

The objective of the Netherlands Brain Bank (NBB) is to supply the international scientific community with clinically and neuropathologically well-documented brain tissue of high quality. Our ultimate goal is to increase the knowledge on how the human brain works and to make treatment of brain diseases possible, by means of creating a better understanding of the human brain and the etiology and pathological mechanisms of neurological and psychiatric diseases.

The specimens collected by the NBB are obtained from donors with a variety of brain diseases. These include Alzheimer's disease, Parkinson's disease, Lewy body disease, vascular dementia, Pick's disease, frontotemporal dementia, multiple sclerosis (MS), multiple system atrophy, progressive supranuclear palsy, and to a lesser extent Down's syndrome, amyotrophic lateral sclerosis, motor neuron disease, Huntington's disease, depression, bipolar disorder and schizophrenia. Furthermore, the NBB can supply samples from controls without neurological or psychiatric history.



Figure 1: NBB's collection of formalin-fixed paraffin embedded tissue blocks (left) and one of the NBB's -80 °C freezers where the frozen tissue is stored (right).

The NBB has an extensive donor program with which donors give informed consent during life for the use of their brain and medical records for research purposes. This enables a rapid transportation of the donor to the VUmc (Amsterdam) and guarantees extremely short post-mortem delays. Because of this procedure, tissue quality is very high and suitable for sensitive techniques applied for genomic and proteomic analyses, stainings and post-mortem cell and tissue cultures.

At every autopsy the pH of the cerebrospinal fluid (CSF) is measured as a marker of agonal state and as an important indicator of RNA integrity. Genotypes, like APO-E, can be provided in many cases.

Together with the material, the researcher receives a clinicopathological report and a summary of all important

medical files, including clinical diagnosis, cause of death and medical drug history. Furthermore, if requested, the samples can be matched for many antemortem parameters (e.g. age, sex or medication) and post mortem parameters (post mortem delay, time and season of death, pH, freezing or fixation procedure, storage time).

### Anatomical areas and tissue treatment

From each donor, the NBB collects approximately 90 different regions and CSF from the brain and sometimes the spinal cord. Depending on the clinical diagnosis and the actual applications of researchers the NBB dissects the brain tissue into anatomically defined structures (Figure 2). According to all protocols except MS, first the external structures such as basal arteries, the pituitary gland and the olfactory bulb are removed. Next, the medulla oblongata is separated from the brainstem and sampled together with the cervical spinal cord. Then, samples from the cerebellum are dissected and the brainstem is cut in half. The right side is put into 4% formaldehyde and from the left side the substantia nigra, locus coeruleus and dorsal vagus nucleus are sampled and frozen.

The cerebrum is cut in half and the right side is fixed in 4% formaldehyde. From the left hemisphere, the cortex is sampled first. From frontal, temporal, parietal and occipital lobes some parts are snap frozen in plastic bags or cryovials in liquid nitrogen and some are treated differently according to current applications. The hippocampus and amygdala are dissected as a whole, sampled as coronal slices and frozen as mentioned above

<b>Type of sample</b>	<b>Storage type</b>
Cortex (F, T, P, O)	Frozen
Hypothalamus	Formalin-fixed
Hippocampus	Paraffin sections
Cerebellum	Culture medium
Locus coeruleus	
Substantia nigra	
Dorsal vagus nucleus	
Striatum	
Spinal cord	
Dorsal root ganglia	
Olfactory bulb	
Normal appearing white matter	
MS lesions (active, inactive)	
MS lesions dissected on MRI guidance	
Cerebrospinal fluid	
Plasma	

Figure 2: Overview of the material that can be supplied or dissected upon request.

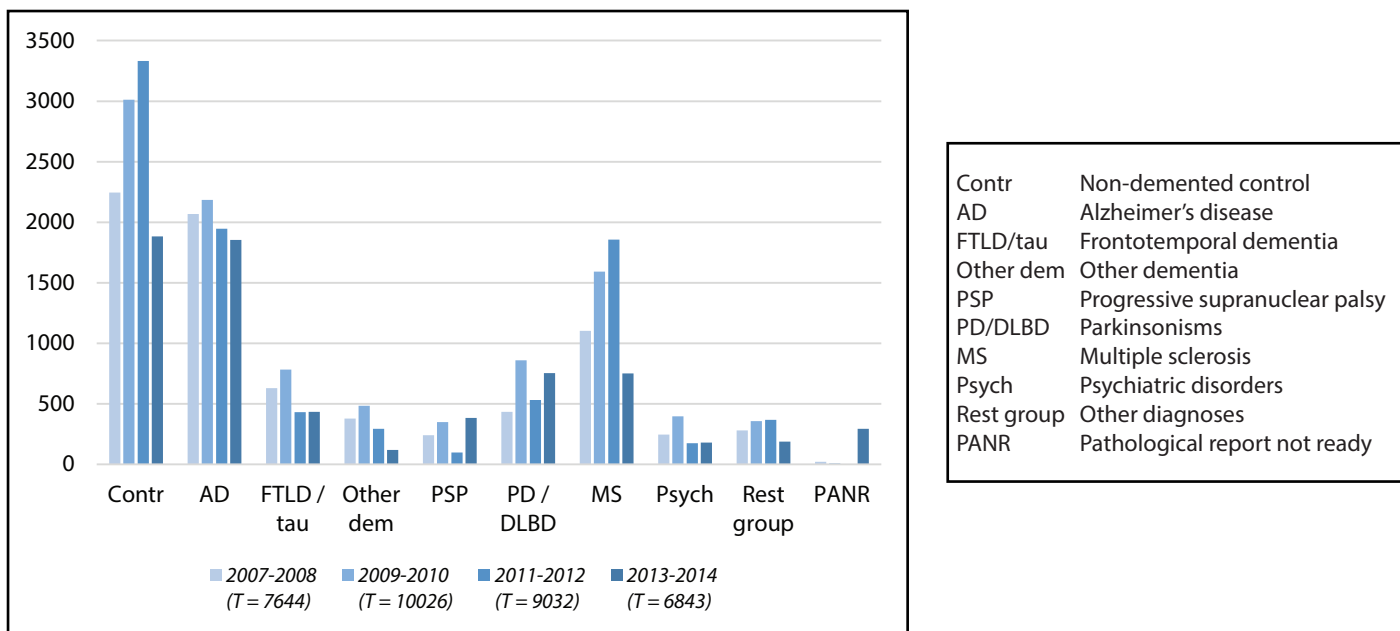


Figure 3: Supplied samples in 2007-2014, specified by neuropathological diagnosis.

for the cortex. Coronal cuts are then made to dissect the hypothalamus, thalamus and the basal nuclei and they are snap frozen in a metal box in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Finally, white and grey matter is harvested for tissue cultures.

When the clinical diagnosis is MS, a different protocol is used. The brain is cut into coronal sections of 1 cm and some of these sections are scanned using MRI technique to identify and dissect macroscopically invisible MS-related alterations. Tissue dissected on MRI-guidance as well as part of the macroscopically visible MS lesions are split. One half is frozen and the other half is fixed in formalin ('mirror blocks'). The remaining macroscopically visible lesions are frozen as a whole.

Of all autopsies, the tissue in formalin is dissected after 4 weeks of fixation into approximately 18 standard regions for diagnostic purposes. These structures are paraffin embedded, histochemically and immunohistochemically stained and offered to the neuropathologist to determine the neuropathological diagnosis. This way the NBB has performed autopsies on hundreds of donors (Figure 3, 4 and 5).

We can provide tissue from these stocks of frozen and formalin fixed (paraffin embedded) tissue (see Figure 1) or can dissect and treat tissue on specific request of the researcher in upcoming autopsies.

### Facts and figures

From 1985 until the present, the NBB has performed more than 4000 autopsies and due to our donor program the NBB has more than 4000 registered donors.

### The procedure of tissue application at the NBB

In order to facilitate researchers in their tissue request, the NBB has introduced the eNBB: the online database

of the NBB, accessible to registered researchers. As a registered user, you can search all NBB samples, with either diagnosis or brain region as a starting point.

To apply for tissue at the NBB, a tissue application form has to be filled in. In the application form, the applicant should indicate what kind of tissue is needed for which research project. The number of donors and diagnosis have to be specified and it has to be indicated how the tissue needs to be treated. In case tissue has to be dissected in future autopsies, please bear in mind that the NBB performs about 100-120 autopsies per year. The Application Form can be downloaded from the eNBB. Applicants can first contact the NBB for specific information on tissue and methods needed to address their research questions.

Every application is reviewed by an independent scientific committee, on availability of the material (diagnosis and anatomical area) and scientific quality. If the application is approved, the NBB will determine the financial contribution for the researcher that is meant to compensate for part of the costs made by the NBB.

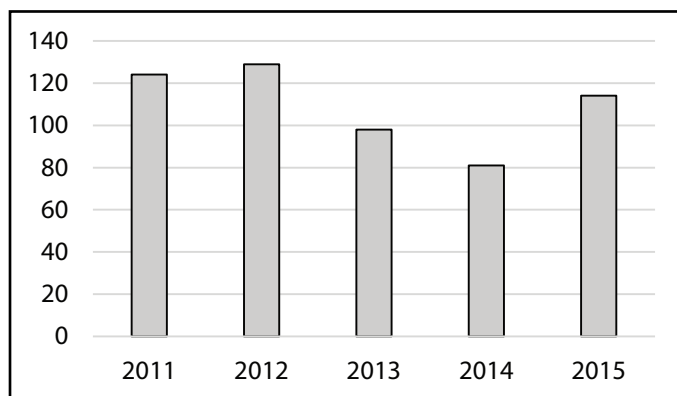


Figure 4: The total number of autopsies in the last 5 years



Figure 5: Brain sections for diagnostic purposes.

When supplementary samples are needed to continue a research project that has already been approved by the NBB's scientific committee, the researcher can send us the Supplementary Application Form, also available on the eNBB, explicitly mentioning the project number (see also the flowchart on page 3).

A supplementary application does not need to be reviewed again by the scientific committee, as long as it concerns similar tissue and the application is compatible with the original one, but the supplementary application does require a new financial contribution.

To ensure that all conditions for the transfer and the use of the material are agreed upon by the receiving and the providing parties, it is essential that a Material Transfer Agreement (MTA) for an indefinite period of time is signed. The MTA must be signed by the person authorized by the university or institute where the researcher is working, to sign official documents (head manager or managing director). There will be one MTA per research institute (legal entity), which will cover all legal terms of delivery. Based on this MTA, all researchers within the research institute may apply for tissue.

After a research application has been approved, an MTS (Material Transfer Statement, a clear summary of the MTA) will be sent to the researcher together with an Implementing Letter (letter of approval). The Implementing Letter specifies the material that will be supplied and the financial contribution. The MTS informs the researcher about the main issues dealt with in the MTA that was signed by his research institute/university. Upon receipt of the Implementing Letter and MTS, both signed by the applicant, the material will be supplied. The financial contribution is necessary because the NBB almost completely depends upon grant money and is based upon the integral cost price of the material. To a small extent it depends on extra services (e.g. MRI-guided dissection or special diagnostic services). It is possible to request an estimation of the financial contribution, which will be based on the requested material.

When there is a cooperation between the applicant and a for-profit organization, there will be different guidelines to determine the contribution.

## Ethical and legal issues

The NBB adheres to strict ethical guidelines such as informed consent, open access, and non-profit policy, as stated in BrainNet Europe's Ethical Code of Conduct for brain banking<sup>1</sup>. This Code was drafted by the NBB and signed in June 2008 by the partners of BrainNet Europe (BNE, a consortium of European brain banks). The Code was published in 2015. It is based on globally accepted bioethical principles, laws and guidelines. The Code of Conduct and the NBB's ethical declaration can be downloaded from [www.brainbank.nl](http://www.brainbank.nl).

## More information

We hope that this brochure contains all necessary information you need before you apply for brain tissue. In case you wish to receive more information, you can visit the NBB's website or contact us concerning your application.

You can contact Dr. Inge Huitinga, director of the NBB, concerning scientific issues such as the best suitable experimental approach. Our technical coordinator Michiel Kooreman is able to give advice on the availability of samples, matching issues and all other related questions.

## Contact

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<sup>1</sup> Klioueva, N. M., Rademaker, M. C., Dexter, D. T., Al-Sarraj, S., Seilhean, D., Streichenberger, N., ... Huitinga, I. (2015). BrainNet Europe's Code of Conduct for brain banking. *Journal of Neural Transmission*, 122(7), 937–940. <http://doi.org/10.1007/s00702014-1353-5>

# Procedure of Material Transfer

