

BEST PRACTICES FOR THE ALZHEIMER'S DISEASE RESEARCH CENTERS

BRAIN GUIDELINES

All laboratories should review current consensus-based recommendations for tissue collection, processing, storage, retrieval, and dissemination as well as for histologic methods and any other tissue-based assays. A relevant document with broader recommendations has been developed by NCI at: <https://biospecimens.cancer.gov/bestpractices/>.

The following guidelines represent current best practices for establishing and maintaining standard operating procedures (SOPs) for a research brain bank focused on Alzheimer's disease (AD) and related neurodegenerative and cerebrovascular diseases.

I. Infrastructure requirements

A. Autopsy considerations

- i. Usual practice for research brain banks is brain autopsy only; however, to the extent possible, including spinal cord or full autopsy should be considered and requested, albeit recognizing that additional resources may be required.
- ii. Staffing of a 24/7 on call autopsy coordinator, autopsy technician(s), and tissue bank technician(s) is recommended so that collection can occur as rapidly as possible after death.

B. Data management

- iii. Autopsy related data are essential to maximize the research usefulness of brain donations.
 - For each case, minimal recommended documentation includes time of death, post-mortem interval, and if available information related to agonal state (e.g., fever, days on ventilator)
 - Additional relevant parameters include study participant's sex, race, ethnicity, *APOE* genotype (if the individual is consented for genotyping), age at death, relevant family history, medication history, diagnosis(es) of brain diseases, other diagnoses, duration of illness(es). Most of these variables should be previously collected through ADRC clinical cores.
- iv. Responsibility for obtaining additional information is commonly carried out independently of brain banking operations; no matter where it is housed, proper database linkages to other ADRC cores (Clinical, Imaging, Biomarker, etc.) should be in place and overseen by the Data Management and Statistics core. Databasing this information may also occur within the brain bank operation, or some other component of the research group. Corroborating data that include biomarker results and genetic data (if available) should be able to be cross-referenced. For all these reasons, having regular meetings between NP core workers and representatives of other ADRC cores is strongly encouraged, because such data may inform the neuropathologic diagnosis. See also **Section IV. B.**, below.
- v. For more on databases, sample storage, and sample tracking infrastructure, see **Section III.**, below.

C. Freezer management

- i. Freezers should be located in designated rooms with independent, high-quality HVAC systems that are relatively safe from potential natural or human-made disasters.
- ii. HVAC units should be placed on outlets with access to a backup generator.
- iii. Freezers, HVAC units, and ambient temperatures should be monitored by a central monitoring system.
- iv. Alarm notifications should be sent to the biobank coordinator via email and phone call, 24/7.
- v. Back-up personnel should be in place for instances when the biobank coordinator is unavailable.
- vi. Back-up, independent monitors should be in place in freezer rooms.
- vii. Routine maintenance is required on freezers to clear ice, clean filter, and condenser. Freezers should be shut down periodically to defrost.
- viii. Maintain a minimum of two back-up freezers for emergencies and yearly shutdowns. Freezers generally have lives of 6-15 years in duration. For a long-term biobank that expands over time, this means that there needs to be resources set aside for freezer replacement and maintenance.
- ix. Measures should be taken to protect the biospecimens' security—controlled access to the building, the rooms, and/or the freezers. (See **Section IV. E.**, below).

- x. When needed, freezers should be able to be relocated to places with comparable infrastructure regarding monitoring, power management, and controlled access.

II. At time of autopsy

A. Safety and regulatory issues

- i. Autopsies must be performed according to local approved IRB protocols (with valid consent protocols), as well as in compliance with all hospital, municipal, state, and federal laws and regulations.
- ii. Explicit permission for genomic studies as part of the autopsy consent (See Best Practices document on Consent and Confidentiality) is encouraged to ensure broad utility of collected materials. Since many of the cases will have pathological diagnoses other than AD per se, it is important to acknowledge non-AD dementias and controls are incorporated as valid study participants.
- iii. Always use at least universal precautions when handling human tissue or body fluids (Administrative and engineering controls should be used in addition to personnel protective equipment).
- iv. 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. Alternatively, some centers refer cases where prion disease is suspected to the National Prion Disease Pathology Surveillance Center.
- v. For COVID-19, institutional, state, and federal recommendations should be followed. Implementing COVID-19 screening questions (i.e. about agonal cough and fever, a COVID-19 test proximal to death, COVID-19 exposure, etc.) on intake forms is recommended but definitely imperfect in terms of predicting infection status. Workflows should be established through discussions with all stakeholders (autopsy technicians, coordinators, study PIs, neuropathologists, etc.), minimizing exposure to all, but especially, at-risk staff.

B. Autopsy protocol

- i. Minimal data should be gathered as described in **Section I.D.**, above.
- ii. Tissue block sampling should follow current NIA-AA guidelines (1, 2). Paraffin-embedded tissue blocks should be archived and stored in a temperature-regulated environment with safeguards against physical damage, temperature changes, severe weather, and natural disasters for as long as possible, but at minimum 10 years depending on research needs or regulations.
- iii. Obtaining a portion of cerebellar hemisphere sufficient to fill a tissue cassette from every case and to store at -80°C as quickly as possible is recommended for potential future DNA preparation (if the consent allows).
- iv. Best practice is to establish protocols to dissect and freeze, as quickly as possible, selected brain regions for potential future biochemical and genomic analyses. A variety of methods can be used and the details depend on the desired use of the tissue. Examples are flash freezing tissue in liquid nitrogen, cold isopentane, or between blocks of dry ice. Freezing the brain intact (i.e. whole hemisphere) is not considered best practice because of difficulty in subsequent dissection.
- v. Support of collaborative research is a best practice. Additional brain samples and additional methods for optimal stabilization for specific assays should follow documented protocols.
- vi. Postmortem cerebrospinal fluid (CSF) may be collected usually from the ventricular system, if adequate resources are available. If it is, then best practice is to centrifuge, aliquot, and freeze at -80°C in appropriate containers based on expected use (see CSF section) and to thaw only once for use. Furthermore, denoting any potential contamination (with blood) is recommended.

III. After autopsy

A. Tissue-relevant procedures and reporting

- i. Histologic and immunohistochemical staining of standard tissue blocks should follow current NIA-AA guidelines (1, 2), which include proscribed sections for A β and phospho-tau immunohistochemical (IHC) evaluation.
- ii. IHC workup should be performed routinely with recommendations to perform TDP-43 staging in amygdala, hippocampus, and middle frontal gyrus (3).
- iii. α -Synuclein IHC workup should also be performed (4, 5) as stated in the NIA-AA guidelines.
- iv. Assessing age-related tau astrogliopathy (ARTAG) using p-Tau IHC in amygdala and middle frontal gyrus is recommended but not obligatory (6).
- v. Notably, at present there is not a list of specific antibodies that are required to be used for IHC on ADRC autopsies. Additional methods including thioflavin-S staining and silver impregnation remain useful for assessing lesion burden and distribution.
- vi. Standard neuropathologic data for all ADRC study participants must be reported to the National Alzheimer Coordinating Center using the most recent Neuropathology Data Form (<https://naccdata.org/data-collection/forms-documentation/np-11>) in a timely manner. Integration with other ADRC cores (specifically those involved with databasing) to aid in this endeavor is encouraged.

B. Storage and inventory

- i. All biospecimens should be stored in appropriately labeled containers with unique identifiers in compliance with HIPAA and in a regulated environment with safeguards against physical damage, temperature changes, severe weather, and natural disasters. Histologic slides used for diagnosis and paraffin-embedded blocks should be archived for as long as possible, but at minimum 10 years depending on research needs or regulations.
- ii. It is best practice that all biospecimens are stored in a manner that meets universal precautions for human infectious material, IRB oversight, employee health safety regulations, permits further neuropathologic evaluation if needed, and optimizes future potential research use.
- iii. Best practice is to **maintain an accurate and appropriately safeguarded inventory** of accrued biospecimens, distributed biospecimens, disposal, and available tissue and fluid resources. **For reporting purposes, tissue requests and sharing should be tracked and documented.** Collaboration with the Data Management and Statistics core is encouraged and allows for optimal linkage of this information to other data sources within the ADRC.
- i. 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. This arrangement must be IRB compliant and meets the need for study participant's confidentiality, security, and informed consent provisions (see Informatics Guideline). Integration with other ADRC cores, such as Administrative and Data Management and Statistics cores are necessary.
- ii. On an ongoing basis, some efforts should be made to audit the integrity of the biobank and the efficiency of storage.

C. Ethical considerations and tissue sharing

- iii. In addition to scientific advisory committees for the research group, a brain bank should regularly convene a **Biospecimen Use Committee** for oversight of biospecimen requests and/or be involved in institutional committees focused on biospecimens. This may be subsumed as part of the ADRC Executive Committee.
- iv. For specimen sharing, data use, and collaborative agreements should include proper acknowledgement of funders etc. for all persons receiving samples and/or data, including those within the same institution, and some efforts should be made to help clarify proper use of the samples/data. It is prudent to use material transfer agreements (MTAs) and/or data use agreements (DUAs), particularly when the biosamples/data are shared with outside institutions. When possible, **results should be returned to be connected with the rest of the data for those samples.**
- v. Shipping of human biospecimens must be done with care, which factors in potential for spoilage, and use appropriate packaging, and in accordance to local and federal regulates. A description of some considerations is present here: <https://biospecimens.cancer.gov/bestpractices>.

IV. Long-term considerations

- A. Digitization of diagnostic slides** should be considered if resources are available. If digital pathology slides are shared, protected health information should be removed from the linked file name or as part of the microscope slide label. For further details on digital slide recommendations please see the Best Practices Digital Neuropathology document.
- B. Participating in the ADRC research volunteer community.** NP Core should integrate with Clinical and ORE Cores to convey the importance of brain autopsy and this may involve direct interactions with research study participants at ADRC conferences, health fairs, etc. In addition, the NP Core should also work with the Research and Education Component to provide opportunities for students and fellows. NIA has the following resources available to assist:
- Brain donation resources for ADRCs
 - <https://www.nia.nih.gov/health/brain-donation-gift-future-generations>
 - View the Brain Donation Social Media Toolkit
 - ADORE brain donation items
- C. The ethics of cost recovery** (See Best Practices Cost Recovery document) needs to be critically assessed when disbursing human biospecimens. There is special potential for these issues to become ethically, and indeed legally, problematic when money changes hands. Acknowledging this, institutions, departments, and individuals will gain support for their biobanks using many different financial models. Within a given biobank, there can be differing protocols for how different subtypes of samples (as well as professional and technical services) are provided for academic and private end-users. There is not a “one-size-fits-all” solution for any of these contexts. Disbursals to private companies may be worthwhile, but attention should be paid to data integrity as biobanks often have access to data from their own samples. Consideration of an on-site collaborator who is granted such access may help to facilitate. The type of sharing of specimens (to non-profit and/or for profit etc.) should be clearly indicated within the autopsy consent. Given the lack of clear and proscriptive rules that apply in this area, biobanks should team with IRBs and oversight consulting groups (e.g., [project-specific executive committee](#), [formal advisory committee \[internal or external\]](#), [research subject advisory council](#), or [other campus-specific consultative/oversight group](#)) to ensure long-term compliance with broader ethical and legal standards.
- D. Procedures and processes to prevent catastrophic loss of stored specimens** are a key priority for repositories, and the best policy is a proactive one. In the event of a disaster, the most likely short-term peril is failure of electricity and/or freezers (See **Section I.C.**, above), either individually or in a room- or building-wide electrical or HVAC failure. Floods, fires, and other sudden causes of damage are also possible. To prevent these events from happening requires a high level of vigilance (e.g., HVAC and freezers alarmed with 24/7 monitoring as described above) and financial backing. Other emergency possibilities include natural or human-made catastrophes such as computer failure or some other scenario of widespread chaos. To optimize outcomes in these situations requires pre-planned emergency SOPs and hardware redundancy (and automatic safe storage).
- E. Long-term biobank protection and optimization**
- Document control software can be used to generate and update SOPs and can be used for administrative (including IRB) requirements.
 - An organizational chart can be a significant tool in supporting existing governance structures through elucidation of roles, responsibilities, chain of command, and requisite reporting relationships.
 - See <https://biospecimens.cancer.gov/bestpractices/>
 - Biobank audits
 - Specimen tracking and inventory quality control (QC) should be built into the biobank plan and included in the IRB.
 - QC and inventory cross-referencing (with the database) can dovetail with sample disbursals
 - Ongoing random sampling can help evaluate and optimize inventory
 - Periodic deeper audits may be executed to verify tissue location and quality, and to optimize tissue allocation for best efficiency.
 - End of the biobank life-cycle (also see Best Practices Disseminating and Discarding document)

- Should a brain bank not have sufficient resources to continue and/or plans are underway to shut it down, the relevant NIH program officer should be consulted to facilitate dissemination of tissue to local and/or qualified ADRC brain banks or to NCRAD or Neurobiobank.
- Different biobanks have their own time windows but some are very long-lasting and plans should be made for eventually handing over control from one group of individuals to others.
- It is important to have it be well understood that the biobank is ultimately the property and responsibility of the parent institution, rather than any individual or department.
- Ethically (that is, to be consistent with the spirit and wording of the original participant consent language) and scientifically, it may be appropriate to move the biobank across institutions under rare and unusual circumstances. This is not a trivial endeavor, may have legal repercussions and should only be done following extensive and transparent consultations with all interested parties.
- It is easiest to integrate these concepts early in the biobank's life-cycle, and reconcile various components of the study design.
 - Involve IRB early in this process.
 - Consent form must reflect the ultimate governance is the University, not that of an individual.
 - Ensure there is a SOP for "unspecified further use" of the samples to the extent allowed by the IRB.

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