-mL urine collection cup with screw cap. The prescreening will be lot tested for heavy metals and phthalates. Only lots that test negative will be used. The urine specimen is collected directly into the container, which is then securely sealed and placed into a resealable plastic bag provided with the kit to provide secondary containment. If the participant collects the specimen before the phlebotomy appointment, it should be placed into the home refrigerator until the time of the visit, to minimize degradation.

B-4b. Blood

Blood will be collected using a 21-gauge x ¾" or 23-gauge x ¾" butterfly needle blood collection set, with 12" tubing and safety-lock for the needle. (*Saliva samples will not be collected by phlebotomists; self-collection of saliva will be used only when blood collection fails.)

All lots of 6-mL K2EDTA Vacutainer should be pre-screened before use in this biorepository by lot-testing for background heavy metals contamination. This screening includes Pb, Cd, Hg, Se, and Mn.

ORDER OF DRAW OF BLOOD COLLECTION TUBES:

1. 10-mL K2 EDTA (B-D 366643 Vacutainer™, lavender Hemogard™ closure, plastic) for DNA, proteins, red cell lipids
2. 6.0-mL K2 EDTA (B-D 367856 Vacutainer™, lavender Hemogard™ closure, plastic) for heavy metals (Pb, Cd, Hg)
3. 10-mL plain (B-D 366430 Vacutainer™, no anticoagulant, silicone-coated, red conventional closure, glass)
4. 1 X 8-mL CPT blood Tube for cell line creation
5. 1 or 2 X 2.5-mL PAXgene™ blood RNA Tube (B-D 762165, plastic, Hemogard™ closure, 6.9 mL additive stabilizer)

Collecting the 6.0-mL EDTA in second order allows the butterfly needle line to be flushed of potential heavy metal contamination beforehand. The PAXgene tubes are always collected last because they contain 6.9 mL of stabilizer.

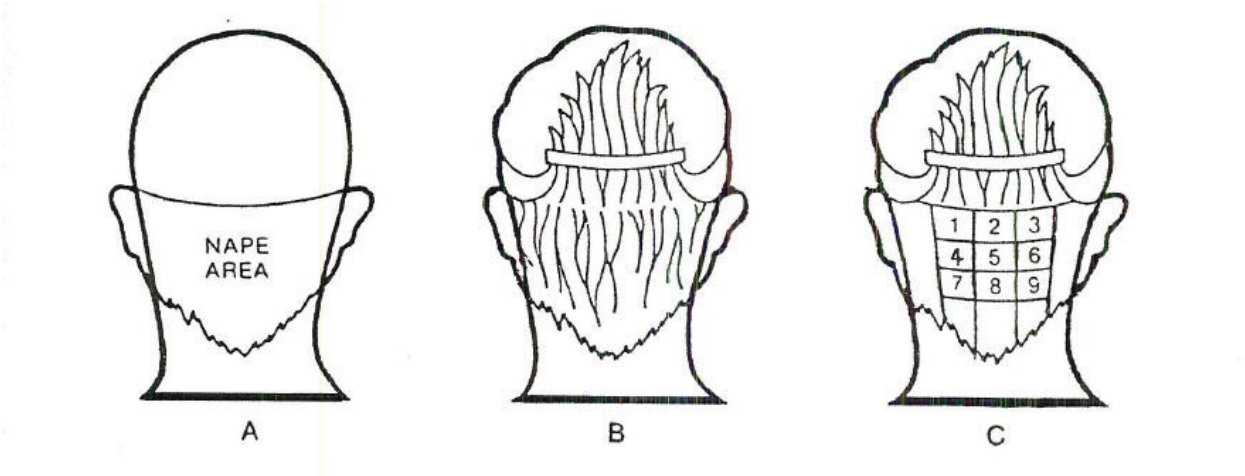
When blood is collected from a participant, the Vacutainers™ are inverted 10 times to mix the anti-coagulant/preservative with the whole blood (or to activate clotting in the red-top tubes with no anticoagulant). Using the butterfly collection device allows the phlebotomist to perform critical thorough mixing of the previous tube while the next tube is being collected, and it minimizes any discomfort of changing tubes while the needle is still in the subject's vein.

After collection from each participant, these specimens will be carefully packed in Styrofoam-insulated shippers with properly conditioned reusable cold-packs to maintain a temperature between 4-8 ℃ for overnight shipping by express courier to Fisher BioServices. Dry ice cannot be used because exposure to freezing temperatures could hemolyze red blood cells and have an adverse effect on plasma or serum – based analyses performed in the future. The specimen will be packaged according to current IATA regulations for clinical samples as "exempt human specimens", packed in leak-proof primary and secondary packaging with enough absorbent material to soak up entire contents of the bag. It must include an itemized list of contents between the secondary packaging and outer packaging. Each shipper should also contain a reusable "data logger" to monitor temperatures during shipment.

B-4c. Hair

Use the specially cleaned surgical scissors, plastic comb, and aluminum hair clips provided in the individual specimen collection kit. Wear disposable nitrile powder-free gloves while handling hair specimens.

Collect the hair samples as follows:



1. Collect samples from the nape area (diagram A).
2. Partition the hair between the ears (diagram B).
3. Fasten the hair above the ears and hold the upper hair out of the way with aluminum clips (diagram B).
4. From each of 8-10 sites at the nape area (diagram C), gather 15-20 strands of hair. Hold the end of the hair, and cut the hair as close to the scalp as possible.
5. For each cutting of long hair, cut off the 2 inches of hair that had been closest to the scalp and store this sample in the labeled resealable container provided.
6. If hair is extremely short, cut as available and note very short hair length in subject record.
7. Discard the remaining cut hair above the lower 2 inches closest to the scalp.
8. Be sure to complete the questionnaire of subject use of hair care products (dyes and permanents often contain heavy metals such as mercury, dandruff shampoos can contain selenium and zinc, ethnic products can also contain heavy metals, etc.).

B-4d. Fingernails

The subject will have been notified in a previous mailing that fingernail clippings will be requested for future study. If he/she has forgotten and has recently trimmed his/her nails too short to permit additional clipping, please note this problem in the subject record.

Use the specially cleaned nail clippers provided in the collection kit. Examine nails to make sure they are free of polish;

- If fingernails have <u>any</u> nail polish on them (even clear), please ask if it can be removed.
- If the participant agrees, use nail polish remover and then soap & water to wash nails prior to clipping. (Document that you had to perform this treatment in your patient record.)
- Do not collect nails from any subject unwilling to remove polish.
- Please record if they had nail polish present prior to collection.

Collect nail samples from all ten (10) fingers.

Catch all the clippings directly into the prescreened resealable labeled container provided, and then seal the container.

If the subject's nails are all too short, leave the collection materials and instructions with the participant. Notify McKing that the subject's nails were too short. McKing will send shipping materials and instructions to the participant.

B-4e. Specimen Processing Form

Specimen Processing Form

Date of collection __ __/__ __/__ __

Place Label Here

PLEASE READ:

- Complete this form with the subject
- Answer all applicable questions
- Questions? Call 1-855-874-6912

URINE

1. **Urine specimen collected?**

☐ Yes ☐ No *(subject declined or unable to void)*

2. If **YES**, record date and time of collection:

__ __/__ __/__ __ __ __:__ __ am/pm

3. If **YES**, did subject collect the specimen when he or she first woke up this morning?

☐ Yes ☐ No

BLOOD *Please note subjects are NOT required to fast.*

1. **Blood specimen collected?** ☐ **Yes** ☐ **No**

If **YES**, please check tubes of blood that were collected:

☐ Tube 1 ☐ Tube 2 ☐ Tube 3 ☐ Tube 4 ☐ Tube 5

Record time of collection: __ __:__ __am/pm

2. When did subject last drink something?

__ __/__ __/__ __ __ __:__ __ am/pm

3. When did subject last have caffeine?

__ __/__ __/__ __ __ __:__ __ am/pm

☐ *Check this box if subject does not consume caffeine*

4. When did subject last have something to eat?

__ __/__ __/__ __ __ __:__ __ am/pm

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

If yes, what is the name of study?

HAIR	**NAILS**
1. **Hair specimen collected?** ☐ Yes ☐ No 2. If **NO**, provide reason: ☐ Hair too short ☐ Subject declined 3. Does subject color his or her hair? ☐ Yes ☐ No 4. Does subject use perm or straighteners on his or her hair? ☐ Yes ☐ No	1. **Nail specimen collected?** ☐ Yes ☐ No 2. If **NO**, provide reason: ☐ Nails too short ☐ Subject declined 3. Does subject use nail polish? ☐ Yes, *date removed* __ __/__ __/__ __ ☐ No

B-5. Email/letter announcement of group event

Come learn more about the National ALS Registry and the National ALS Biorepository at the _____ meeting on date at time. We will be meeting at _____. Representatives from the Registry and Biorepository will be there to answer questions. People enrolled in the Registry can provide a saliva/blood sample (select appropriate sample type) if they are interested.

Please contact _____ at _____ if you have questions.

Hope to see you there.

B-6a. Biorepository COVID-19 Safety Procedures and Restart Plan

This document was created to inform the OMB and IRB office of the plan that was created to minimize risk and exposure and safely restart participant sample collections.

Introduction

On March 11, 2020, the Registry paused Biorepository specimen collections to protect the health of the Biorepository participants and members of their household. The Biorepository staff canceled all collection appointments scheduled until further review of the pandemic. Restarting the Biorepository collections will require that precautions be put in place to ensure that staff and participants and their caretakers/household members are protected from COVID-19. This plan is based on interim CDC guidance for non-COVID public health activities that require face-to-face interaction during the COVID-19 pandemic and will be used to outline practices for conducting Biorepository project activities.

Changes in the Biorepository procedures are proposed to reduce or eliminate close contact (within 6 feet) between project staff and participants when possible to prevent the spread of during collection activities. COVID-19 The precautions and personal protective equipment (PPE) recommended in this plan will be reevaluated monthly and more often as needed to ensure they are still in line with CDC COVID-19 guid and may be revised to add or remove precautions as needed. elines

Objectives

- Minimize risk of exposure, illness, and spread of disease among staff working with the Biorepository;
- M of their households; andsinimize risk of exposure, illness, and spread of disease among Biorepository participants and member
- Preserve essential functions of the Biorepository.

Schedule Considerations

Scheduling study activities, the Phlebotomy companies will consider before appointments are finalized: Biorepository staff and

- The level of local COVID-19 transmission (number of new cases, emergency department visits, and percent positive for testing in each community as available)
- Not scheduling appointments in areas with higher-than-average transmission
- S tate and local guidance/mandates

COVID-19 Safety Precautions for the National ALS Biorepository

Staffing. these activitiesfor already established by their offices or agencies. The Biorepository staff are not expected to provide in-person support procedures staff with follow the s, thephysician's officein The collections that take place by home healthcare workers and processing.sample and s,

collectionpecimenoversee collection activities relating to the project operations office, salso to collect specimens at each location. The Biorepository staff will assigned: Biorepository staff will oversee the phlebotomists

All phlebotomists will be provided clear information about new procedures and PPE .hface shield that covers eyes, nose, and moutand a maskface face covering or disposable cloth COVID-19 which include a , we are adding extra measures due tothe usual PPE used during collectionsIn addition to . and gloves full PPE, including long sleeves, long pants (or scrubs), closed-toe shoes, erequirements by their employer (as described in table below). Phlebotomists will go through collection training and all PPE will be provided to staff prior to initiation of activities. Phlebotomists utiliz(Personal Protective Equipment)

F The Biorepository staff will include at least two disposable face masks in each collection kit that is shipped to the participant for the participant or their family members to wear during appointment. surgical mask.disposable will wear a the phlebotomist mask due to medical conditions, disposable faceshould not be placed on anyone who has trouble breathing, or is unconscious, incapacitated or otherwise unable to remove the mask without assistance. If participants are unable to wear a cloth face covering or and disposable face masks in-home appointment. Cloth face coverings the during or a disposable face mask cloth face covering , wear apresent for the specimen collection appointment both the phlebotomists, the participants, and household membersWe recommend . in case you are infectedsace coverings are required and meant to protect other

Screening of Project Phlebotomists, Participants, and Household Members

<u>Phlebotomist screening</u>

Before entering the collection location, phlebotomists will be screened for symptoms any symptoms or travelled in violation in the past 14 days.in violation of any federal, state, or local guidance or orders, or had close contact with an individual who hasPhlebotomist will not be assigned to any collections if they answer yes to having any of the COVID-19 symptoms or traveled associated with COVID-19.

Questions on presence of any of the following in the past 14 days: asking about thesymptoms will includeCOVID-19

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose
- Nausea or vomiting
- Diarrhea

If any phlebotomist develops COVID-19 symptoms they will be instructed not to come to work, to inform their supervisor who will notify the Biorepository staff. If a phlebotomist develops symptoms consistent with COVID-19, Biorepository staff will pause that phlebotomists' collections. The phlebotomist must subsequently test negative for COVID-19, before resuming collection activities. If the phlebotomist tests positive, Biorepository staff will notify any participants who have been in contact with the positive individual. any

Participant and Household member screening

The They will also be instructed to notify Biorepository staff to cancel their appointment if they are experiencing COVID-19 symptoms (as defined in the list above). Participants will be asked about symptoms in a reminder call the day before their scheduled appointment. .face mask during their appointmentface covering or disposable articipants and household members will be informed of screening procedures and be required to wear a cloth , phen appointments are scheduledonce the screening questions have been answered. W appointment theircomfortable scheduling The participants will be asked if they are they have experienced any of the symptoms above or have they had close (within 6 feet) prolonged (10 minutes or more) contact with someone who has had any of the symptoms. In the last 14 days have you traveled in violation of any federal, state, or local guidance or orders, or had close contact with an individual who has.in the past 14 days, staff will ask the participant if Biorepository

Additional actions that will be transmission include:COVID-19 to reduce the potential for enforced

- Proper hand washing.
- Providing masks for participants who do not have their own face covering upon entry into the home or designated collection location.disposable face

Travel Guidelines

Biorepository staff normally present during the blood collection and do not interact with the participants during the collection visit. Phlebotomists are the only staff members that meet in person with the participants and other household members. are not

During travel Biorepository staff will be directed to the considerations to the CDC travel in the US guidelines at . Staff will also be provided with the guidance below.
https://www.cdc.gov/coronavirus/2019-ncov/travelers/travel-in-the-us.html

Protect yourself and others during your trip:

- Clean your hands often.
    - Wash your hands often with soap and water for at least 20 seconds especially after you have been in a public place, or after blowing your nose, coughing, or sneezing.
    - If soap and water are not readily available, . Cover all surfaces of your hands and rub your hands together until they feel dry.**use a hand sanitizer that contains at least 60% alcohol**
- **Avoid touching your eyes, nose, and mouth.**
- Avoid close contact with others.

  - Keep 6 feet of physical distance from others.
  - Avoiding close contact is especially important if you . from COVID-19are at higher risk of getting very sick
- Wear a cloth face covering in public.or face mask
- Cover coughs and sneezes.
- Pick up food at drive-throughs, curbside restaurant service, or stores. Do not dine in restaurants if that is prohibited by state or local guidance.

1.1.1.

Protective Measures

Specific project activities are shown in the table below with recommended PPE, and additional precautions. All PPE for project phlebotomists will be provided by their organization. See table below.

Activity	Description	PPE	Additional Precautions
Travel	Time spent in ride share/public transportation, in airport, on airplane, time spent in public venues while traveling	Disposable mask face	• Frequent handwashing • Cloth face coverings • General travel precautions • Ride sharing and public transportation
COVID-19 Screening	Talk to participants outside of their home or collection location, ask symptom screening questions	Disposable mask, disposable glovesface	• Confirm participants and care givers/family members are symptom and fever free before entering the collection location
Biological sample collection	Collect blood samples	Phlebotomists: Cloth face covering or and mouth. , that cover eyes, nosee shieldsPhlebotomists will be utilizing full PPE (Personal Protective Equipment), including long sleeves, long pants (or scrubs), closed-toe shoes, gloves and fac, mask, disposable glovesface	• Frequent handwashing • Blood.precautions and plasma collection

B-6b. National ALS Biorepository COVID-19 Safety Information Sheet

Th.participantsto ent that is sinformation packet along with other information will be included in the document This that would like to take part in the ALS Biorepository and get samples safely collected during the COVID-19 pandemic. to participants general information will provideis document

Introduction

On ,to ensure that staff precautionsand have added estarting Biorepository collections We are now r. sthe Biorepository and members of their householdthose taking part in March 11, 2020, the Registry paused Biorepository specimen collections to protect the health of participants, . members are protected from COVID-19. This is based on CDC guidance for non-COVID public health activities that require face-to-face interaction during the COVID-19 pandemichousehold and their caretakers/

What you need to know

Changes in the Biorepository procedures ;tohave been made

- Minimize risk of exposure, illness, and spread of disease among staff working with the Biorepository;
- M of their households; andsBiorepository and memberthose taking part in the inimize risk of exposure, illness, and spread of disease among
- Maintain essential functions of the Biorepository.

COVID-19 Safety

F. before collections will be provided personal protective equipmentPhlebotomists will go through training and all The Biorepository staff will include at least two disposable face masks in each collection kit that is shipped to your home. surgical mask.disposable will wear a . Phlebotomists in case you are infectedsace coverings are required and meant to protect other

COVID-19 Screening

Before each appointment, participants, household members, and phlebotomists will be asked if they have in the past 14 days. symptomsCOVID-19 experienced any

Wabout symptoms in a reminder call the day before their scheduled appointment. again They will also be instructed to notify Biorepository staff to cancel their appointment if they are experiencing COVID-19 symptoms. Participants will be asked .screening procedurestold about the and household members will be , those taking part hen appointments are scheduled

Before entering the .in violation of any federal, state, or local guidance or ordersPhlebotomist will not be assigned to any collections if they answer yes to having any of the COVID-19 symptoms or traveled associated with COVID-19. phlebotomists will be screened for symptoms home,

Feel free to contact a member of the National ALS Biorepository by phone at 1-855-874-6912 or by email at if you have any questions or concerns. alsbiorepository@secure.mcking.com HYPERLINK "mailto:alsbiorepository@secure.mcking.com"

---

lwagner@secure.mcking.com

-
-
    -
    -
-
-
-
-
-
-
    -
    -
-
-
-
-
    -
    -
    -
    -

-
-

-
    -
    -
    -
    -

    -
    -
    -

-
-
-

-
-

-

-
-

-
-
-
-
-

-
-
  -
  -
-
-
-
-
  - o
-
-
-
-

-
-
  -
  -
-
-
  - o
  - o
  - o
  - o
-
-
-

-
-
  -
  -
-
-
-
-
-
-
-
-
-

-
-
-
-
-
-

-
-

-
-

-
-
-

-

    - o
    - o
    - o
    - o

b)

-
-

c)

-

-
-

-
-
-

-
-

-
-

-
-
-

-
-
  -
  -
-
-
-
-
-
-
-
-
  - o
  - o
  - o
  - o

-
-
-

1.
2.
3.
4.

-
-
-
    -
-
-
-
-
-
-
-
-

lwagner@secure.mcking.com

lwagner@secure.mcking.com

lwagner@secure.mcking.com wwwn.cdc.gov/als/alsBioRegistry.aspx	

1. 
2.

-
-
-
-
-

-
-

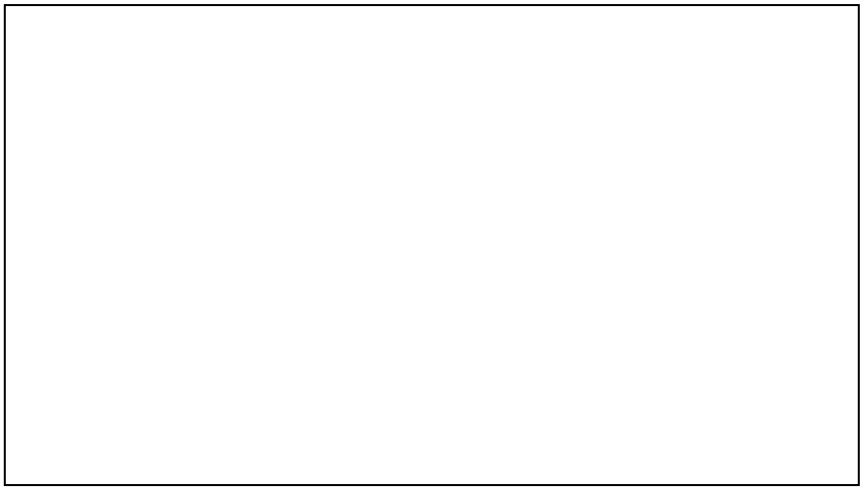
-
-
-
-

A.

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.
18.
19.
20.
21.
22.
23.
24.
25.
26.
27.
28.
29.
30.
31.
32.
33.
34.
35.

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.

1.
2.
3.
4.
5.



1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.
18.
19.
20.
21.
22.
23.
24.
25.
26.
27.
28.
29.
30.
31.
32.
33.
34.
35.

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.

1.
2.
3.
4.
5.