

NATIONAL INSTITUTE ON AGING

**BIOSPECIMEN
BEST PRACTICE
GUIDELINES**

**FOR THE
ALZHEIMER'S DISEASE CENTERS**

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PLASMA, SERUM AND BLOOD CONSTITUENTS GUIDELINE

I. Acquisition of blood biospecimens

- A. Typically, blood will be drawn after application of a tourniquet, by use of a small gauge needle, from an antecubital vein, and by an appropriately trained professional.
- B. To allow the widest range of possible studies, blood ideally may be drawn from fasting subjects. Time of day should be noted. Early morning specimens may be best for the broadest range of studies. Fasting conditions may be preferred for some studies. However, non-fasting specimens may be useful for measuring some biomarkers.
- C. If funding allows, consideration may be given to drawing a serum sample together with the biomarker sample(s) to measure glucose, BUN and creatinine levels, which may influence levels of many biomarkers.
- D. For most purposes, blood will be drawn using a vacutainer system, into a series of tubes whose volume is typically 5 to 10 ml. Use of glass tubes may lead to low values for certain analytes, such as beta amyloid protein. For the broadest purposes, use of polypropylene vacutainer tubes, as well as subsequent storage tubes also made of polypropylene, is recommended.
- E. The choice of anticoagulant is important. Vacutainer tubes are manufactured with anticoagulants added. Options include EDTA or heparin when preparing plasma. No anticoagulant is added to prepare serum. Previous research suggests that EDTA is preferred since others may interfere with amyloid beta peptide detection.³
- F. For certain types of studies, in particular those that involve isolation of platelets, attention must be paid to how strongly a tourniquet is applied, and whether to use a larger caliber needle and a syringe to draw blood rather than a vacutainer system, to minimize the chances of activating the platelets during blood draw.
- G. If blood biomarker analyses will include platelets or white blood cells, a complete blood count may also be obtained.

II. Preparation and storage of blood biospecimens

- A. Standardized and uniform techniques of samples processing is recommended.
- B. Rapid processing of samples is optimal. Centrifugation may be carried out within 30 minutes of blood draw, or ideally within 10 to 15 minutes, followed by aliquoting samples into the storage tubes, with quick freeze at the bedside or in the clinic on dry ice, and then transfer to a -80 degree freezer.
- C. It is unknown whether a room temperature centrifuge versus a refrigerated (4 degrees) one makes a difference for the preparation of serum or plasma. Refrigerated centrifuges are better for platelet preparation.
- D. Whether it is necessary to add protease inhibitors to samples after aliquoting is not certain. This may be worth consideration if plasma or serum samples are to be used for proteomic analyses.
- E. Small aliquots (generally not larger than 0.5 ml) are recommended for storage.
- F. Uniform, non-redundant annotation of samples is recommended.
- G. Document the volume of plasma or serum that was obtained, because this may be variable. The quality of serum or plasma can be influenced by hemolysis, and red or pink tingeing of plasma or serum is an indicator that significant hemolysis has occurred, which is likely to render the samples less useful for biomarker studies.
- H. Appropriate and complete documentation surrounding biospecimen collection, processing, and storage are essential and will influence the quality of research data to be obtained.
- I. Avoid unnecessary thawing and refreezing of samples. For plasma or serum samples, consider aliquoting in small volumes, e.g. 100 to 200 microliters, if these are to be used for a large number of analyses.
- J. Centers at which samples are stored may consider having a back-up plan for freezer failure (e.g. dry ice or liquid nitrogen). An appropriate alarm system to support freezers for longtime storage is recommended.
- K. For some studies in which blood is drawn for biomarker analyses, as described above, additional analyses of DNA may be planned. This will often entail drawing an additional tube of blood to obtain a

buffy coat for extraction of genomic DNA. Please refer to best practice guidelines for DNA preparation and storage.

III. Sharing and dissemination of blood samples

- A. The repository is a national resource, to be shared for the purpose of answering valid scientific questions, and typically is not supported for the development and use by a single investigator.
- B. Specific evaluation criteria for specimen requests that are documented and consistently applied by a Center-designated committee to all such requests is recommended (see Dissemination / Discarding Guideline).
- C. Blood biospecimen sharing is recommended to be limited to the smallest amount of sample(s) that will adequately answer the research question under investigation
- D. Sharing and associated labeling of specimens that is consistent with the description in the informed consent process is recommended

References

1. Tworoger SS, Hankinson SE. Collection, Processing, and Storage of Biological Samples in Epidemiologic Studies: Sex Hormones, Carotenoids, Inflammatory Markers, and Proteomics as Examples. *Cancer Epidemiology Biomarkers & Prevention* 2006;15: 1578-1581.
2. Rai AJ, Gelfand CA, Haywood BC et al. HUPO Plasma Proteome Project specimen collection and handling: Towards the standardization of parameters for plasma proteome samples. *Proteomics* 2005;5:3262 - 3277
3. Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E. Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma. *Amyloid*. 2000;7:245-58.
4. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

CEREBROSPINAL FLUID GUIDELINE**I. Acquisition of Cerebrospinal Fluid (CSF) Biospecimens**

- A. Two DVD resources available
 - 1. Physician education on lumbar puncture (LP) technique
 - 2. Research subject education on spinal tap procedure
- B. Fasting is recommended prior to the procedure; consider taking a serum sample simultaneously for glucose measurement
- C. Use 25 g needle for deep local anesthesia rather than the needle provided in kit
- D. Atraumatic spinal needle (e.g., Sprotte 24 g atraumatic spinal needle) are recommended for the lumbar puncture to minimize risk of post-LP headache (<1%) [ref – AAN clinical practice guideline]. Other spinal needles that have been used are a 22 g Sprotte atraumatic needle or a 25 gauge Quincke needle, although their use is associated with a somewhat higher post-LP headache risk (up to 5%)
- E. CSF may be withdrawn under negative pressure with sterile polypropylene syringes; up to 30 cc CSF may be withdrawn. Using a 22 gauge needle permits CSF to flow under gravity
- F. First 2 cc of CSF withdrawn is sent for local laboratory analysis (cell counts, protein, glucose measurements) – requires 2 ml for clinical testing. These 2 mls can be placed in the plastic (polystyrene) tubes that come in the commercial LP kit. The polystyrene tubes can ONLY be used for the sample sent to the clinical lab.
- G. Clinical Exemplar – to avoid a post-lumbar puncture headache, the following actions are strongly recommended:
 - 1. Participant rest in recumbent position for one hour post-LP (a common clinical practice)
 - 2. Encourage liberal fluid intake
 - 3. Subject should avoid exertion (exercise, housework, gardening, sexual activity, lifting/bending, etc.) for 24-48 hours following lumbar puncture
 - 4. Stress importance that participant maintain usual caffeine intake to prevent caffeine-withdrawal headache

II. Preparation and Storage of Cerebrospinal Fluid Biospecimens

- A. Standardized processing techniques using ONLY polypropylene tubes are recommended for all samples. CSF for research purposes should NEVER come in contact with polystyrene (clear hard plastic) or glass, since this could result in falsely low measurement levels of various proteins.³
- B. Rapid processing of samples is optimal; aliquoting followed by quick freeze at bedside on dry ice is recommended, followed by transfer to -80°C freezer
- C. Optional guideline – Quick freezing of CSF samples at bedside is not possible if CSF is to be spun to remove RBCs; unfrozen samples are spun to remove RBCs. However, this does not remove plasma proteins, and samples with greater than 10 RBCs before spinning may still be unusable for proteomics
- D. Small aliquots (generally no larger than 0.5 ml) are recommended for storage
- E. Optional guideline – Depending on research objectives, additives or preservatives (e.g., reduced glutathione or protease inhibitors such as aprotinin) may be added to specific specimen tubes before storage
- F. Uniform, non-redundant annotation of samples is recommended
- G. Document the exact volume of fluid obtained at each tap since this can be variable
- H. Optional guideline - maintain gradient of collected specimens as appropriate to specific research purpose(s)
- I. Appropriate and complete documentation surrounding biospecimen collection, processing, and storage are essential and relevant to the quality of research data to be obtained
- J. Avoid unnecessary thawing and refreezing of samples
- K. A back-up plan for freezer failure (e.g., CO₂ or liquid nitrogen) is recommended

III. Sharing and Dissemination of Cerebrospinal Fluid Samples

- A. The repository is a national resource to be shared for the purpose of answering valid scientific

- questions, and is not supported for the development and use by a single investigator
- B. Specific evaluation criteria for specimen requests that are documented and consistently applied by a Center-designated committee to all such requests is recommended (see Dissemination / Discarding Guideline)
 - C. CSF biospecimen sharing is recommended to be limited to the smallest amount of sample(s) that will adequately answer the research question under investigation
 - D. Sharing and associated labeling of specimens that is consistent with the description in the informed consent process is recommended

References

1. Evans RW, Armon C, Frohman EM, and Goodin DS. Assessment: Prevention of post-lumbar puncture headaches: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 2000;55;909-914.
2. Armon C, and Evans RW. Addendum to assessment: Prevention of post-lumbar puncture headaches: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 2005;65;510-512.
3. Hesse C, Larsson H, Fredman P, Minthon L, Andreasen N, Davidsson P, Blennow K. Measurement of Apolipoprotein E (apoE) in cerebrospinal fluid. *Neurochem Res.* 2000;25:511-7.
4. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

BRAIN AND DNA / RNA GUIDELINE

Note: All protocols should be *standardized*

I. Collection and Processing**A. Brain****1. Collection**

- a. Both diseased and normal brains must be collected.
- b. Brain must be collected according to local IRB and state legal codes, using appropriate consent forms, with adherence to HIPAA regulations.^{1,2}
- c. 24/7 on call autopsy coordinator, autopsy technician(s), and tissue bank technician(s) is optimal so collection may occur as rapidly as possible after death.
- d. Tissue bank directors are recommended to be aware of the number and amount of banked specimens needed to meet their researchers' needs, without overwhelming the storage capacity of the bank.
- e. Dissection protocols *should be tailored to the needs of prospective users*.
- f. Agonal state information (e.g., fever, hypotension, hypoxia, oxygen supplementation, cyanosis, O₂ saturation, medications in the last days to weeks of life), if available, can be recorded at the discretion of the Center, and shared as requested.³
- g. Postmortem interval that is recorded and entered into the database (number of hours from death until autopsy or until specimen is fixed / frozen, depending on which measurement is standard in the specific laboratory) is recommended.
- h. Collection of postmortem cerebrospinal/ventricular fluid is optional.^{4,5} If collected, it is recommended to be stored in small aliquots to minimize instability related to thaw procedures at the time of retrieval.

2. Processing

- a. A portion of brain tissue that is frozen and stored for biochemical and molecular/genetic studies is recommended.
 - i. Regions and amount frozen and freezing technique *should be determined by needs of researchers* utilizing center's tissues; (see Storage and Retrieval section below).
 - ii. Examples
 - aa. Selected small samples (few grams)—snap-freeze in liquid nitrogen with or without isopentane
 - bb. Selected slices—quick-freeze between blocks of dry ice
 - cc. Freezing of one intact hemisphere is not recommended as this method does not provide maximal brain region accessibility for biospecimen retrieval and distribution.
- b. Remainder of brain that is fixed
 - i. Buffered formalin, 10% or 20%
 - ii. Paraformaldehyde, 3-4%, followed by sucrose/sodium azide cryopreservation
- c. Paraffin blocks that are to be prepared from fixed tissue for diagnostic evaluation and permanent storage are recommended.

B. DNA / RNA / Protein

1. Whether prepared from blood samples or from brain tissue, must be collected according to local IRB and state legal codes, using appropriate informed consent forms, with adherence to HIPAA regulations^{1,2} (see Informed Consent, Confidentiality and Privacy Guideline).
2. Bioanalysis for quality control (e.g., standard assays for integrity of RNA) is recommended, if funding permits, using as little of the specimen as possible.⁶
3. Whether or not performing bioanalysis if financially feasible, measurement of brain pH at autopsy is recommended—either by using a surface pH electrode or by measuring pH of either cerebrospinal/ventricular fluid or homogenized brain using a standard pH electrode. pH has been

reported to be remarkably stable in postmortem brain and correlates better with RNA quality than does postmortem interval.⁷

4. Protein is best preserved by rapid postmortem body cooling and freezing of samples up to 50 hrs postmortem.⁸

II. Safety Provisions

- A. Laboratories must have safety plans.
- B. Laboratory personnel
 1. Immunization for hepatitis B is recommended.
 2. Must be trained in safety procedures related to handling of human tissue
 3. Must observe universal precautions; all specimens must be handled as if infectious
- C. Biospecimens
 1. It is recommended that a disclaimer accompany all biospecimen disbursements, even if tested negative for HIV and hepatitis B and C, which PIs sign and return to Core leaders. The disclaimer would indicate that they understand that absence of infectivity of biospecimens cannot be guaranteed, that laboratory personnel have been trained in procedures related to handling of human tissue, and that universal precautions will be observed.
 2. HIV and hepatitis B and C
 - a. Testing of blood for hepatitis and HIV may be performed, if desired. However, as there can be both false positives and negatives, a negative test for hepatitis or HIV does not guarantee absence of infectivity.
 - b. Cases with a history of hepatitis B or C or HIV infection may be excluded from brain donation unless a study specifically requires this type of tissue.
 - c. It is recommended that frozen brain, blood, and DNA not be distributed from cases positive for hepatitis or HIV, unless a study specifically requires this type of tissue. These may be kept and labeled as either hepatitis or HIV positive for such needs. Fixed tissue may be distributed with specific hepatitis and HIV warnings as above.
 3. CJD: Participation in the National CJD Surveillance Program is recommended (see website for protocol: <http://www.cjdsurveillance.com/>). To ensure inactivation of prions, tissue blocks may be disinfected by placing in full-strength (99%) formic acid for one hour following complete formalin fixation and followed by at least 48 hours of additional formalin fixation. This procedure may be reserved for cases of short-duration dementia or those clinically suspected of harboring prion disease; some centers may use this protocol for all dementia cases because of the possibility that any case may have unsuspected CJD.^{9,10,11}

III. Annotating

- A. It is recommended that all biospecimens be de-identified and given a unique identifier that follows the specimen from acquisition through processing and storage to retrieval and distribution.

IV. Storage and Retrieval

A. Storage

1. It is recommended that a portion of brain tissue be frozen and stored for biochemical and molecular/genetic studies and the remainder of the brain be fixed for preparation of paraffin blocks, etc., which are kept permanently.
2. Stabilization
 - a. Note: Consideration given to storage bags/containers that protect the integrity of the contents is recommended.
 - b. Freezers that are monitored by automated security alarm systems that contact laboratory director and personnel by telephone or pager when failure occurs are recommended.
 - c. Freezers with back-up systems (e.g., CO₂ or LN₂) or spare freezers for emergency situations are recommended.
3. Temperature recommendations
 - a. Formalin-fixed: room temperature (20-25°C)
 - b. Paraformaldehyde-fixed, sucrose/sodium azide preserved: refrigerator (2-8°C)

- c. Frozen: -70-80°C or liquid nitrogen vapor

D. Retrieval

1. Biospecimen requests must be approved by the appropriate decision-making body (see Dissemination / Discarding Guideline).
2. Effective annotation that results in minimal effort expenditure to retrieve samples is recommended.
3. Tracking and storage methods that minimize disruption of stable state during retrieval to ensure biospecimen quality is recommended.
4. Inventory database is recommended to track specific position of each biospecimen.
5. Investigators receiving biospecimens must be warned to observe universal precautions; all specimens must be handled as if infectious.

References

1. Federal Register Department of Health and Human Services, Title 45, Code of Federal Regulations, Parts 160 and 164
2. Root J. Field guide to HIPAA implementation, rev. ed. American Medical Association Press, 2004.
3. Morrison-Bogorad M, Zimmerman AL, Pardue S. Heat-shock 70 messenger RNA levels in human brain: correlation with agonal fever. *J Neurochem* 63:235-246;1995.
4. Morihara T, Kudo T, Ikura Y, Kashiwagi Y, Miyamae Y, Nakamura Y, Tanaka T, Shinozaki K, Nishikawa T, Takeda M. Increased tau protein level in postmortem cerebrospinal fluid. *Psychiatry & Clinical Neurosciences* 52:107-110;1998.
5. Rosler N, Wichart I, Jellinger KA. Ex vivo lumbar and post mortem ventricular cerebrospinal fluid substance P-immunoreactivity in Alzheimer's disease patients. *Neuroscience Letters* 299:117-120;2001.
6. National Biospecimen Network Blueprint, Andrew Friede, Ruth Grossman, Rachel Hunt, Rose Maria Li, and Susan Stern, eds. (Constella Group, Inc., Durham, NC, 2003); Appendix M, Advanced Analysis Techniques
7. Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV, Lopez JF, Avelar A, Shokoohi V, Chung T, Mesarwi O, Jones EG, Watson SJ, Akil H, Bunney WE Jr., Myers RM. Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. *Human Molecular Genetics* 13:609-616;2004.
8. Ferrer I, Santpere G, Arzberger T, et al. Brain protein preservation largely depends on the postmortem storage temperature: implications for study of proteins in human neurologic diseases and management of brain banks: a BrainNet Europe study. *J Neuropathol Exp Neurol* 66:35-46;2007.
9. Rosenberg R, White CL III, Brown P, Gadjusek C, Volpe JJ, Posner J, Dyck PJ. Precautions in handling tissues, fluids, and other contaminated materials from patients with documented or suspected Creutzfeldt-Jakob disease. *Annals of Neurology* 19:75-77; 1986.
10. Brown P, Wolff A, Gadjusek C. A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. *Neurology* 40:887-890;1990.
11. WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies, Report of a WHO Consultation, Geneva, Switzerland, 23-26 March 1999.
<http://www.who.int/emc-documents/tse/docs/whocdscsraph2003.pdf>.
12. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

INFORMATICS GUIDELINE

- I. Data Procedures:** It is recommended that each Center have specific requirements and practices in place to ensure the optimal collection of the Uniform Data Set and the Neuropath Data Set, when available. We note below information, protocols, and procedures that are likely to be applicable to enhance the efficiency and integrity of the data gathering process.
- II. Sharing and Distribution**
- A. Specific evaluation criteria for specimen and associated data requests that are documented and consistently applied by a Center-designated committee to all such requests is recommended (see Dissemination / Discarding Guideline).
 - B. Center-specific guidelines that incorporate best practices for the dissemination of identifiable, de-identified and anonymous data, including genetic information, are recommended to be established and adhered to for all data requests from academic and non-academic collaborators.
 - C. Policies and procedures for requesting ADC resources may be published on each Center's website.
 - D. **Documentation and archiving of all requests and their review outcome is recommended.**
- III. Database Structure**
- A. Maintenance of a local database is recommended to facilitate the local research needs of each Center.
 - B. A complete and current set of documentation/manuals is recommended for the database. These should include at least a data element dictionary and an operations manual. Operating system documentation, usually available from the vendor, may also be maintained.
- IV. Quality Control, Tracking, Security, and Data Mining**
- A. Database repository are recommended to be installed on secured servers/network systems that have automated backup capabilities on at least a once per 24 hr basis. Long-term archival storage of data at an off-site storage facility is also recommended.
 - B. Network security may be established through consideration of (a) an institutional network firewall; (b) database password, user, group and role-based security; (c) application-level security with passwords and login required to access an application; (d) server-level access passwords. Password changes are recommended to be enforced regularly, and procedures to protect the secrecy and log-on codes, including the use of nondisplay/masked features, are recommended.
 - C. Database access is recommended to be limited to key authorized users and only from trusted Internet addresses.
 - D. Tiered-access that allows definition of "authority levels" for accessing and updating of data, particularly identifiable and genetic information is recommended.
 - E. Centers are encouraged to track all successful and failed attempts at database access, and updates to the database logged and associated with a user ID. Accounts are recommended to be locked-out after several consecutive unsuccessful login attempts, and the system administrator notified.
 - F. Range checks and error checks are recommended for data, and out of range violations should be flagged back to a user and disallowed as a quality control measure.
 - G. Authorized data transfer is recommended to be protected via encryption capabilities.
 - H. Centers are strongly discouraged from downloading databases to laptops that contain subject identifiable data, as this poses a significant security risk. All identifiable data must be kept securely behind a firewall.
 - I. All databases must comply with HIPAA (Health Insurance Portability and Accountability Act of 1996) regulations to appropriately protect access to individually identifiable protected health information. All databases must comply with the Federal Information Processing Standards, if applicable.
 - J. Data mining infrastructure is recommended to be designed to meet local research needs, but may be optimally designed for the ease of interrogation of stored datasets, in response to inquiries about integrity, content, and specific research-facilitating queries.

V. System Support, Maintenance and Evolution

- A. A designated team of institutional Information Systems personnel and system administrators are recommended to be in place for routine technical maintenance and trouble-shooting issues.
- B. Software development and data mining capabilities are recommended to evolve under the direction of a committee that includes database users and investigators, bioinformaticians, statisticians and software engineers.

References:

1. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

INFORMED CONSENT, CONFIDENTIALITY AND PRIVACY GUIDELINE

I. General guidelines for informed consent, confidentiality and privacy related to biospecimens:

- A. The three principles of the Belmont Report must be respected in the informed consent process
 1. Beneficence
 - a. Risks and benefits of the research should be discussed
 2. Respect for persons
 - a. Achieving informed consent for the research
 - b. Voluntariness of consent is acknowledged
 - c. Issues of privacy and confidentiality should be covered as appropriate to the research
 3. Justice
 - a. Equitable selection of subjects - all subjects who may potentially benefit from the research should be included in the research opportunity; should share the benefits and burdens
- B. All research on biospecimens must comply with the applicable privacy and human subjects protections regulations (45CFR46, 21CFR 50 and 56; 45CFR 160-164 – HIPAA)
- C. Local procedure will dictate how human subjects and non-human subjects determinations are done, as they pertain to biospecimens
- D. Differing access and retention of private identifiable information related to biospecimens will determine the appropriate level of review for the research by the Institutional Review Board
- E. Informed consent for biospecimens is recommended to be executed at the appropriate and thoughtful time with respect to other demands of the research participant
- F. The capacity to provide informed consent for current and or future research use of biospecimens must be assessed for each research participant as per local IRB requirements and guidelines.
- G. Surrogate consent for research may be utilized in compliance with federal law and state statutes

II. Recommended components of the informed consent document and process:

- A. The procedure for biospecimen acquisition, short-term and long-term linkage to identifiers, and all intended purposes/uses (known at the time of consent) of the specimens
 1. Brain autopsy consent is required as per local IRB guidelines – this research may be exempt from federal regulations since the subjects are deceased. Brain autopsy is best accomplished through early educational initiatives with patients and their collaterals
 2. Blood and spinal fluid collected prospectively on living individuals definitely constitutes human subject research and must be reviewed and approved through local IRB processes.
- B. Where appropriate, it is recommended to provide options for the research participant, in the informed consent document, to choose to participate or not participate in various aspects of the biospecimen project (*e.g.*, lumbar puncture, storage of DNA, etc.).
- C. When applicable to the project, it is recommended to use a one-time consent mechanism to cover the prospective and future intent of the research project on biospecimens.
- D. It is recommended that intended data sharing include
 1. General use of samples and data for future research
 2. If appropriate, DNA samples can be shared with NCRAD (National Cell Repository for Alzheimer's Disease @ Indiana University); the NCRAD acquisitions committee decides
 3. If appropriate, genetic data will be shared with NIAGADS (Genetic Analysis Data Set @ Washington University); all routine genetic analyses, other than ApoE, should be sent to NIAGADS
 4. Potential for sharing of data and specimens with non-government, non-academic investigators should be addressed, with potential for development of commercial products
 5. ApoE genotyping results and stored DNA to be shared
 6. Local standardized specimen language for sharing of specimens, commercial value, etc., must be included in the site-specific informed consent document
 7. Risks of privacy and confidentiality, as they exist per protocol, and as applicable to the retention of identifiers with the specimens
 8. Acknowledgement of the absence of direct benefits to participants
 9. Acknowledgement of potential benefit to society
 10. A policy / procedure for handling of withdrawn consent

11. Storage of data generated from biospecimens is recommended to be addressed with appropriate regard to security measures (encryption, coding, limited access to database, etc.)

References

1. Wendler, D. One-time general consent for research on biological samples. *British Medical Journal* 332:544-547;2006.
2. OHRP, Guidance on research involving coded private information or biological specimens. August 10, 2004.
3. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

DISSEMINATING AND DISCARDING GUIDELINE

I. Disseminating: All Alzheimer's Disease Centers (ADCs) are required to have Resource Sharing Plans in accordance with the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice.² Such Resource Sharing Plans are recommended to include or address the following issues:

A. General Issues

1. Establish clear, explicit guidelines for sample distribution (and clinical data sharing) consistent with ethical principles, prevailing laws, and, if applicable, consent form language. Flexible guidelines are recommended so that ADCs may respond to changing scientific needs. It is recommended that the guidelines address the following processes and procedures involved in tissue dissemination: the request process, review procedures, decision-making, distribution and future tracking.
2. Ensure that investigators have timely, equitable, and appropriate access to human biospecimens and associated clinical data stored at NIH supported biorepositories without undue administrative burden. Access is to be guided by policies and procedures that address the following:
 - a. Scientific validity of the research proposal
 - b. Assessment of burden or demand on the tissue resource and staff
 - c. Investigator's agreement covering confidentiality, use, disposition, and security of biospecimens and associated data
 - d. Investigator's written agreement in a Material Transfer Agreement to comply with the NIH Research Tool Guidelines²
 - e. Investigator and institutional research qualifications
 - f. Ethical oversight where required by Federal regulations or local institutional requirements
 - g. Adequate funding for the biorepository

In addition to the above, the following points are recommended for consideration while assessing access privileges:

Biospecimens and associated clinical data are to be appropriately matched with the specific scientific investigations for which they are intended. Investigators requesting access need to be informed about site-specific information in order to correctly interpret their findings, e.g., how tissue was prepared, how the clinical diagnosis was derived, how subjects were recruited (volunteers, clinic patients, etc.). Additionally, if requested and available, agonal state information may be shared.^{3,4}

The local decision-making body should take local principles into account. Ethical considerations including conflicts of interest should come first among principles that guide the decision-making process.

Biorepositories should establish an appeals process for addressing disputes over allocation decisions.

3. Apply guidelines to all new collections and, whenever possible, to existing collections.
4. If applicable and where monetary charges are necessary, charge only to recover costs as appropriate to retrieve and disseminate specimens (see Cost Recovery Guidelines).
5. Within the biorepository, use a system of data access with defined levels of access privileges. Restrict access to research subjects' identities and medical, genetic, social, and personal histories to necessary biorepository staff members who need such access as part of their duty or to persons permitted access by law. Monitor personnel compliance with access restrictions.
6. Store human biospecimens only for research purposes according to approved protocols, not to serve individual biospecimen donor's needs or wishes.

B. Retrieval of Specimens: Before disseminating, it is recommended that biospecimens be retrieved from storage according to biorepository SOPs that safeguard biospecimen quality. Before retrieval, systems verification that the request has received approval from the appropriate committee is necessary. It is recommended that SOPs include a checklist to confirm completion of the retrieval

process. It is recommended that deviations during retrieval be documented, such as inventory inconsistencies, damaged containers, thawing, or refreezing, etc.

C. Shipping conditions

1. Refrigerated or Frozen Specimens: To regulate temperature of frozen specimens during shipping, consider time, distance, climate, season, method of transportation and regulations as well as type of biospecimens and their intended use. Note that the number of biospecimens per container also affects whether or not temperature can be maintained during transport. For extremely valuable specimens consider sending a test shipment of frozen water samples, for example, to check adequacy of shipping method and potential obstacles. Devices that monitor temperature during shipping are available. In addition, placing samples in sealed bags with a desiccant can be used to control humidity. Allow for at least 24 hour transport time. When necessary, it is recommended that temperature sensitive material shipments be sent using a courier with resources to replenish the refrigerant in case of shipping delay. It is also recommended that shipment days be chosen to avoid inherent problems with weekend arrival of specimens.

For refrigerated samples use gel packs conditioned at -15 degrees Celsius or phase change material rated for refrigerated transport. For frozen samples use appropriate insulation and gel packs (conditioned at or below -20°C); for frozen temperatures at -70°C use dry ice pellets or sheets. Note that dry ice is considered a hazardous material for shipping. To maintain temperatures at or below -150°C use liquid nitrogen dry shipper. Use insulated packaging to protect specimens from extreme ambient temperatures.

2. Paraffin blocks and slides: Ship at room temperature in insulated package via overnight carrier. Samples on glass or plastic slides should be cushioned inside sturdy outer package. Triple package liquid samples.

D. Documentation

1. Notification by the shipping laboratory to the recipient is recommended before shipping to confirm that the recipient can accept the package and properly store. Package tracking by sender and recipient with shipping log is suggested, to include the following information: invoice number, recipient, date shipped/received, courier name and package tracking number, sample description, number shipped/received, condition on arrival, study name and number, key investigator's name.
2. Standardized paperwork to accompany the shipment may include shipping manifest, list of sample ID numbers, and description of samples electronically with optional hard copy included in shipment. A feedback questionnaire regarding quality of samples received may be intermittently included for QA/QC.

E. Regulatory considerations

1. Consult ISBER Best Practices (ISBER 2005) for information regarding international transport regulations and classifying biospecimens for shipment. For packaged biospecimens, International Air Transport Association (IATA 2004) regulations require (1) Primary inner receptacle (2) secondary packaging (3) rigid outer package. Consult OSHA regulations to determine if substance requires biohazard label.

F. Training

1. Training of personnel for shipment of biospecimens is strongly recommended with updating at least every 2 years. Dangerous goods training may be required for some biorepository personnel. A record of training is recommended to be maintained by the laboratory, as per institutional policies.

II. Discarding:

- A. Prior to discarding biospecimens that have potential research use, the center is advised to contact NACC (National Alzheimer's Coordinating Center) www.alz.washington.edu/ or naccmail@u.washington.edu with information regarding the biospecimens that are being considered for disposal. NACC will send electronic notifications to a list serve of ADC Center Directors,

Neuropathology Core leaders, and Clinical Core leaders regarding the immediate availability of the biospecimens. In addition, the availability of the biospecimens will be posted on the NACC secure website for ADC investigators. The following information is necessary for dissemination by NACC:

1. The number and types of biospecimens
 2. If available, the manner of collection, fixation, storage, and the accessibility to clinical and pathologic phenotypic information.
 3. The length of time that the specimens will be available needs to be provided to NACC. Note that a 30 day minimum is recommended for notification prior to disposal. If this time period is exceeded, low-priority biospecimens that have not been requested for distribution may be discarded (see below).
 4. The phone number and/or email information of the primary contact person is required for questions, requests, and information regarding the biospecimens.
- B.** Disposal of biospecimens can be considered under certain circumstances including but not limited to reduced integrity, exhausted capacity or insufficient funds for long-term maintenance or storage of low priority biospecimens and inability to distribute low priority biospecimens. Determination of the integrity of biospecimens is at the discretion of the center. Low priority biospecimens may vary from center to center but may include biospecimens from end-stage AD without adequate clinical or neuropathologic characterization, or last evaluation many years prior to death. The discarding of high priority biospecimens (eg. biospecimens from subjects with no cognitive impairment, mild cognitive impairment, atypical dementias, and from well characterized cohorts) is discouraged. Continued efforts at distribution of high priority biospecimens through NACC and/or personal resources are strongly encouraged.
- C.** The discarding of clinical biospecimens should conform to the CAP guidelines. The discarding of research specimens is also subject to any written agreement at the time of enrollment in the study and institutional policy.

References

1. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.
2. Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice http://ott.od.nih.gov/policy/rt_guide_final.html.
3. Morrison-Bogorad M, Zimmerman AL, Pardue S. Heat-shock 70 messenger RNA levels in human brain: correlation with agonal fever. *J Neurochem* 63:235-246;1995.
4. Pardue S, Wangb S, Miller MM, Morrison-Bogorad M. Elevated levels of inducible heat shock 70 proteins in human brain. *Neurobiology of Aging* 28:314–324;2007.

COST RECOVERY GUIDELINE:

General Comment: Alzheimer's Disease Centers, with their cores, are funded to provide the infrastructure to support both Center-affiliated research projects and non-affiliated research projects. In general, Centers do not charge investigators for receiving biospecimens to the extent that NIH Center budgets cover these costs. However, there are caveats to this general rule. In competing renewal applications, cores generate and justify their budgets according to their best estimates of the future demands for their resources. These expectations are based on future plans of the Center components (other cores and projects), planned affiliations and collaborations, and on past experience (e.g. autopsy rate for the last 5 years). Budgetary projections may not match reality of the demands placed on the Center. Tissue requests may be so large that an individual lab would be forced to abandon other Center duties in order to prepare the samples. In these situations it is allowable and recommended that Centers develop cost recovery policies to deal with demands when they exceed the budgeted support of the Center. The following recommendations may guide these policies:

- A. Cost recovery refers to the recovery of the expenses of the labor, supplies, and services required to operate biospecimen programs that are not already included in the Center budget (prepare, document, ship, etc.) biospecimens. It does NOT refer to the sale of the specimen itself.
- B. Cost recovery policies should be in accordance with Office of Management and Budget (OMB) Circular A21 principles. All institutions should work closely with their own Sponsored Projects Accounting offices and their NIH Grant Management Specialists in developing policies and procedures that distinguish between 'cost recovery' and 'program income' and deal with issues of overhead costs, etc.
- C. 'Cost recovery' connotes 'no profit' from the activity; however the consultation referenced in Item 2 is critical in establishing the limitations on cost recovery efforts.
- D. Cost recovery procedures that are based on fair estimates of the actual effort (salary and fringe benefits) and costs (supplies, shipping, etc.) associated with sharing tissue and that are reviewed and revised annually are recommended.
- E. Cost recovery income that is directed toward support of the operations of the unit expending the effort is recommended.
- F. A tiered system of charges may be considered. That is, the charge would depend on the funding source (federally funded, non-profit foundation or industry) of the research for which the biospecimens are requested. An example of such a system has been developed for the National Cell Repository for Alzheimer's Disease (*NCRAD*).
- G. Cost recovery may not be limited to the laboratory providing the specimen. Often the burden to data managers is significant and cost recovery may also apply to the Data core.

INTELLECTUAL PROPERTY GUIDELINE

Note: This guideline refers to inventions, and is not meant to address authorship issues.

- A. Recognize that biorepository faculty and staff members as custodians of biospecimens are not *a priori* considered inventors under patent law for inventions made using materials distributed by the biorepository. In general, the staff should be informed that one whose sole contribution to an invention consists of the routine collection, handling, storage, and disbursement of biospecimens might not rise to the level of “inventor” of an invention. Inventorship is determined by patent law and must be considered on a case-by-case basis by trained legal personnel.
- B. If true research collaboration is contemplated with the involved biorepository faculty and staff, the nature of that collaboration may qualify for Intellectual Property rights and can be detailed in other Intellectual Property contractual arrangements or by a MTA.
- C. Recognize that biorepositories have no inherent rights to future Intellectual Property, including reach-through rights in inventions made by investigators using samples obtained from the biorepository. Recipient scientists may want assurance that their Intellectual Property rights are protected should they develop inventions from use of banked tissue and data. MTAs can secure these rights.
- D. Ensure through MTAs that research data developed using biospecimens are made available to the research community.

References:

1. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.

MATERIAL TRANSFER GUIDELINE:

The NIH has established principles and policies for the sharing of biospecimens in “Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice”¹ in order to achieve the widest possible dissemination of “unique research resources” to promote research progress. The tissues obtained from carefully-characterized clinical populations, such as from ADCs, represent important resources to be considered in the same way. NIH has also formally endorsed the use of material transfer documents known either as “material transfer agreements” (MTA) or “simple letters of agreement” (SLA).^{2, 3} These documents, executed usually between institutions not individual scientists, define the terms and protections of the exchange. The SLA is a simpler version of the MTA, less detailed and usually does not contain indemnification language.

In the past, tissue samples were often exchanged without MTAs because they did not represent a unique resource that had been created, engineered or invented. The need for intellectual property and financial protections did not apply (e.g. an investigator would be unlikely to ‘patent’ blood samples). However, MTAs offer other protections and restrictions which make them useful. Most academic and government research institutions have technology management offices that govern/negotiate the material transfers of their faculty and staff scientists. Such offices often have institutional templates which can be customized for the individual material transfer.

In addition to individual institutional documents, the NIH published the final version of the Uniform Biological Material Transfer Agreement (UBMTA)⁴ and a Simple Letter Agreement for the Transfer of Non-Proprietary Biological Material in 1995. Institutions can sign the UBMTA Master Agreement (to date, 320 have signed) and transfer materials under the terms of the UBMTA upon execution of an Implementing Letter for the particular transfer. The Association of University Technology Managers (AUTM) serves as the repository for the signed UBMTA Master Agreements from those institutions wishing to use the UBMTA for some or all of their exchanges of biological materials.

The following outline contains items/issues that are often addressed in MTAs. Although individual academic institutions determine the final elements of the MTA, the Task Force makes recommendations below each item:

- A. Identify the parties and specific material to be transferred – By formally identifying the provider and recipient, there can be no later accusations of misappropriation or misdirection of materials.
 1. Strongly recommend
- B. Protect intellectual property – An MTA can establish the investigator’s claim to their intellectual work. The MTA can ensure that the investigator is rewarded if commercial development results in income.
 1. Consult with your institutional guidelines on intellectual property.
- C. Issues of academic freedom and integrity – An MTA may include language to limit publication rights/conditions or require collaborations or authorship requirements that are unwelcome or unjustified. However, issues regarding publications, IP, authorship do not need to be part the MTA and can be contracted separately.
 1. Few limitations need be placed on publication rights and conditions. Confidential review by the biospecimen provider of publications prior to submission (usually 30 days) can afford the opportunity to ensure that nothing confidential or proprietary is disclosed. Potential scientific collaborations are always encouraged but need not be mandated as part of the agreement contract unless justified. For example, when unique information generated by the provider is requested to accompany the tissue, the collaboration may be formalized in the MTA.
- D. Protect against improper or unsafe use of the material – An MTA informs the recipient of their institution’s responsibility if their handling of the material results in injury or damages. Indemnification may also be part of the MTA.
 1. It is recommended that both institutions be fully informed and aware of the assignment of responsibility and liability.
- E. Provide and document explicit warnings – If the material requires special warnings they can be part of the MTA language.

1. If warnings are justified, they can be included in the MTA. However, the MTA need not be the only place such warnings are provided (e.g., a warning should be included for tissue that has not been tested for infectious agents).
- F. Restrict any further dissemination without notice – A recipient scientist cannot provide materials received under the MTA to a secondary investigator without written approval.
 1. The ADCs and NIH wish to account for the use and productivity of biospecimens they share. Unacknowledged third-party sharing of material thwarts such accountability and should be prohibited without explicit permission of the provider.
- G. Restrict and define the scope of how the material may be used – An MTA can contain language that limits the use of the material. For example, the use and development of the material may be restricted to non-profit research or teaching uses.
 1. It is recommended that the least restrictive language be used in sharing tissue.
- H. Compliance with federal, state and local laws regarding confidentiality of research participants can also be assured in MTAs (e.g. HIPAA).
 1. Such assurances are required.
- I. MTAs/SLAs can be modified for unique situations and requirements.
 1. If your Center has special requirements, they can be added. For example, the following items can be part of the MTA language:
 - a. Provide a copy of any publication which contains experimental results obtained from the use of the Material
 - b. Agree to protect the confidentiality of research participants by not attempting to identify them.
 - c. Provide a brief progress report XX months from the receipt of the requested tissue.
 - d. Acknowledge your Center grant in any presentation or publication that may result from this research:
 - i. P50AGXXXX or P30AGXXXX.
 - ii. Should funding result from this research now or in the future, please notify the Alzheimer's Disease Center with details so we may report productivity derived from our resources to NIA.

References:

1. Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources: Final Notice http://ott.od.nih.gov/policy/rt_guide_final.html
2. The NIH Office of Technology Transfer (OTT), <http://ott.od.nih.gov/>
3. The NINDS Technology Transfer Office, <http://tto.ninds.nih.gov/Mta.asp>
4. Association of University Technology Managers, http://www.autm.net/aboutTT/aboutTT_umbta.cfm
5. National Cancer Institute, NCI best practices for biospecimen resources. June, 2007.