

NATIONAL INSTITUTE ON AGING

BIOSPECIMEN BEST PRACTICE GUIDELINES FOR THE ALZHEIMER’S DISEASE CENTERS

VERSION 3.0 (24JUNE 2014)

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BLOOD AND URINE GUIDELINE

I. Preparation and storage of blood biospecimens

- A. A number of factors related to the physiology of the human research participant have been demonstrated to impact blood biomarker results (*e.g.*, age, gender, ethnicity, exercise, overall health, food and beverages consumed prior to collection, medications, time of day of blood draw)[1-3]. Attempts should be made to record as much information related to these variables as possible in order for appropriate adjustments to be made during analysis of results[1, 2].
- B. The majority of Alzheimer’s disease studies globally utilize fasting blood collection and this is recommended. Whether fasting or non-fasting, time since last meal should be collected.
- C. It has been estimated that up to 46% of laboratory errors come from pre-analytic processing[4]. Factors related to blood collection devices (needle gauge, tube lubricants, tube walls) can impact blood marker levels[1, 5, 6]. Standardized and uniform techniques of samples processing are recommended[1, 2, 7].
- D. Detailed step-by-step procedures for collection of blood samples are available in the CLSI H3-A6 [8]. Broad recommendations for standardization of sample collection are
 - a. Blood should be collected from the median cubital vein as opposed to other, more fragile, veins
 - b. Alcohol used to clean the skin should be allowed to evaporate before venipuncture
 - c. A tourniquet applied 3-4 inches above the site of venipuncture should be loosened once blood starts to flow
 - d. The position of the patient (sitting, standing, lying down) should be noted
 - e. Blood is generally drawn with a vacutainer system
 - f. Tubes for plasma should be adequately filled with blood to ensure the optimal blood/additive ratio
 - g. For most studies, the needle gauge of 19-23 is preferable with 21g being the most common [5, 8].
 - h. Order of blood draw should be as follows (skip tubes not being utilized)(CLSI H3-A6; Qiagen):
 - 1. Blood Culture Tube
 - 2. Coagulation tube
 - 3. Serum with or without clot activator or gel
 - 4. Heparin with or without gel separator
 - 5. EDTA with or without separator
 - 6. Glycolytic inhibitor
 - 7. PAXgene blood RNA tube
- E. Rapid processing of samples is optimal (total processing time < 2hrs from “stick-to-freezer”). Detailed procedures for processing blood specimens are provided by CLSI H18-A4 [7]. General recommendations follow, although individual steps may need to be modified for specific markers:
 - a. Serum/plasma should be physically separated from contact with cells as soon as possible (<2hrs). Do not store aliquots from serum/plasma that have been in contact with cells for > 2hrs.
 - b. Serum should be clotted in vertical position before centrifugation (30-60 min) if patient is not on anti-coagulant therapy.
 - c. Plasma tube should be gently inverted 5-10 times.
 - d. Relative centrifugal force (RCF; g-force) should be utilized over revolutions per minute in SOPs and publications.
 - e. Centrifugation at 2000g for 10 min with horizontal rotors preferable. Centrifugation at room temperature versus a refrigerated (4°C) can make a difference for certain markers in both serum and plasma. Refrigerated centrifuges are better for platelet preparation.
 - f. Other factors that should be documented:
 - 1. Type of collection tube (manufacturer’s name, type of anticoagulant, etc.)
 - 2. Time from collection to centrifugation

3. Temperature between collection and centrifugation
 4. Presence and type of separator
 5. Temperature of centrifugation
 6. Number of centrifugations (single or double)
- F. Other considerations. Whether it is necessary to add protease inhibitors to samples after aliquoting is not certain and depends upon the nature of the study. This may be worth consideration if plasma or serum samples are to be used for proteomic analyses and must be noted.
- G. Post centrifugation considerations that should be documented:
- a. Type of secondary container (tube, straw)
 - b. Time between centrifugation and freezing
 - c. Storage temperature
 - d. Number of freeze/thaw cycles
 - e. Duration of storage
 - f. Storage location of aliquot vials
- H. Aliquots should be made in siliconized polypropylene tubes (or straws) using polypropylene tips for pipets.
- I. Small aliquots (generally not larger than 0.5 ml) are recommended for storage. 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. Long-term storage should be at -80°C or liquid nitrogen. If storage on dry ice is utilized for shipment, the headspace should be vented or the sample should be allowed to sit in -80°C freezer for 9 hr prior to thaw[9].
- K. Document the volume of plasma or serum that was obtained.
- L. The quality of serum or plasma can be influenced by hemolysis, red or pink tingeing of plasma or serum is an indicator that significant hemolysis has occurred, and, lipemia, a milky white substance floating in the plasma or serum, which may render the samples less useful for many biomarker studies should be determined on case-by-case basis.
- M. For some studies in which blood is drawn for biomarker analyses, as described above, additional analyses of DNA may be planned. This may entail processing the buffy coat from the plasma tube(s) or the collection of an additional tube. Please refer to best practice guidelines for DNA preparation and storage.
- N. Important points for PAXgene collection and processing should be noted:
- a. Store PAXgene™ Blood RNA Tubes at room temperature (18°C to 25°C) before use.
 - b. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5 mL of blood into the tube.
 - c. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times (see also training video regarding inverting tubes at <http://www.preanalytix.com/videos/PAXgenePhlebotomy.mpg>).
 - d. Incubate the PAXgene™ Blood RNA Tube UPRIGHT at room temperature (18°C to 25°C) for 24 hours. Record time/date of draw.
 - e. REPEAT STEPS 4 to 7 for each of the three PAXgene™ Blood RNA Tubes to be collected per subject.
 - f. After 24 hours at room temperature, transfer the three PAXgene tubes to -80°C (or -20°C) freezer. Record time and date of freezing.
- O. Centers should refer to OBBR [2] guidelines maintenance and long term storage recommendations.[2]

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3. Vanderstichele, H., et al., *Standardization of measurement of β -amyloid((1-42)) in cerebrospinal fluid and plasma*. *Amyloid*, 2000. **7**(4): p. 245-258.
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8. CLSI, *Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard - Sixth Edition*. H3-A6. **27**(26).
9. Murphy BM, S.S., Mueller BM, van der Geer P, Manning MC, & Fitchmum MI, *Protein instability following transport on dry ice*. *Nature Methods*, 2013. **10**(4): p. 278-98.

II. Preparation and storage of urine biospecimens

- A. A number of factors can impact biomarker results that have yet to be fully investigated and characterized (e.g., binding of urine proteins to catheters and other collection devices). Additionally, factors related to the physiology of the human research participant will impact biomarker results (e.g., overall health, food and beverages consumed prior to collection, medications, time of day of collection) and attempts should be made to record as detailed information related to these variables as possible in order for appropriate adjustments to be made during analysis of results [1-4].
- B. The Clinical and Laboratory Standards Institute (CLSI GP16-A3) provide detailed guidelines for collection and processing of urine samples and should be referred to for more comprehensive instruction. The current guidelines have been adapted from CLSI GP16-A3, the Human Kidney and Urine Proteome Project guidelines, and the Standard Protocol for Urine Collection developed by the Human Urine and Kidney Proteome Project, HUKPP and the European Urine and Kidney Proteomics, EuroKUP (See Metabolomics and Proteomics Guideline).
- C. Collect mid-stream of 2nd morning urine (preferable) or morning random-catch urine in sterile urine collector
- D. Centrifuge at 1000g for 10 min to remove cells and debris.
- E. Aliquot supernatant avoiding disturbing the pellets at small aliquots (10, 50 or 1.5 mL).
- F. Store at -80°C (preferably) or -20°C. Time from collection to storage (or assay) should be less than 3 hours. For samples not analyzed or stored within 3 hours, storage at 4°C or on ice and addition of 10mM NaN₃ (or 0.2M Boric acid) is added to inhibit bacterial growth.
- G. Minimal data set to be collected includes:
 - a. Freezing time.
 - b. Sample number
 - c. Storage temperature
 - d. Date of collection

- e. Time of collection
 - f. Time until freezing
 - g. Aliquot volume and number of aliquots
 - h. Height
 - i. Weight
 - j. GFR (or eGFR)
 - k. Urinary protein amount (and calculation method)
 - l. Urine creatinine (and calculation method)
 - m. Hematuria
 - n. Serum creatinine (and calculation method)
 - o. Urine pH
 - p. Serum protein
 - q. Serum cholesterol
 - r. Storage location aliquot vials
- H. Centers should refer to OBBR guidelines for maintenance and long term storage recommendations.[5]

References

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2. HKUPP., *Standard Protocol for Urine Collection and Storage.*
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4. NIDDK, *Best Practices for Sample Storage: A report from the Workshop on Urine Biospecimen handling.*
5. National Cancer Institute, NCI best practices for biospecimen resources, 2011 (NCI Best Practices website: <http://biospecimens.cancer.gov/practices/>; PDF of the NCI Biospecimens Best Practice: <http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf>).

CEREBROSPINAL FLUID GUIDELINE

I. Acquisition of Cerebrospinal Fluid (CSF) Biospecimens

A. Two DVD resources available:

a. Physician education on lumbar puncture (LP) technique is made available by the University of Washington Alzheimer's Disease Research Center. Contact Elaine Peskind, MD, at peskind@uw.edu

b. Research subject education on spinal tap procedure is made available by the Alzheimer's Disease Cooperative Study. Contact Jeffree Itrich at jitrich@ucsd.edu

B. Fasting is recommended prior to the procedure; CSF should be collected at a consistent time in the morning (*e.g.*, 0800-1100h)

C. Consider taking matching plasma and serum samples for simultaneous measurement of CSF and blood biomarkers. If this is not feasible, consider taking a serum sample for simultaneous measurement of blood glucose.

D. Use 25 g needle for deep local anesthesia rather than the needle provided in kit.

E. Atraumatic spinal needle (*e.g.*, Sprotte 24 g atraumatic spinal needle) is recommended for the LP to minimize risk of post-LP headache (<1%).^{1,2,3} 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%)

F. CSF may be withdrawn under negative pressure with sterile polypropylene syringes; up to 30 ml CSF may be withdrawn without increased risk of adverse events. Using a 22 gauge needle permits CSF to flow under gravity. The extension tubing provided in LP kits should not be used.

G. There is some controversy concerning the use of negative pressure for withdrawal of CSF. Clinical judgment should be used in evaluating risk of exceedingly rare vs. more common adverse events.

H. In addition to sterile gloves, the clinician should employ a mask and safety goggles or eyeglasses.

I. First 2 ml of CSF withdrawn is sent for local laboratory analysis (cell counts, protein, glucose measurements). These 2 ml 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.

J. Clinical Exemplar – to avoid a post-lumbar puncture headache, the following actions are strongly recommended:

a. Participant rest in recumbent position for one hour post-LP (a common clinical practice)

b. Encourage liberal fluid intake

c. Subject should avoid exertion (exercise, housework, gardening, sexual activity, lifting/bending, etc.) for 24-48 hours following lumbar puncture

d. 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.⁴

B. Rapid processing of samples is optimal; aliquoting followed by quick freeze at bedside on dry ice is recommended, followed by transfer to ultralow (e.g. -80°C) freezer. Freezing at -20 °C is not adequate for proper storage of CSF for any length of time.

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. (See Metabolomics and Proteomics Guideline).

D. Small aliquots (generally no larger than 0.5 ml) are recommended for storage. Smaller aliquots are adequate for most analytes; e.g., 300-500ul is adequate for A β species recovery. However, a consistent aliquot volume should be used throughout. Please ensure that the tube being used is “low-bind” and is appropriately sized for your aliquots. Reduce air space in tubes as much as possible.

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. (Also, see Metabolomics and Proteomics Guideline).

F. Uniform, non-redundant annotation of samples is recommended.

G. Document the exact volume of fluid obtained at each tap, as 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 are 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.
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4. 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.
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BRAIN GUIDELINE

All laboratories should follow documented standardized protocols for tissue collection, processing, storage, retrieval, and dissemination as well as for histologic methods and any other tissue-based assays. The following presents guidelines for current best practices for a research brain bank focused on Alzheimer's disease (AD) and related neurodegenerative diseases.

I. Prior to autopsy

A. Usual practice for research brain banks is brain autopsy only; however, to the extent possible, full autopsy should be considered and requested.

B. 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.

C. Autopsies should include individuals with documentation of research quality clinical work up and a diagnosis of AD dementia, MCI, related neurodegenerative diseases, or cognitively normal.

D. Detailed clinical information is essential to maximize the research usefulness of brain donations. Responsibility for obtaining this information is largely outside of brain banking operations; however, databasing this information may occur within the brain bank or some other component of the research group:

- Sex, cause of death, age at death, date of last clinical assessment, relevant family history, medication history, diagnosis(es) of brain diseases, other diagnoses, duration of illness(es), relevant neuroimaging or other laboratory findings, and agonal conditions, *e.g.*, fever, O₂ saturation, etc.

II. At time of autopsy

A. Autopsies must be performed according to local consenting and IRB protocols, as well as in compliance with all hospital, municipal, state, and federal laws and regulations.

B. The goals are to ensure the safety of all personnel, to make the correct neuropathologic diagnosis(es), and to obtain and process brain regions in a manner that maximizes their research utility.

- a. Always use universal precautions when handling human tissue or body fluids.
- b. If prion disease is a consideration, then follow protocols published by the National Prion Disease Pathology Surveillance Center. (<http://www.cjdsurveillance.com/>). 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.
- c. Minimal tissue block dissection should follow current NIA-AA guidelines^{1,2}. Paraffin-embedded tissue blocks should be archived indefinitely.
- d. Best practice is to obtain a portion of cerebellar hemisphere sufficient to fill a tissue cassette from every case and to store at -80°C as quickly as possible for potential future DNA preparation.
- e. Best practice is to establish protocols to dissect and freeze as quickly as possible selected brain regions for potential future biochemical analyses. A variety of methods can be used and the details depend on the desired

use of the tissue. Examples are flash freezing a few grams of tissue in liquid nitrogen, with or without isopentane, or between blocks of dry ice. Freezing tissue slabs is not considered best practice because of difficulty in subsequent dissection.

- f. Support of collaborative research is a best practice. Additional brain samples and additional methods for optimal stabilization for specific assays should follow documented protocols.
- g. Post mortem cerebrospinal fluid (CSF) may be collected, usually from the ventricular system. If it is, then best practice is to freeze at -80°C in appropriate containers based on expected use (see CSF section) and to thaw only once for use.

C. Standard metrics should be collected from each autopsy; at a minimum, this should include post mortem interval (PMI), the time measured in hours between death and stabilization of the tissue. Measures of tissue integrity also should be considered; however, none has achieved consensus status. These include tissue pH as well as a variety of measures of molecular degeneration. Measurement of brain pH is recommended—either by using a surface pH electrode or by measuring pH of either ventricular fluid or homogenized brain using a standard pH electrode.

III. After autopsy

A. Histologic and immunohistochemical staining of standard tissue blocks should follow current NIA-AA guidelines^{1,2}. Histologic slides should be archived for at least 10 years or longer depending on research needs or regulations.

B. Fixed tissue as well as frozen tissue and CSF also should be retained for at least 10 years, or longer depending on research needs or regulations.

C. All biospecimens should be stored in appropriately labeled containers with unique identifiers and in a regulated environment with safeguards against physical damage, temperature changes, severe weather, and natural disasters.

D. It is best practice that all biospecimens are stored in a manner that meets universal precautions, IRB oversight, and employee health safety regulations; permits further neuropathologic evaluation if needed; and optimizes future potential research use.

E. Best practice is to maintain an accurate and appropriately safeguarded inventory of accrued biospecimens, distributed biospecimens, and available tissue and fluid resources.

F. Biospecimen resource inventory should be linked with a database(s) that contains outcomes of neuropathologic evaluation, clinical information, and results from other investigations, *e.g.*, genetic information, in a manner that is IRB compliant and meets the need for subject confidentiality, security, and informed consent provisions (see Informatics Guideline).

G. In addition to scientific advisory committees for the research group, a brain bank should regularly convene a Biospecimen Use Committee.

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2. Hyman BT, Phelps, CH, Beach, TG, Bigio, EH, Cairns, NJ, Carrillo, MC, Dickson, DW, Duyckaerts, C, Frosch, MP, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's Dement.* 2012; 8:1-13.PMC3266529

DNA /RNA / PROTEIN GUIDELINE

I. General

A. Whether prepared from biofluids or from 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).

B. 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.³

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

II. Safety Provisions

A. Laboratories must have safety plans.

B. Laboratory personnel

- a. Immunization for hepatitis B is recommended.
- b. Must be trained in safety procedures related to handling of human tissue
- c. Must observe universal precautions; all specimens must be handled as if infectious

C. Biospecimens

a. 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.

b. HIV and hepatitis B and C

1. 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.
2. 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.
3. 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.

III. Annotating

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

a. 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.

b. Stabilization

1. Note: Consideration given to storage bags/containers that protect the integrity of the contents is recommended.
2. Freezers that are monitored by automated security alarm systems that contact laboratory director and personnel by telephone or pager when failure occurs are recommended.
3. Freezers with back-up systems (*e.g.*, CO₂ or LN₂) or spare freezers for emergency situations are recommended.

c. Temperature recommendations

1. Formalin-fixed: room temperature (20-25°C)
2. Paraformaldehyde-fixed, sucrose/sodium azide preserved: refrigerator (2-8°C)
3. Frozen: -70-80°C or liquid nitrogen vapor

B. Retrieval

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

References

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INDUCED PLURIPOTENT STEM CELLS (iPSCs)

A detailed compendium of iPSC protocols, prepared by Scott Noggle, Ph.D., Director of the New York Stem Cell Foundation Laboratories in New York NY and provided in collaboration with Sam Gandy, M.D., Ph.D., containing:

- Growth Medium
- MEF-Conditioned Medium (CM)
- Producing MEF Feeder Cells
- Matrigel Plate Coating
- Fibroblast Cultures From Skin Biopsies
- Fibroblast Cultures From Skin Biopsies Using Dry-Down Technique
- iPSC Induction Protocols - Retrovirus
- iPSC Induction Protocols – Sendai Virus
- Passaging Methods for HES and iPS Cell Lines
- Enzymatic Passaging of HES and iPS Cells
- Feeder-Free Protocols
- Freezing iPS Cells
- Alternate Protocol: Freezing by Vitrification in Cryovials
- Karyotyping
- Teratoma and Embryoid Body Assays
- Immunofluorescent Procedures & Markers
- Real-Time RT-PCR Protocols & Markers
- Flow Cytometry Analysis of HES and iPS Cells
- Magnetic Sorting of iPS Cells
- 2D Neuronal Directed Differentiation For iPS/HES Cells From Feeders
- Adapted Sasai EB Preparation for Freezing and Sectioning
- Endoderm Directed Differentiation – Beta Cells
- Mycoplasma Testing
- Nanostring Protocols

METABOLOMICS AND PROTEOMICS GUIDELINE

Part I: Metabolomics

Select sources and references:

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8. Metabolon, Sample Preparation, General Sample Guidelines for Metabolon Studies, 2013

Recommendations are centered on reducing bias in experimental design, implementation, and analysis based on a review of the literature and input from metabolomics companies. In many instances recommendations are based on expert opinion and not necessarily on evidence, which is incomplete.

I. Experimental design: metabolomics-related considerations

Generally follow proposed “rules of evidence” for biomarker study design, implementation, and interpretation as delineated in ([Ransohoff 2004](#); [Ransohoff 2005](#)). This includes employment of explicit pre-defined, standardized collection, processing, and storage standard operating procedures and quality-control parameters.

A. Obtain and record IRB approval, geographical location, institution, race and ethnic background (based on FDA and Office of National statistics criteria), medical history, age at sample collection, weight, height, and/or BMI, gender, dietary restrictions (if applicable). Record potential confounders of smoking, blood pressure, diet (e.g. vegetarian, vegan etc.), alcohol consumption, medications.

B. Define number of groups, subjects/gender/group, inclusion criteria exclusion criteria, fasting status. For interventional trials record compound, route, dose, dose volume, duration of dosing, vehicle.

C. Dietary effects are a substantial source of bias for metabolomic studies, thus it may be advisable to use fasted patients and/or record time of last meal ([Teahan, Gamble et al. 2006](#)).

D. To reduce technical bias between groups collect, process, and analyze samples from cases and controls in parallel or in a randomized manner. Blind the assay operator to the sample diagnosis.

E. Uniformly specify type and brand of collection systems (vacutainer, syringe, atraumatic technique for lumbar puncture etc. as applicable), collection tubes, aliquot tubes, and pipette tips to be used in a specific study. Polypropylene tubes may be preferable. Siliconized tubes and tips may be considered. Plasma anticoagulant guidelines vary, with EDTA advocated for plasma and whole blood by some metabolomics providers.

II. Metabolomics-related sample collection

Blood

A. For general blood processing parameters, see Blood and Urine section outlined above.

a. For metabolomics, EDTA plasma tubes are preferred by several authors because EDTA not only inhibits coagulation but also Mg-dependent enzymes such as hexokinase in erythrocytes(Kamlage, Maldonado et al. 2014).

b. For metabolomics, there appears to be uncertainty on whether blood samples benefit from storage on ice until centrifugation. This may reduce metabolite variation (Teahan, Gamble et al. 2006; Bernini, Bertini et al. 2011). However, others have noted that cold can activate platelet function thereby affecting their metabolism (references in (Kamlage, Maldonado et al. 2014)).

c. The plasma supernatant should be carefully removed after centrifugation without touching the buffy coat layer (Kamlage, Maldonado et al. 2014).

d. Plasma processing: Removal of the red blood cells makes plasma more vulnerable to chemical oxidative processes. Plasma (or serum) should be frozen immediately(Bernini, Bertini et al. 2011). Some metabolites significantly change abundance at 0.5 hours at room temperature and at 2 hours at 4°C (Kamlage, Maldonado et al. 2014).

e. Consider measuring relevant clinical chemistries as covariates. For blood analyses, consider measuring routine blood chemistry and complete blood counts (Urea, creatinine, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total protein, albumin, erythrocyte count, hemoglobin, hematocrit, platelets, white blood count, sodium, potassium, bilirubin, ALT, ALP, GGT).

Urine

A. For general urine processing parameters, see Blood and Urine Guidelines section outlined above.

B. For urine analysis, consider measuring routine urine chemistry (osmolality, ketones, pH, protein, glucose, bilirubin, blood, sediment and color).

Part II: Proteomics

Proteomics - the large scale study of proteins, including their identification, characterization and quantification - is sensitive to various pre-analytical variables including biological and technical factors.

I. Biological factors

As outlined above, a variety of biological effects can conceivably affect the proteome, including immutable factors and potentially modifiable lifestyle factors [1-3] and should be recorded.

II. Technical factors also see consensus guideline (12) for pre-analytical factors involved in CSF AD biomarker measurements:

A. Technical factors affecting the proteome of cerebrospinal fluid (CSF)

a. Lumbar puncture (see also CSF Guideline)

The composition and subsequent proteomic profile of CSF can be influenced by several factors, including the volume of CSF collected, the rostro-caudal gradient in CSF, the influence of circadian rhythms on CSF production and absorption, and blood contamination due to a traumatic tap [3-5]. For the collection of CSF via lumbar puncture (LP) the following is recommended:

- The time of sample collection should be standardized across the study - morning specimens are recommended
- Whether the subject has fasted or not should ideally be standardized across the study – sample collection before breakfast after an overnight fast is recommended
- The site of the LP should be recorded and kept consistent (vertebral body L3 – L5 is recommended)
- A 25 gauge needle should be used for deep local anesthesia
- The use of atraumatic spinal needles (e.g., Sprotte 24 gauge atraumatic spinal needle) is recommended for LP to minimize risk of post-LP headache
- The first 1 – 2 ml of CSF may be discarded to help prevent contamination (especially if it is a noticeably bloody tap)
- The volume of CSF collected should be recorded and kept consistent (at least 12 ml after the start of collection with the first 2 ml to be used for basic laboratory analysis such as total protein levels, cell counts and glucose measurement)
- CSF should be collected in polypropylene tubes without any additives, the fraction number should be recorded on each tube
- Blood contamination of CSF should be avoided and assessed (samples may be centrifuged to remove blood cells – see next section. However, this does not remove other blood components (e.g., those from lysed blood cells) and it is recommended that CSF hemoglobin levels should be measured as an indication of blood contamination)

b. CSF processing

The addition of protease inhibitors (PIs) and phosphatase inhibitors to the sample at the time of CSF collection may prevent/limit degradation and dephosphorylation of proteins. However, these inhibitors may interfere with the analytical method or targets being evaluated. For example, protease inhibitors like AEBSF that act through

covalent mechanisms may cause artificial modification of proteins and low molecular weight PIs may mask the presence of species at similar mass ranges [2]. Therefore, in general it is recommended that protease and phosphatase inhibitors NOT be added to samples at the time of collection. If the addition of these inhibitors has been shown to have no effect on a specific analytical method and target, the researchers performing the experiment may decide to add them to aliquots after the first thaw.

The proteomic profile of CSF can be influenced by several variables related to sample processing [3-5]. The following is recommended:

- CSF samples should ideally be processed immediately or within 15 minutes of collection
- CSF samples may be frozen directly (see next section) or centrifuged to remove cells at 2000 g for 10 minutes at room temperature (18 - 25 °C)
- CSF samples should be aliquoted in polypropylene tubes in appropriately small volumes (no more than 0.5 ml is recommended) to minimize freeze-thaw cycles, facilitate the distribution of samples and offer several replicates handled in an identical way

c. CSF storage

It is well known that temperature significantly affects protein stability. Therefore, temperature for short and long-term storage can affect the quality of CSF samples and subsequent proteomic data [3-5]. The following is recommended:

- CSF aliquots should be rapidly frozen on dry ice within 30 minutes of sample collection, followed by storage at – 80 °C in a monitored freezer with minimal temperature fluctuations, or in liquid nitrogen
- The number of freeze-thaw cycles should be minimized - ideally samples should be used without refreezing
- When transporting samples between facilities, an adequate amount of dry ice should be included for 24 hours beyond the estimated duration of the trip in case of delay
- An accurate record of all temperature storage values and times should be kept for archived samples

B. Technical factors affecting the proteome of blood

a. Serum vs plasma

With regard to blood, the choice of plasma vs serum can have a significant effect on the outcome of the proteomic study as the protein profiles of plasma and serum have been reported to be dissimilar [6]. Serum would typically be preferred for some tests because of interferences of anticoagulants in plasma. However, during serum processing various ex vivo processes occur which lead to neo-generation of many peptides. In addition, certain proteins may bind to the clot in an uncontrolled manner, causing a concomitant decrease in free protein concentration. Finally, serum shows many intense peptide signals that impede the detection of endogenous peptides. Therefore, the use of plasma is generally recommended for most proteomic studies [2].

b. Proteomic-specific blood sample collection, processing and storage

Refer to Blood and Urine section above for broad guidelines of collection, processing and storage of samples.

- The addition of protease and phosphatase inhibitors to the sample at the time of blood draw may prevent/limit degradation and dephosphorylation of proteins. However, these inhibitors may interfere with the analytical method or targets being evaluated [2]. Therefore, in general it is recommended that protease and phosphatase inhibitors NOT be added to samples at the time of collection. If the addition of these inhibitors has been shown to have no effect on a specific analytical method and target, the researchers performing the experiment may decide to add them to aliquots after the first thaw.
- For plasma, the incubation time before centrifugation should be minimized and the processing protocol standardized [1, 7, 8]. The following is recommended:
 - Samples should be centrifuged within 30 minutes of collection at 1500 g for 15 minutes at 4 °C
 - The plasma layer will be at the top of the tube, the mononuclear cells and platelets will be in a whitish layer (buffy coat) just under the plasma and the erythrocytes will be at the bottom of the tube
 - Carefully transfer the plasma with an appropriate pipette to a clean polypropylene collection tube without disturbing the buffy coat layer
 - Visually check if the sample hemolyzed (red or pink color) - if yes, sample cannot be used
 - Platelets should be removed from plasma by using a low protein binding sterile filter (0.2 µM) or by a sequential centrifugation (centrifuge plasma at 3200 g for 15 minutes at 4 °C, carefully pipet off the platelet poor plasma into a new clean polypropylene tube - platelets should appear as a creamy-white pellet below the plasma. Centrifuge platelet poor plasma again at 3200 g for 15 minutes at 4°C, carefully pipet off the platelet free plasma into a new clean polypropylene tube)

c. Proteomic-specific urine sample collection, processing and storage

- The addition of and phosphatase inhibitors is NOT recommended at this time for nonproteinuric urine (unless exosome analysis is planned), as normal urine has a much lower amount of proteases compared to plasma and inhibitors have the potential to interfere with the analytical method or targets being evaluated [9].
- With regard to urine sample processing, in addition to Urine Guideline discussed above, the following is recommended:
 - Samples may be centrifuged a second time at high speed to reduce microbial load (10 000 g for 25 – 30 minutes at 4 °C is recommended)
 - Urine aliquots should be rapidly frozen on dry ice within 60 minutes of sample collection, followed by storage at – 80 °C in a monitored freezer with minimal temperature fluctuations, or in liquid nitrogen

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INFORMATICS GUIDELINE

The future of Alzheimer's disease research relies heavily upon research in the basic sciences, and flexible and robust informatics systems for biospecimen resources are vital to collaborative research efforts and progress. Best practices for biospecimen resource data system structure, function, and operational procedures are outlined below.

I. Database Structure

A. ADC local databases or biospecimen informatics systems should track, or have linkage capabilities to systems that track, a single biospecimen through all aspects of collection, processing, storage, dissemination, return, depletion, and disposal.

B. Biospecimen informatics systems should track associated clinical data and/or link to external sources of clinical data, where applicable.

C. At a minimum, it is recommended that biospecimen acquisition date and current availability status be tracked and linked to the Neuropathology Data Set , the Uniform Data Set , and the Biomarker and Imaging Database, where applicable.

a. Informatics systems should have linkage capabilities such that the physical tube or label of specimen containers or slides is linked to additional data on that specimen in the system.

b. It is recommended that each biospecimen be assigned a unique identifier in the form of a barcode and/or other identifying number.

c. Specimen ID format and database structure should be capable of tracking derivatives, aliquots, and mother/daughter sample relationships.

II. Data Procedures

A. Informatics systems should be capable of generating a report or files of current biospecimen availability that could be uploaded to NACC to keep the data current.

B. Informatics systems should be flexible and adaptable to add new biospecimen collection or processing protocols and data upload specifications as new specimen types are collected.

III. Sharing and dissemination of data

A. Center-specific guidelines that incorporate best practices for the dissemination of identifiable, de-identified and anonymous data, including genetic and biomarker data, are recommended to be established and adhered to for all data requests from academic and non-academic collaborators.

B. Policies and procedures for requesting ADC resources should be published on each Center's website.

C. ADCs should document and archive researcher requests for biospecimens and clinical data as well as the review outcome, and if possible, resulting publications with attribution to their grant.

IV. Quality Control, Security and Regulations

A. It is recommended that informatics systems document and monitor measures of biospecimen quality.

B. 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.

C. 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.

D. Database write access is recommended to be limited to key authorized users and only from trusted Internet addresses.

E. Tiered-access should be specified to allow definition of “authority levels” for accessing and updating of data, particularly identifiable and genetic information is recommended.

F. Range checks and logical error checks are recommended for data, and as a quality control measure, errors should be flagged back to a user and disallowed entry into the database until repaired.

G. Authorized data transfer is recommended to be protected via strong encryption capabilities through a secure Web or FTP site; data transfer via email is unacceptable. A minimum of a 128 bit encryption suite is recommended for web sites.

H. 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.

I. All information on a patient should be linked within an ADC by a common ID. This should be accomplished through an overall relational database design that allows creation of this ID at patient entry into the ADC.

V. System Support and design

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 locally under the direction of a committee that may include database users and investigators, bioinformaticians, statisticians and software engineers.

References:

1. National Cancer Institute, NCI best practices for biospecimen resources, 2011 (NCI Best Practices website: <http://biospecimens.cancer.gov/practices/>; PDF of the NCI Biospecimens Best Practice: <http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf>)

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

a. Beneficence

1. Risks and benefits of the research should be discussed
2. Wherever possible, research risks should be minimized such as by linking data gathering to clinical care and procedures.
3. Research risks should be appropriate to the importance of the knowledge that can reasonably be expected to be gained from the research.

b. Respect for persons

1. Achieving informed consent for the research
2. Voluntariness of consent is achieved (e.g. giving potential subjects time to consider their decision)
3. Issues of privacy and confidentiality should be covered as appropriate to the research
4. 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.
5. Surrogate consent for research may be utilized in compliance with federal law, state statutes and IRB requirements and guidelines.

c. Justice

1. 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 on the research participant

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
- a. 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
 - b. 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
- a. General use of samples and data for future research
 - b. If appropriate, DNA samples can be shared with NCRAD (National Cell Repository for Alzheimer’s Disease @ Indiana University); the NCRAD sample acquisition committee provides guidance on whether particular studies or sample sets should be brought into NCRAD for distribution
 - c. 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
 - d. Potential for sharing of data and specimens with non-government, non-academic investigators should be addressed, with potential for development of commercial products
 - e. ApoE genotyping results and stored DNA to be shared
 - f. Local standardized specimen language for sharing of specimens, commercial value, etc., must be included in the site-specific informed consent document
 - g. Risks of privacy and confidentiality, as they exist per protocol, and as applicable to the retention of identifiers with the specimens
 - h. Acknowledgement of the absence of direct benefits to participants
 - i. Acknowledgement of potential benefit to society
 - j. A policy / procedure for handling of withdrawn consent
 - k. Storage of data generated from biospecimens is recommended to be addressed with appropriate regard to security measures (encryption, coding, limited access to database, etc.)

I. Whether subjects will have access to their individual results or to group results (such as via a newsletter or presentation).

References

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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

a. 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.

b. 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:

1. Scientific validity of the research proposal
2. Assessment of burden or demand on the tissue resource and staff
3. Investigator’s agreement covering confidentiality, use, disposition, and security of biospecimens and associated data
4. Investigator’s written agreement in a Material Transfer Agreement to comply with the NIH Research Tool Guidelines²
5. Investigator and institutional research qualifications
6. Ethical oversight where required by Federal regulations or local institutional requirements
7. 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, and 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.

- c. Apply guidelines to all new collections and, whenever possible, to existing collections.
- d. If applicable and where monetary charges are necessary, charge only to recover costs as appropriate to retrieve and disseminate specimens (see Cost Recovery Guidelines).
- e. 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.
- f. 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

a. 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. Use enough dry ice (or refrigerant) to 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 dry ice (or refrigerant) in case of shipping delay. It is also recommended that shipment days be chosen to avoid inherent problems with weekend arrival of specimens and the same consideration for holidays.

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.

b. 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

a. 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.

b. 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 the shipment. A feedback questionnaire regarding quality of samples received may be intermittently included for QA/QC.

E. Regulatory considerations

a. Consult ISBER Best Practices (ISBER 2012) for information regarding international transport regulations and classifying biospecimens for shipment. For packaged biospecimens, International Air Transport Association (IATA 2014) 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

a. 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:

a. The number and types of biospecimens

b. If available, the manner of collection, fixation, storage, and the accessibility to clinical and pathologic phenotypic information.

c. 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).

d. 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 (*e.g.*, 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.

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COST RECOVERY GUIDELINE:

General Comment/Justification:

Alzheimer’s Disease Centers (ADCs), with their cores, are funded to provide the infrastructure to support both Center-affiliated research projects and non-affiliated research projects. In general, ADCs do not charge investigators for receiving biospecimens to the extent that NIH Center budgets cover these costs. However, there are exceptions 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 laboratory would be forced to abandon other Center duties in order to prepare the samples. In these situations it is appropriate, allowable, and recommended that Centers develop cost recovery policies to deal with demands when they exceed the budgeted support of the Center. A guiding principle is that no cost recovery program should diminish the essential goal of providing human biospecimens to fuel research into Alzheimer’s disease. The best practice guidelines outlined here are intended to apply to ADC specimens and not necessarily specimens collected through other mechanisms. The following recommendations may guide each Center’s development of cost recovery policies.

Definitions:

Program Income is defined as **gross** income—earned by a grantee, a consortium participant, or a contractor under a grant—that was directly generated by the grant-supported activity or earned as a result of the award. Program income includes, but is not limited to, **income from fees for services performed, and charges for research resources**. The amount of program income earned and the amount expended must be reported on the annual financial report. Any costs associated with the generation of the gross amount of program income that are not charged to the grant should be deducted from the gross program income earned, and the net program income should be the amount reported.¹

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. Cost recovery does **not** refer to the sale of the specimen itself. There can be no overlap between specific expenses paid by the grant and paid through program income/cost recovery.

Program Income Alternatives: NIH allows four program income alternatives (additive, deductive, combination and matching). The NIA P30 and P50 Alzheimer Disease Center grant mechanisms dictate use of the combination alternative in their Notice of Grant Award under the section titled: “Treatment of Program Income.” The combination alternative states that in each budget year: General Program Income is to be treated as follows: The first \$25,000 under the Additional costs Alternative; the remainder under the Deduction Alternative. This statement means that with careful accounting and reporting, Centers may receive and retain payment for their biospecimen services up to \$25,000 after subtracting expenses not covered by the grant. Any income (after subtracting expenses) received over that \$25,000 will result in the grant award being reduced by the amount over \$25,000.

Recommendations for Best Practices:

Cost recovery policies are subject to scrutiny and should be developed in accordance with Office of Management and Budget (OMB) Circular A21 principles (see Note at end of section). All institutions should work closely with their own

Sponsored Projects Accounting offices and their NIH Grant Management Specialists in developing policies and procedures to comply with the NIH Grants Policy Statement.

Determining and Setting the charges for Biospecimen services:

1. Charges or fee schedules for biospecimen services should be based on fair estimates of the actual effort (salary and fringe benefits) and costs (supplies, shipping, etc.) associated with sharing tissue. These charges should include all costs associated with the service that are not covered by the grant award and should be reviewed at least annually and revised to assure that charges are covering excess costs.
2. Sound cost accounting principles should be employed when setting charges. Personnel costs (salary and fringes), supplies, shipping, equipment rental, and other services (e.g., genome sequencing) are examples of costs that can be considered when setting charges.
3. Overhead can ONLY be included in cost recovery charges when applied to services provided to an external client (not to users within your institution).
4. Cost recovery may not be limited to the laboratory providing the specimen. Often the burden on data managers to identify appropriate samples based on inclusion-exclusion criteria is significant and cost recovery may also apply to the Data Management Core.
5. Cost recovery income should be directed toward support of the operations of the unit expending the effort, i.e., the income should not subsidize an unrelated function or laboratory.
6. A tiered system of charges may be considered. That is, the charge may 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).

Tracking and Reporting of Program Income

Perhaps the simplest method of tracking program income is creation of a program income fund where costs in excess of what the grant can cover (personnel, supplies, etc.) are charged and income from services rendered can be applied. The balance at the end of the budget year will determine which "alternative" is used: Additional or Deductive. The activity on this fund would be the basis of reporting program income on the institution's annual financial report to NIA. If an ADC has more than one core laboratory generating program income or if a laboratory is funded for similar activities from multiple grants that stipulate different program income alternatives, tracking will be more complicated. The \$25,000 limit on net income is for the entire grant

Reference

- (1) NIH Grants Policy Statement (10/11), Part II: Terms and Conditions of NIH Grant Awards, Subpart A: General, 8 ADMINISTRATIVE REQUIREMENTS, 8.3.2 Program Income.

Note:

At the time of preparing this document the OMB is preparing to release the Uniform Grant Guidance policy statement that establishes uniform cost principles and audit requirements for all Federal awards to non-Federal entities and administrative requirements for all Federal grants and cooperative agreements. Once this policy is released it will supersede the (OMB) Circular A21 principles.

INTELLECTUAL PROPERTY GUIDELINE

Note: This guideline refers to inventions, and is not meant to address authorship issues. The need for intellectual property protections is likely low for biospecimen transfers from Alzheimer’s Disease Centers where tissue collection has been standardized. Such tissue does not represent a unique resource that was created, engineered or invented (e.g. an investigator would be unlikely to ‘patent’ blood samples). The NIA endorses the least restrictive policies when it comes to sharing biospecimens.¹

A. Recognize that as custodians of biospecimens, biorepository faculty and staff members 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 define and 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, 2011 (NCI Best Practices website: <http://biospecimens.cancer.gov/practices/>; PDF of the NCI Biospecimens Best Practice: <http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf>)

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; it is less detailed and usually does not contain indemnification language.

Why MTA/SLA? 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 that make them useful. Most academic and government research institutions have technology management offices that govern/negotiate the material transfer agreements of their faculty and staff scientists. Such offices often have institutional templates, which usually can be customized for the individual material transfer (see Item I. below).

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, 510 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.

Regulatory Issues Related to Material Transfer: Compliance with federal, state and local laws regarding confidentiality of research participants can also be assured in MTAs. Institutions now routinely review the Institutional Review Board approvals under which tissue was collected to determine if the sharing is permitted. The data that accompanies tissue may often contain Protected Health Information even when the usual identifiers (name, SS#, etc.) are removed. Providing coded data (a non-informative identification number) and a date (date of blood draw, date of expiration, date of birth, etc.) may require use of data use agreement (executed between institutions) under the Health Insurance Portability and Accountability Act (HIPAA). If no dates are required to analyze the tissue, then a simpler code access agreement (executed between individual scientists) may be possible (and usually will render the use of the material as non-human research).

Recommendations: The following outline contains items/issues that are often addressed in MTAs. Although individual academic institutions determine the final elements of the MTA, the NIA Biospecimens 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.
- 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. 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.

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. 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 (e.g., a warning should be included for tissue that has not been tested for infectious agents). However, the MTA need not be the only place such warnings are provided.

F. Restrict any further dissemination without notice – A recipient scientist cannot provide materials received under the MTA to a secondary investigator without written approval. 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. However, it is recommended that the least restrictive language be used in sharing tissue.

I. MTAs/SLAs can be modified for unique situations and requirements. 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 that contains experimental results obtained from the use of the Material. Any publication using this Material must follow NIH Public Access Policies (e.g. a PubMed Central ID)
- 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:
 1. Acknowledge the Center grant number P50AGXXXXX or P30AGXXXXX in all publications using the Material.
 2. Adhere to NIH Public Access Policies and provide PubMed Central ID numbers for all publications.
 3. 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://www.gpo.gov/fdsys/pkg/FR-1999-12-23/pdf/99-33292.pdf>
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/Technology_Transfer_Resources/8395.htm
5. National Cancer Institute, NCI best practices for biospecimen resources, 2011 (NCI Best Practices website: <http://biospecimens.cancer.gov/practices/>; PDF of the NCI Biospecimens Best Practice: <http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf>)