DPUK Work Package 13: Brain Donation

Agreed Standards for Collection, Classification & Storage of Brain Tissue

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1. Introduction

This report relates to Dementias Platform UK (DPUK) Objective 3 of Work Package 13: Brain Donation. Specifically, objective 3 is to develop agreed standards for the collection, classification and storage of brains tissue. Harmonisation of procedures across brain banks enables i) comparable standards of quality and diagnostic categorisation to be employed, ii) implementation of comparable sampling, preparation and storage techniques and iii) provision of consistent key tissue quality and pathology data. Consistent with the diversity of researcher requests, it is also necessary to preserve a sufficient degree of operational flexibility in accordance with the nature of the disease and user demand. In such instances, a rationale and explanation of any differences can be provided, or where appropriate, a view presented as to when convergence might be achieved in the future.

The policies outlined below are those agreed by the 4 Medical Research Council (MRC) funded brain banks as part of a milestoned funding package and are used as a minimum protocol by all the banks in the MRC UK Brain Bank Network (UK BBN). Minimal standards for collection are presented in relation to i) Brain tissue, ii) Clinical and Neuropathological information and iii) Neuropathology (Classification & storage).

MRC UK Brain Bank Network	BDR Network*	MRC Funded
South West Dementia Brain Bank	✓	
Edinburgh Brain and Tissue Banks		✓
Newcastle Brain Tissue Resource	✓	✓
Thomas Willis Oxford Brain Collection	✓	✓
London Neurodegenerative Diseases Brain Bank	✓	✓
Cambridge Brain Bank		
Manchester Brain Bank	✓	
Queen Square Brain Bank		
Sheffield Brain Tissue Bank		
Multiple Sclerosis and Parkinson's Tissue Bank		

*A further BDR centre is located at the University of Cardiff. This site is responsible for participant recruitment and assessment in Wales but is not a brain bank.

2. Brain tissue: Processing & dissection protocols & diagnostic procedures

This encompasses agreed standardised protocols for sampling, tissue processing, and diagnostic procedures where possible. The protocol was developed by the MRC Brain Banks and Brains for Dementia Research (BDR) Network and was submitted to, and approved by, the MRC Neuroscience Mental Health Board (2014).

In summary:
(a) All banks are considering moving towards nitrogen vapour for freezing to preserve cytoarchitecture to support cell-specific studies.
(b) All banks adhere to a core sampling protocol in harmony with the BDR network.
(c) All banks are using identical quality measures which are available to researchers on the MRC network website (pH, RNA integrity numbers).
(d) Differences exist in the extent of sampling of paraffin-embedded blocks. This relates largely to the restriction of whole brain retention in one of the banks (Edinburgh).

(e) All banks provide neuropathological diagnoses based on internationally accepted, standardised criteria thus allowing for consistency in nosological classification when samples from different banks are requested.

2.1 Sampling protocols

Sampling protocols are disease specific but there is a clear ethos between the banks supporting a standardized core set of tissue regions available from all cases, the core samples reflecting BDR recommended core samples. All banks sample both fresh brain tissue and formalin-fixed brain tissue.

2.1.1. Core small samples

Core small samples (particularly suited to DNA extraction, in small tubes

a. Frontal (BA46)
b. Temporal (BA21)
c. Parietal (BA5/7)
d. Occipital (BA19)
e. cerebellum

2.1.2 Core frozen samples

a. Frontal (BA46)
b. Cingulate (BA32/24)
c. Anterior hippocampus
d. Striatum
e. Superior temporal (BA41/42)
f. Posterior hippocampus
g. Thalamus
h. Posterior parietal lobule (BA39)
i. Primary visual cortex (BA17)
j. Cerebellar cortex
k. Midbrain
l. Pons
m. Medulla
n. Spinal cord (cervical, thoracic, lumbar)

Discussion is taking place with UK Brain Bank Network colleagues to resolve issue of which side to sample

2.1.3 Core diagnostic samples (FFPE)

Core diagnostic samples (FFPE) taken from cerebral/cerebellar hemispheres

a. Frontal (BA46)
b. Cingulate (BA32/24)
c. Superior temporal (BA41/42)
d. Amygdala
e. Anterior hippocampus
f. Posterior hippocampus

g. Striatum
h. Thalamus
i. Posterior parietal lobule (BA39)
j. Primary visual cortex (BA17)
k. Cerebellar cortex
l. Midbrain
m. Pons
n. Medulla
o. Spinal cord (cervical, thoracic, lumbar)

2.1.4 Immunohistochemical on core blocks for staging

Immunohistochemical on core blocks for staging; tau (AT8), β-amyloid (4G8), α-synuclein (novocastra), TDP43 (TARDBP Protein Tech Group)

In 3 banks, the whole brain is bisected with one cerebral hemisphere being sampled fresh. The fresh hemisphere is cut in coronal sections and all coronal slices including core frozen samples are then frozen using either nitrogen vapour or brass plates, and then placed in -80C freezer.

All banks are considering moving to nitrogen vapour for freezing for preservation of cytoarchitecture, anticipating future single cell technologies.

The opposite hemisphere is fixed in 10% neutral buffered formalin for between 4-6 weeks. Coronal sections are cut and the core diagnostic blocks are taken. There is no evidence to suggest that a variation between 4-6 weeks results in any meaningful differences to tissue quality for research purposes.

In 1 bank, the whole brain is cut fresh due to the consent available for the clinical cohorts being studied (organs are returned to the body for burial and no whole brains are retained). The core frozen blocks are sampled from the left hemisphere, and frozen using nitrogen vapour before being placed in -80C freezer. The core diagnostic blocks are sampled from the right hemisphere. These blocks are placed in 10% buffered formalin for at least 1 week and then processed. Again, there is no scientific evidence to suggest the shorter fixation period has any detrimental effect on standardized scientific protocols. The shorter period in fixative purely reflects the smaller volume of the tissue being fixed.

All the banks have more extensive sampling protocols for both frozen and FFPE tissues, the protocols often reflecting the needs of local research groups or specific requests received from national or international research groups. However, it was felt that the core samples would satisfy a majority of tissue research requests, and this will continue to be audited and reported by all banks.

2.2 Tissue processing protocols

For all banks brain tissue is fixed in 10% neutral buffered formalin. 3 banks fix whole hemispheres for approximately 4-6 weeks prior to sampling and processing, whereas Edinburgh fixes tissues in formalin for only 3-4 days as the tissue samples are considerably smaller and require much shorter fixation (2x2cm cubed tissue samples as opposed to a

whole hemisphere). Samples are then processed on a 3-day cycle. This is a standard cycle passing through alcohol (dehydration) and chemicals for removal of lipid. The protocols used for tissue freezing all currently use brass/ copper plates and liquid nitrogen for snap freezing. Nitrogen vapour is being trialed to compare cytoarchitecture preservation between techniques (2 banks).

2.3 Diagnostic protocols

All banks use standard diagnostic protocols when assessing brains. Diagnosis is based on assessment of the 13 core FFPE blocks, using standard grading criteria outlined below. Each block is stained with haematoxylin and eosin (H&E) and luxol fast blue/cresyl violet (LFB/CV). Immunohistochemistry is assessed using same antibody clones in each banks: tau (AT8); β-amyloid (4G8); α-synuclein (Novocastra); TDP43 (TARDBP Protein Tech Group).

For Alzheimer's disease- NIA-AA criteria (Montine TJ et al., 2012, Acta Neuropathol 123:1-11), Neurofibrillary tangle Braak stages (Braak H et al., 2006, Acta Neuropathol 112:389-404; Alafuzoff I et al., 2008, Brain Pathol 18:484-496)

For Lewy body disease- McKeith criteria for Lewy body disease (McKeith IG et al., 2005, Neurology 65:1863-1872) including modification by Leverenz (Leverenz JB et al., 2008, Brain Pathol18:220-224)

For FTD cases- Frontotemporal lobar degeneration (FTLD) is assessed according to: McKenzie IRA et al 2010, Acta Neuropathol 119:1-4; in case of FTLD with TDP-43 pathology according to: McKenzie IRA et al 2011, Acta Neuropathol 122:111-113

For white matter pathology- White matter pallor and small vessel disease in white and deep gray matter is scored as described in: Smallwood A et al., 2012, Neuropathol Appl Neurobiol 38:337-343

2.4 Tissue quality parameters:

Tissue pH and RNA quality as assessed by RIN values are used as tissue quality parameters across all the MRC Brain banks. pH is assessed using a standardized hand-held pH meter (the same meter is used by each bank) at the time of post mortem. pH values are taken from the frontal regions.

RIN values are determined in-house in 3 banks, and using an internal collaboration in London. An Agilent Bioanalyser system is used to determine this value. All banks use previously frozen (at -80C) brain tissue to assess RIN value, and prospectively only cerebellar tissue will be used.

3. Demographic, Clinical & Neuropathological information

Tissue from over 10,000 brains, from all ten UK brain banks is now catalogued in one central database – the joint MRC Brain Banks Network database. This is led by the Director of the UK Brain Bank Network, Professor Seth Love and a Database Manager Richard Cain. As this database will correspond closely to the information available through the DPUK data portal, this shared protocol may serve as a useful template that could be adapted for DPUK use. Indeed, a potential long-term option under consideration is a centrally hosted cloud-based system which links to the DPUK database, thus avoiding duplication of data entry for individual banks.

The UK Brain Bank Network has agreed that a minimal dataset be provided for all tissue samples. This includes ICD10 codes for all clinical diagnoses and a bespoke system for collecting and coding information on neuropathological findings (https://brainbanknetwork.cse.bris.ac.uk/UserPages/Pathology_Nested). This initiative will help to improve the detail and accuracy of recording of neuropathological findings within the database, to facilitate more precise and detailed searches, and to allow the database itself to be used as a research tool. Additional data may be available for participants included in specific cohorts and additional data requests can be made directly to cohort PIs. At present, genetic information is included in amongst the neuropathology findings but in the near future, genetic data will be relocated to a separate part of the database. Specific variables currently include:

Brain Bank
Network ID
Internal ID
Gender
Age
Post Mortem Delay
Report Date
Donation Date
Cause of Death
Brain pH
CFS pH
Clinical Diagnosis (ICD-10 codes)
Neuropathological Diagnosis (See ANNEX 1: Neuropathology hierarchy)
Assessments
Genotype

Additions and amendments to the minimum dataset are ongoing and reflect the needs of the scientific community accessing tissue samples. For example, in order to improve the value and usefulness of the dataset in relation to vascular dementia research, a number of largely cerebrovascular disease risk factors are currently under consideration for recording. These include: body mass; height; drug history (particularly in relation to antihypertensive drugs, antipsychotic drugs, antiepileptic drugs, cholinesterase inhibitors, memantine, L-dopa, dopamine agonists, COMPT inhibitors, MAO-B inhibitors); blood pressure data; smoking history; diabetes; hypercholesterolaemia.

4. Neuropathology (Classification & Storage)

The potential for an alternative neuropathological approach to sampling for brain tissue collected through DPUK was discussed at the MRC UKBBN Management Meeting (April 2015). A sub group (David Mann, Colin Smith & Johannes Attems) were asked to take forward separate discussions to formulate proposals as to the minimum sampling and assessment for DPUK brains.

In order to address the question of what regions of brain tissue should be stored for research use, Professor Seth Love (Director of UK BBN) obtained data from BDR and CFAS cohorts relating to the brain regions requested by researchers over a 3 year period ('Brain regions distributed by TRID' & 'Data for MRC-SBTB'). Requests during this time encompassed every possible brain region and on the basis of this evidence, Professor Love concludes:

i) Any restriction on the storage of tissue would have an adverse impact on the ability of brain banks to serve researchers.

ii) In time, it may be possible to streamline the assessment of brains by use of biochemical approaches (e.g. for measurement of α-synuclein, Aβ and phospho-tau) but those have relatively restricted application and, for the moment, remain unvalidated. The possibility of applying for funding for a study of this type of biochemical approach to post-mortem brain assessment has recently been the subject of discussion between the Director of the Network and the Director of BDR (Professor Paul Francis) and is being explored further.

iii) If anything, the emphasis seems to be on more extensive assessment of brains, particularly of those thought clinically to be normal.

One initiative pertinent to the possible centralised storage of brain tissue in the future is the development of the NIHR National Biosample Centre with facilities in Milton Keynes and Oxford. The purpose of the centre is to provide a high quality, high capacity service for biomedical researchers engaged in studies that include the collection, processing, storage and analysis of biological samples. Initial impressions indicate that the centre could be a useful resource for any at-scale storage of frozen aliquots of defined anatomical regions (but not necessarily for full brains). At this early stage, the centre is very flexible in terms of what could be accommodated. Currently, the centre offers block storage and will potentially provide a cutting service in the longer term. One advantage of this type of centralised storage facility would be the negation of the need for freezer back-ups, sample tracking and quality control systems at an individual brain bank level.