Institute of Health Carlos III

Red Nacional de Biobancos
Spanish National Biobank Network

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SOP
Collecting and Processing of Neural Tissue Samples for a Biobank

Brain Bank Working Group

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Protocol for Collecting and Processing Neural Tissues

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Authors' note

The protocols, methods and techniques described below must be considered as recommended and approximate, and it is desirable that they be performed in a similar manner in all biobanks of the neural tissue biobank network.

It should be pointed out that processing and neuropathological diagnoses are not the result of the mere implementation of these protocols. On the contrary, examination by a specialist in neuropathology is crucial for proper interpretation and comprehensiveness of the findings.

For that reason, *the management, processing, monitoring and neuropathological diagnosis of neural tissue banks must be performed by an experienced* ***specialist in neuropathology***.

1. DISSECTION OF THE NERVOUS SYSTEM

1.1. Extraction of the brain

This is done using the classical method of opening the cranial cavity and cutting under the foramen magnum, taking the upper part of the cervical spine.

Special care must be taken when severing the optic nerves and the cranial nerves previously, so that part of them remains in the brain, and also for obtaining the olfactory bulbs and nerves intact.

The pituitary must be obtained separately. It must be obtained separate from other regions (e.g. Gasser's ganglion) when deemed appropriate.

Collecting cerebrospinal fluid **(CSF)** can be accomplished from the middle ventricle by puncture from the basal side of the brain and inserting a plastic pipette, while raising the chiasm slightly to allow passage of the pipette without damaging it. The biggest problem when obtaining CSF is contamination with blood. Centrifuging the CSF does not help as this removes possible cellular components of the blood but preserves contaminating plasma proteins, so that the sample cannot be used for further studies.

External macroscopic observation and recording fresh brain weight are mandatory.

***Photographs of the fresh brain** must be taken.*

Brain pH must be measured by inserting pH strips into the brain.

The pH of the cerebrospinal fluid (CSF) may be measured.

***Fresh brain weight and the pH of the fresh brain or CSF** must be recorded.*

1.2. Dissection of the spinal cord.

Dissection by a posterior approach or, more properly, by an anterior approach taking special care to obtain the entire length of the spinal cord from the high cervical region to the cauda equina.

Dissection of the dorsal root ganglia and of appropriate segments of anterior and posterior roots.

Complete dissection of the cauda equina.

*External macroscopic observation is mandatory. **Photographs** of the fresh spinal cord must be taken.*

1.3. Dissection of the sense organs

Only in case of express authorization: eyeballs.

2. SECTIONING OF BRAIN AND SPINAL CORD

There are various ways of removing the brain, some of which depend on the pathology and the lateralization of the neurological process. Protocols must be adapted to the particular conditions of the neurological disease. It makes little sense, for example, to freeze coronal sections of one hemisphere for biochemical studies and to fix the other one in formaldehyde when dealing with a unilateral stroke.

The following protocol is considered standard.

- Separate the brainstem and cerebellum from the brain hemispheres with a high incision above the superior colliculi
- Separate the cerebellum from the brainstem by severing the cerebellar peduncles
- Separate the cerebellar hemispheres with a midsagittal cut through the vermis
- Separate the cerebral hemispheres by cutting exactly along the midline of the corpus callosum

In general terms, sectioning is as follows:

- The left brain hemisphere is fixed in 4% buffered formalin, while the right hemisphere is processed for subsequent freezing
- The left cerebellar hemisphere is fixed in 4% buffered formalin, and the right cerebellar hemisphere is processed for subsequent freezing
- The brain stem is cut into approximately 3 mm thick sections; the first one is fixed in 4% buffered formalin, the next one is frozen, and so forth
- The spinal cord is sectioned into segments of about 5 mm. The first section is fixed in 4% buffered formalin, the next one is frozen, and so forth
- Anterior and posterior roots are identified in the cauda equina and samples are taken of each of the regions for morphological study; they are formalin-fixed or frozen
- The additional samples: dorsal root ganglia, Gasser's ganglion, pituitary (and possibly olfactory bulbs and tracts if they were broken during removal of the brain) are processed to obtain frozen samples and formalin-fixed samples

Alternatively, the brainstem can be cut sagittally into approximately three mm thick sections, using sections from one side for formalin fixation and the ones from the other side for freezing. In this way, no tiers are lost for morphological and molecular study. This method also allows obtaining at least two areas of the substantia nigra, a rostral one and a caudal one.

Alternatively, some brain banks prefer freezing one or the other hemisphere on alternate days, so that in half the cases the right hemisphere is frozen and in the other half the left.

3. FORMALIN FIXATION AND SUBSEQUENT EXAMINATION AND PROCESSING

Fixation time in 4% buffered formalin must be sufficient, but not too long. Buffered formalin must be prepared at reasonable intervals to avoid deterioration (once a month). It is recommended not to let more than three weeks pass before sectioning the samples. Prolonged times in formalin reduce the accessibility of certain antibodies even when of antigen clearing methods are used.

Normally classic coronal sections of maximally 1 cm thick are made and placed successively on a flat surface from the frontal pole to the occipital pole. ***Microscopic examination of the brain (and the spinal cord) is now completed. Photographs of the fixed brain*** are taken.

Samples are taken for making small and large (hemispheric) paraffin blocks and sections may be made (in laboratories that are equipped for this) for collodion embedding.

3.1. Small paraffin blocks (routine blocks)

These are considered routine or indispensable procedures and they are numbered in all cases. It is convenient to use the same type of numbering in all neural tissue biobanks or, if applicable, to know the equivalences between two banks.

N1 Medulla oblongata

N2 Mesencephalon (including substantia nigra)

N3 Pons (including locus ceruleus)

N4 Cerebellar vermis (superior)

N5 Cerebellum and dentate nucleus

N6 Right anterior hippocampus and parahippocampal gyrus

N7 Left anterior hippocampus and parahippocampal gyrus

N8 Anterior striatum (caudate/putamen), includes the accumbens

N9 Frontal lobe (cortex/white matter) area 8

N10 Occipital lobe (primary visual area and visual association area)

N11 Amygdala

N12 Basal nucleus of Meynert/globus pallidus

N13 Hypothalamus/mammillary bodies

N14 Anterior cingulate cortex

N15 Right posterior hippocampus

N16 Left posterior hippocampus

N17 Parietal cortex (cortex/white matter) at the level of the splenium

N18a Anterior temporal cortex (middle and superior temporal gyri)

N18b Posterior temporal cortex (middle and superior gyri) at the level of the geniculate nucleus

N19 Posterior cingulate cortex

N20a Medial-anterior thalamus and subthalamic nucleus

N20b Posterior thalamus

N21 Hypophysis

N22 Olfactory bulb and tract

N23 Cervical spinal cord (N23a, b, c)

N24 Thoracic spinal cord (N24a, b, c)

N25 Lumbar spinal cord (N25a, b, c). Sacral spinal cord N25d

N26 Precentral frontal cortex (primary motor area)

N27 White matter of the oval center

N28 Dorsal root ganglia (N28a, b, c)

N29 Cauda equina (N29a: anterior; N29p: posterior)

3.2. Small paraffin blocks (reserve blocks)

Since they are considered reserve material they are also included here, but in principle they are not sectioned.

Tissue is better and longer preserved in a paraffin block than in formalin. For this reason, it is desirable to process most of the brain in paraffin with a short fixation time in buffered formalin and then preserve the paraffin blocks.

N30 Supraorbital frontal cortex

N31 Frontal cortex area 8

N32 Primary motor cortex area (N32m) and primary somatosensory area (N32s)

N33 Posterior temporal cortex

N34 Parietal cortex (angular gyrus)

N35 Anterior insular cortex

N36 Posterior insular cortex

N37 Hippocampus

N38 Caudate and putamen: medial regions

N39 Anterior (N39a) and posterior (N39p) thalamic nuclei

N40 Nucleus of Meynert (remainder)

N41 Medulla oblongata (remainder, N41a, b, c)

N42 Pons (remainder, N42a, b, c)

N43 Mesencephalon (remainder, N43a, b, c)

N44 Spinal cord (remaining sections N44a, b, c)

N45 Dorsal root ganglia (remainders, N45 a, b)

N46 Rest of the optic tracts and chiasm

Sequential numbering for other regions (i.e. identified cranial pairs)

3.3. Hemispheric blocks embedded in paraffin

Hemispheric blocks are essential for gaining a realistic view of the white matter of the brain.

A minimum of two blocks must be taken:

- Frontal
- Occipital

As many blocks can be added as considered appropriate to gain a better visualization of the white matter of other areas

4. FREEZING OF THE SAMPLES

Freezing of samples must be done immediately after extraction and sectioning of the brain as indicated under point 2.

Embedding more or less types of samples depends on the capacity of each biobank, since priority should always be given to the speed of the process.

4.1. Minimum standard condition of suboptimal performance

This is based on freezing 8-10 mm thick coronal brain hemisphere sections on a plate cooled on dry ice. The blocks are stored in airtight plastic bags that are numbered consecutively, starting with 1 which corresponds to the frontal-most section.

Coronal brain sections are frozen and then stored, as well as the alternating sections of the entire brainstem and those of the spinal cord, dorsal root ganglia and sections of the cauda equina. Storing must be done immediately and continuously at -80ºC.

Drawbacks: The major drawback of this type of storage is the dissection of samples of specific regions once the coronal brain section is frozen. It is frozen material that has to be broken up by blows of a chisel or similar tools; the pieces are irregular and the contours are imprecise. It is difficult to know exactly which region is being obtained.

Moreover, the remaining fragments are often difficult to identify, so that the rest of the coronal sample sometimes loses its orientation.

The possibility of thawing the sample for better dissection and subsequent re-freezing of the required sample and the remaining sample must be considered prohibited. Thawing and freezing changes the molecules, so that thawed and frozen material is not reliable anymore for any molecular or biochemical research.

4.2. Intermediate condition

Immediately after making the coronal brain sections, those regions that are in highest demand for molecular studies of the most common neurodegenerative diseases must be dissected, frozen on a cold plate on dry ice and stored in numbered plastic bags to make identification possible. The rest of the brain and cerebellum are preserved as indicated in the previous section.

Thus, the samples dissected, frozen and stored in separate bags are:

- Hippocampus (several sections from anterior to posterior: 1, 2, 3)
- Entorhinal cortex (from anterior to posterior: 1, 2, 3)
- Amygdala
- Caudate
- Putamen
- Globi pallidi
- Basal nucleus of Meynert

- Medial hypothalamus
- Medial thalamus
- Olfactory bulb
- Cerebellar vermis
- Substantia nigra
- Rest of the mesencephalon
- Pons (1,2, 3)
- Medulla oblongata (1, 2, 3)
- Spinal cord (cervical 1, 2, 3; thoracic 1, 2, 3; lumbar 1, 2, 3; sacral)
- Choroid plexuses

The remaining samples from coronal sections of the brain and cerebellum are stored as described in the previous section (4.1) in bags with consecutive numbering, starting with the most anterior sample as 1.

4.3. Optimal condition for the number of dissected and frozen samples

Dissection is done of the different regions from slices, selecting tissue samples of about 2x2 cm. These samples are rapidly frozen on a charged steel plate cooled on dry ice and the samples are stored in labeled and numbered zip lock bags:

The following regions are recommended:

AC1 Middle frontal gyrus (BA 8/9)
AC2 Middle and superior temporal gyrus (BA21)
AC3 Anterior hippocampus and parahippocampal gyrus
AC4 Superior parietal gyrus (BA40)
AC5 Hemi-mesencephalon at the level of cranial pair III
AC6 Superior frontal gyrus with cingulate gyrus (BA 24 and 32)
AC7 Occipital cortex including the calcarine fissure
AC8 Striatum, including the nucleus basalis of Meynert and the hypothalamus
AC9 Amygdala and anterior entorhinal cortex
AC10 Thalamus and subthalamic nucleus
AC11 Pons through the middle of the locus ceruleus
AC12 Medulla oblongata through the maximum diameter of the inferior olive and hypoglossal nucleus
AC13 Cerebellum with a complete section of the dentate nucleus
AC14 Deep white matter of the frontal lobe
AC15 Deep white matter of the occipital lobe
AC16 Head of the caudate nucleus
AC17 Medial thalamus (anterior to the lateral geniculate body)

AC18	Anterior thalamus and posterior part of the Putamen and Globus Pallidus
AC19	Posterior thalamus (posterior to the lateral geniculate body)
AC20	Anterior frontal lobe (BA 10)
AC21	Frontal Lobe (BA9)
AC22	Frontal Lobe (BA8)
AC23	Frontal Lobe (BA6)
AC24	Inferior lateral Frontal Lobe (BA46)
AC25	Inferior Frontal Lobe (BA47)
AC26	Frontal Lobe (BA45)
AC27	Cingulate gyrus, anterior (BA24)
AC28	Cingulate gyrus, posterior (BA 32)
AC29	Motor area. Precentral gyrus (BA4)
AC30	Primary somatosensory area. Postcentral gyrus (BA 3/1/2/5)
AC31	Superior parietal lobe (BA7)
AC32	Lateral parietal lobe (BA39)
AC33	Inferior parietal lobe (BA37)
AC34	Anterior temporal lobe (BA38)
AC35	Superior temporal gyrus -posterior part- (BA22)
AC36	Middle temporal gyrus -posterior part- (BA21)
AC37	Inferior temporal gyrus -posterior part- (BA20)
AC38	Anterior hippocampus and parahippocampal gyrus (BA28, 36)
AC39	Posterior hippocampus and parahippocampal gyrus (BA28, 36)
AC40	Calcarine cortex BA17
AC41	Occipital lobe BA18, 19 superior
AC42	Occipital lobe BA 18, 19 inferior
AC43	White matter of the frontal lobe X2 (anterior to that at the level of the amygdala)
AC44	Parietal white matter at the level of the splenium
AC45	Occipital white matter at the level of the calcarine cortex
AC46	Temporal white matter at the level of the lateral geniculate body
AC47	Cerebellar vermis
AC48	Rest of the cerebellar hemisphere
AC49	Rest of the pons
AC50	Rest of the medulla oblongata
AC51	Spinal cord, cervical level
AC52	Spinal cord, thoracic level
AC53	Spinal cord, lumbar level
AC54	Olfactory bulb
AC55	Dura mater

AC56 Subependymal white matter, anterior horn of the lateral ventricle

AC57 Hypophysis

AC58 Dorsal sensory ganglion

Advantages and limitations: The biggest advantage of this procedure is to have sections selected and already prepared for molecular and biochemical studies. The biggest limitation is to have to do all the processing in a short time, which requires the active and efficient presence of many people for dissecting, labeling and storing. The entire process must be done quickly without a significant time difference between the first and the last blocks.

5. CRYOPROTECTED SAMPLES

These samples are useful for cryostat sections processed for free-floating immunohistochemistry studies, and for certain *in situ* hybridizations.

Sections (10x10x2mm) are fixed in 4% phosphate-buffered paraformaldehyde for 24 hours and then cryoprotected in 30% saccharose in water until they sink (about 24 hours). The sections are then frozen and stored in numbered plastic zip lock bags at -80ºC until use.

Samples that can be preserved under these conditions are variable; the most common regions are:

- Hippocampus
- Amygdala
- Mesencephalon
- Pons
- Medulla oblongata
- Striatum
- Frontal cortex (area 8)

6. COLLECTING AND PROCESSING SAMPLES FOR ELECTRON MICROSCOPY

Small blocks for electron microscopy should be obtained only in cases of a short post-mortem time (two or three hours maximum) and a pre-mortem state without major problems related to hypoxia, acidosis and severe metabolic disturbances.

It is also important to have objective criteria for subsequent sectioning of samples for embedding, while maintaining the optimum orientation of the blocks.

Maximum size of the tissue blocks must be 1 mm^3

6.1. Fixation for conventional electron microscopy

Fixation should be done in 2.5% glutaraldehyde in phosphate buffer for 24 hours, followed by washing in phosphate buffer-saccharose (pH 7, 200-400 mOsm), post-fixation in 1% osmium tetroxide for 2 hours, washing and embedding in resin.

6.2. Fixation for electron microscopy (EM) that allows for EM-immunohistochemistry

Fixation should be done in 4% paraformaldehyde-0.1% glutaraldehyde for 24 hours, followed by washing in phosphate buffer-saccharose, following the procedure described above.

7. OTHER SAMPLES RELATED TO NEURAL TISSUE BIOBANKS

It is useful to collect other samples related to the nervous system during autopsy.

Cerebrospinal fluid (CSF): The biggest problem is that obtained CSF is almost always contaminated with blood at the time of the autopsy. It is possible to obtain CSF before opening the skull, although this is difficult. Intraventricular puncture after removal of the calotte bears clear risks of contamination. CSF must be stored immediately at -80ºC.

Serum: can be extracted from a vessel, centrifuged, aliquoted into 2 ml cryotubes and stored frozen at -80ºC.

RNA from peripheral blood cells: blood can be extracted and stored in PAXgene Blood RNA tubes and processed afterwards to obtain RNA from blood cells.

DNA: in some banks DNA is extracted from a region of the brain (e.g. cerebellum) and stored for studies of known risk factors for neurodegenerative diseases (e.g. ApoE).

Obtaining such samples, although they are related to the nervous system, must be specified in the donation document signed by the donor or his/her legal representatives.

8. BASIC METHODS FOR NEUROPATHOLOGICAL MORPHOLOGICAL DIAGNOSIS IN BIOBANKS

Rigorous microscopic neuropathological examination is essential in the everyday functioning of neural tissue biobanks. To this end, such studies must be standardized and similar methodologies must be used in the different biobanks.

Routine neuropathological examination is performed on the small paraffin blocks indicated in section 3.1 and on the hemispheric blocks indicated in section 3.3.

8.1. Application of routine techniques

The techniques can be modified according to the pathology, but **routine examinations of adults with or without neurodegenerative disease** (those considered as controls because they did not present with neurological disorders are also processed in this way) can follow the protocol below:

All sections (N1-N29) and the hemispherical sections are stained with **hematoxylin and eosin**, and with a myelin staining method (usually **Klüver-Barrera**)

In all cases, but not in all sections, immunohistochemistry techniques are applied for glial fibrillary acidic protein (**GFAP**), microglia (usually **CD68**), phosphorylated tau (often antibody **AT8**), **tau3R** and **tau4R**, **ubiquitin**, **α-synuclein**, **αB-crystallin**, phosphorylated neurofilaments (usually antibody **RT97**), **TDP-43** and **β-amyloid**.

A suggestion for a minimum application of immunohistochemistry in routine examination is given below:

Blocks are according to the numbering given in section 3.1 and stains for each of the regions:

N1	α-synuclein, AT8
N2	α-synuclein, AT8
N3	α-synuclein, AT8
N4	RT97
N6	α-synuclein, β-amyloid, AT8, tau4R, tau3R, ubiquitin
N7	α-synuclein, β-amyloid, AT8, tau4R, tau3R, ubiquitin
N8	AT8
N9	AT8, ubiquitin, TDP-43, RT97, β-amyloid
N10	α-synuclein, β-amyloid, AT8
N11	αB-crystallin, α-synuclein, AT8
N15	β-amyloid, AT8, TDP-43
N16	β-amyloid, AT8, TDP-43
N17	β-amyloid
N22	AT8, α-synuclein

N23 RT97, TDP-43

N24 RT97, TDP-43

N25 RT97, TDP-43

N29 RT97

Application of GFAP and CD68 must be assessed depending on the alterations found in the first observation.

8.2. Stages and applications in specific pathologies

8.2.1 Stages of the disease

The necessary techniques must be applied to the appropriate regions to identify the stage of the disease.

8.2.2. Specific pathologies

The study of different regions and techniques varies according to the pathologies, and their use depends solely on the neuropathology specialist who examines the case.

By way of example, markers should be used for:

- Prion protein (clones 3F4 and 1E4) with and without pre-incubation with proteinase K when suspecting prion diseases
- Lymphocyte and mononuclear phagocyte system markers in inflammatory diseases
- Different amyloid markers other than β-amyloid and PRP when β-amyloid and PRP stains are negative in the presence of amyloid angiopathy
- Extension of the study with TDP-43 and phosphorylated TDP-43 in non-tau frontotemporal dementias (FTDs) and with ubiquitin-immunoreactive inclusions
- The same applies to motor neuron disease with or without associated FTD, and to amyotrophic lateral sclerosis (ALS)
- Anti-FUS antibody in ubiquitin-positive/TDP-43 negative FTD, NIFID, and TDP-43 negative ALS
- Anti-polyglutamine antibodies in trinucleotide repeat disorders, and specific markers for diseases with excess repeats: e.g. huntingtin, ataxin 1, 2, 3
- Stains for lipids and carbohydrates, and autofluorescence studies in certain metabolic disorders
- Stains for pigments, iron, or calcium in certain deposits
- Extracellular matrix markers, and collagen and Notch antibodies in certain vascular diseases

9. BASIC METHODS FOR MOLECULAR NEUROPATHOLOGICAL DIAGNOSIS IN BIOBANKS

The techniques mentioned are usually sufficient to make the neuropathological diagnosis. However, there is an exception that should be mentioned.

9.1. Prion diseases

Although there are morphological predictions regarding the subtypes of Creutzfeldt-Jakob disease based solely on the morphology and distribution of lesions together with the type of deposit of the prion protein, it is important to know the PrP banding pattern with and without proteinase K treatment, as analyzed by gel electrophoresis and western blotting.

It is also advisable to know the composition of codon 129. The classifications of subtypes of Creutzfeldt-Jakob disease are based on morphological, genetic and biochemical criteria.

9.2. Metabolic diseases

Manifested most frequently in childhood, it is difficult to diagnose this group of diseases based on mere neuropathological examination, even when they are accompanied by the identification of specific deposit profiles on electron microscopic examination.

Neuropathological examination is, however, essential to substantiate a diagnostic suspicion or to orient biochemical, enzymatic and genetic studies required to reach the correct diagnosis of the disease.

10. SPECIFIC ASPECTS. PEDIATRIC BIOBANKS AND BIOBANKS FOR PSYCHIATRIC DISEASES

10.1. Pediatric biobanks

Pediatric neural tissue biobanks constitute a special but not exceptional case. Irrespective of the legal aspects of donation, they are determined by the type of pathology and the size and consistency of the brains, especially in the perinatal period.

10.1.1. Examination and processing of the central nervous system

This must be adapted to the size and fragility of the sample.

- Microscopic examination is essential for the identification of malformations and for the interpretation of lesions related to perinatal damage
- Processing and collection of samples for formalin fixation and paraffin embedding may be easier to do, since large section can be made that allow the study of an entire hemisphere or, where appropriate, the study of several brain regions in a single block
- Obtaining samples for freezing is more complex because it is not easy to separate certain regions. For example, obtaining samples according to paragraph 4.3 is impossible in practice

10.1.2. Biochemical and genetic studies

These are often required to confirm or make an accurate diagnosis.

10.2. Biobanks for psychiatric diseases

A bank of "neurological" diseases and a bank of "psychiatric" diseases are not necessarily different. Two aspects might be pointed out, though:

- ✓ Neuropathological diagnoses must especially be taken into account at the time of transferring samples to third parties, since additional or underlying diseases in older people can distort possible research results. Not infrequently, older patients whether admitted to psychiatric facilities or not have additional pathologies of neurodegenerative diseases. Studies involving such cases must take into account that one should control not only controls for age and gender, but also very specifically for associated diseases (e.g., stage II, IV or V Alzheimer's disease). Obviously this is not unique to the study of psychiatric diseases, but associated degenerative diseases are often not given enough attention.

- ✓ Dissection and storage of as many frozen samples as feasible is highly desirable in the case of psychiatric diseases. More important results can be expected from biochemical and molecular studies than from morphological studies.

11. REGISTRATION OF SAMPLES IN BIOBANKS

The register should contain those data that may be necessary for the correct identification of the desired sample type for a particular research project and for tracing a given sample in the bank.

The register of the samples should include the following data:

- Personal data
  - ✓ identification or code of the center (biobank)
  - ✓ identification of the patient
  - ✓ gender
  - ✓ age

- **Clinical neurological diagnoses (link with the CLINICAL DATA PROTOCOL)**
- Hypertension, diabetes, hypercholesterolemia, obesity
- Agonic state
- Preservation of the corpse (temperature, cooling)
- Post-mortem delay (time between death and removal of neural tissues)
- pH of the brain at the time of extraction
- Types and conditions of the samples (samples in formalin and paraffin, frozen samples, cryoprotected samples, samples for electron microscopy (regions))
- Numbering of paraffin blocks
- Numbering of frozen blocks
- Other samples: CSF, serum, peripheral RNA, DNA, other
- Macroscopic examination (including weight of the brain)
- Microscopic examination

- **Neuropathological diagnoses:** it should be noted that in most adult cases it is rare to find a single pathology. This is important in the research field because combined pathologies can distort the observations of a study and invalidate the results. **Stage of the disease** according to the established classifications and that are detailed in an *addendum* to this protocol

Detailed registration of the samples allows optimizing the transfer of a given sample and to know at all times what type of sample is available for study. Total use of a given sample should be recorded as transferred and completed, and therefore not available.

ANNEX I

CLINICAL DATA PROTOCOL

- Personal data
  - identification or code of the center (biobank)
  - identification of the patient
  - gender
  - age
  - weight, height and body mass index
  - date of death (day/month/year)
  - post-mortem delay (time between death and processing of neural tissues)
  - preservation of the corpse (ambient temperature or cooling)
- Consent
  - clinical autopsy (unused material after diagnostic study)
  - forensic autopsy
  - living donor program
  - consent for genetic studies
  - limitations for certain studies
- Familial, genetic and environmental factors
  - family history
  - genetic studies
  - gestation (if applicable)
    - ✓ known obstetric complications
    - ✓ infections during the prenatal period
  - environmental factors
    - ✓ smoking habits
    - ✓ alcohol abuse
    - ✓ other drug abuse
    - ✓ other (high-risk working conditions, insecticides)
  - treatments
    - ✓ type and duration
    - ✓ detailed treatment regimens during the last two months
- Risk factors: traumatism, hypertension, obesity, hypercholesterolemia, diabetes
- History of infectious or contagious disease
- Systemic disease
- Main clinical neurological signs and symptoms and chronology:
  - date of last neurological examination

- focal signs or lateralization
- mental impairment and dementia: Mini Mental State Examination, memory, language, praxis, gnosis, attention, executive functions, cognitive fluctuations
- psychiatric symptoms: depression, anxiety, delirium, agitation, apathy, irritability, aberrant motor behavior, disinhibition, hallucinations (NPI: neuropsychiatric assessment in dementia)
- motor signs:
  - ✓ parkinsonism
  - ✓ ataxia
  - ✓ chorea
  - ✓ myoclonus
  - ✓ amyotrophy
  - ✓ pyramidalism
- sensory signs
- autonomic signs
- epilepsy
- sleep disorders
- history of unexplained falls
- syncope
- abnormal eye movements
- other

• Course of the disease:
- acute
- subacute
- chronic
- relapsing/remitting

• Relevant complementary examinations or tests
- biochemistry
- neuroimaging
- neurophysiology
- other

• Clinical diagnoses

• Agonic state: intensive care, vegetative state

• Clinical cause of death

ANNEX II

PROTOCOL FOR NEUROPATHOLOGICAL DIAGNOSES

- Nomenclature is given in English to facilitate the exchange of information with other centers
- Listing of single pathologies does not imply the possibility of multiple diagnoses in the same case. Rather, an ordered final list of several pathologies is quite likely
- Furthermore, it is recommended to include all neuropathological diagnoses in the final summary of the neuropathological diagnosis

No neuropathological lesions

A. Degenerative diseases of the central nervous system

Alzheimer disease and amyloid angiopathies

Alzheimer disease stage I/II = NFT Braak stage I-II

Alzheimer disease stage III/IV = NFT Braak stage III-IV

Alzheimer disease stage V/VI = NFT Braak stage V/VI

Alzheimer disease β-amyloid: stages A, B, C

Alzheimer disease only amyloid plaques

Amyloid angiopathies non-Alzheimer

Familial Alzheimer disease

Down syndrome

Tauopathies

Pick disease

Corticobasal degeneration

Progressive supranuclear palsy

Argyrophilic grain disease

Myotonic dystrophy type I

Frontotemporal lobar degeneration tau+ Familial

frontotemporal lobar degeneration tau+

Other tauopathies including atypical tauopathies (tauopathy with globular inclusions in oligodendrocytes)

Frontotemporal lobar degeneration tau-

Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43+

Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43-, FUS+

Frontotemporal lobar degeneration with motor neuron disease

Frontotemporal lobar degeneration with no inclusions

NIFID

BIBD (basophilic inclusion body disease)

Alpha-synucleinopathies

Lewy body disease stage 1/2

Lewy body disease stage 3/4

Lewy body disease stage 5/6

Amygdala-predominant Lewy body disease

Atypical Lewy body disease

Familial Parkinson disease ***including PD without Lewy bodies***

Multiple system atrophy

Prionopathies

Creutzfeldt-Jakob disease

Variant Creutzfeldt-Jakob disease

Familial Creutzfeldt-Jakob disease: genetic Creutzfeldt-Jakob disease

Fatal familial insomnia

Gerstmann-Sträussler-Scheinker syndrome

Huntington disease

Other degenerative diseases of the basal ganglia

Spinocerebellar ataxias

Autosomal dominant spinocerebellar atrophy 1 (SCA1)

Autosomal dominant spinocerebellar atrophy 2 (SCA2)

Autosomal dominant spinocerebellar atrophy 3 (SCA3)

Other autosomal dominant spinocerebellar atrophies

Dentatorubropallidoluysian atrophy (DRPLA)

Friedreich ataxia

Other autosomal recessive and sporadic ataxias

Motor neuron diseases

Sporadic amyotrophic lateral sclerosis (ALS)

Familial ALS

Lower motor neuron diseases

Upper motor neuron diseases

Primary axonal diseases with spheroids

Hallervorden-Spatz disease

Neuroaxonal dystrophies

TDP-43 pathology (other than disease-specific: FTLD-TDP-43+, ALS)

B. Cerebrovascular and circulatory diseases

Global ischemia

Focal ischemia: unique infarction

Lacunae

Status cribosus

Multi-infarct encephalopathy

Atherosclerosis (AT)

Small vessel disease (SVD)

Binswanger encephalopathy

CADASIL

Haemorrhage, extradural and subdural

Haemorrhage, subarachnoid

Haemorrhage, intraparenchymal

Aneurisms and vascular malformations

Vasculitis

Degenerative vascular diseases (other than amyloid angiopathy, AT and SVD)

Gas and fat embolism

C. Nutritional and toxic diseases

Wernicke encephalopathy

Pellagra

Vitamin B12 and folate deficiency

Late effects of alcohol in adults

Fetal alcohol syndrome

Marchiafava-Bignami

Central pontine and extrapontine myelinolysis

Hepatic encephalopathy

Other toxics

D. Metabolic diseases

Lipidoses (excepting Krabbe and metachromatic leukodystrophy)

Neuronal ceroid lipofuscinoses

Mucopolysaccharidoses

Polyglucosan diseases

Glycogenoses

Mucolipidoses

Leukodystrophies

Adrenoleukodystrophy and other peroxisomal diseases

Disorders of amino acids

Mitochondrial disorders

E. Inflammatory diseases

Multiple sclerosis

Acute disseminated encephalomyelitis

Guillain-Barre syndrome

F. Viral infectious diseases

Herpes virus infection

Cytomegalovirus infection

Progressive multifocal encephalitis

Retrovirus infection: HIV

Other viral diseases

G. Non-viral infections

Bacterial meningitis

Septic encephalitis and abscess

Whipple disease

Mycobacterial diseases: tuberculosis

Nocardiosis, actinomycosis

Neurosyphilis

Other bacterial diseases

Toxoplasmosis

Other protozoal infections

Arthropod infections

Metazoan infections

Fungal infections

H. Primary brain and spinal cord malformations including disorders of cell migration

I. Secondary malformations and destructive pathologies in the perinatal period

J. Trauma

K. Tumors

Primary tumors of the nervous system

Secondary malignancies

L. Paraneoplastic diseases

M. Muscular diseases

N. Diseases of the peripheral nerves

Inflammatory neuropathy

Amyloid neuropathy

Leprosy neuropathy

Neuropathy associated to lysosomal storage diseases

Axonal neuropathy

Segmental demyelination

Mixed axonal and myelin neuropathy

Hypertrophic neuropathy (onion bulb formation)

J. Undetermined and non-specific lesions

Hippocampal sclerosis

Subcortical gliosis of unknown origin

Demyelination of the white matter of the centrum semiovale

Calcifications

K. Disorders or lesions not categorized in the previous paragraphs

ANNEX III

DIAGNOSTIC CODES

1. CLINICAL DIAGNOSTIC CODES

	NEUROLOGICAL DIAGNOSES
0NEU	no neurological symptoms described
0PSYCH	no psychiatric symptoms described
AIDS	AIDS
OH	Alcoholism
AD	Alzheimer's disease
ATAX	Ataxia
INFARCT	Cerebrovascular disease, infarct
HAEM	Cerebrovascular disease, haemorrhage
EDEM	Cerebral edema
CBD	Corticobasal degeneration
CJD	Creutzfeldt-Jakob disease
DLB	Dementia with Lewy Bodies
D+MND	Dementia with Motor Neuron Disease
FTD	Dementia, frontotemporal
D-VASC	Dementia vascular
D	Dementia, not specified
DRPLA	Dentatorubral-pallidoluysian atrophy
DRUG	Drug abuse disorder, other
EPI	Epilepsy
HD	Huntington's disease
INF-VIR	Infection of the nervous system, viral
INF-BAC	Infection of the nervous system, bacterial
INF-FUNG	Infection of the nervous system, fungal
INF-OTH	Infection of the nervous system, other
MET	Metabolic disease inherited
MND/ALS	Motor Neuron Disease (MND)
MS	Multiple sclerosis
MSA	Multiple system atrophy
NEUDEG	Neurodegeneration, other
PD	Parkinson's disease
PiD	Pick's disease

PSP	Progressive Supranuclear Palsy
PSYC	Psychosis
SCHZ	Schizophrenia
TR	Trauma
TUM1	Tumor, primary
TUM2	Tumor, secondary
OTHER	Other disease category than listed

2. NEUROPATHOLOGICAL DIAGNOSTIC CODES

CODE	NEUROPATHOLOGICAL DIAGNOSES
0	**0. No neuropathological lesions**
	A. Degenerative diseases of the central nervous system
AD	**Alzheimer disease and amyloid angiopathies**
ADI	Alzheimer disease stage I - NFT Braak stage I
ADII	Alzheimer disease stage II
ADIII	Alzheimer disease stage III
ADIV	Alzheimer disease stage IV
ADV	Alzheimer disease stage V
ADVI	Alzheimer disease stage VI
	Stages amyloid plaques: A, B, C
ADPL	Alzheimer disease only amyloid plaques
AMAN	Amyloid angiopathies non-alzheimer
FAD	Familial Alzheimer disease
DOWN	Down syndrome
	Tauopathies
PiD	Pick disease
CBD	Corticobasal degeneration
PSP	Progressive supranuclear palsy
AGD	Argyrophilic grain disease
MyDysI	Myotonic dystrophy type I
FTLD tau	Frontotemporal lobar degeneration tau+
fFTLD tau	Familial frontotemporal lobar degeneration tau+
TAUP oth	Other tauopathies including atypical tauopathies (tauopathy with globular inclusions in oligodendrocytes)
	Frontotemporal lobar degeneration tau-
FTLD-TDP-43	Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43+

FTLD-FUS	Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43-, FUS+
FTLD-MND	Frontotemporal lobar degeneration with motor neuron disease
FTLD-0	Frontotemporal lobar degeneration with no inclusions
NIFID	Neuronal intermediate filament inclusion disease
BIBD	basophilic inclusion body disease)
	Alpha-synucleinopathies
LBDI	Lewy body disease stage 1
LBDII	Lewy body disease stage 2
LBDIII	Lewy body disease stage 3
LBDIV	Lewy body disease stage 4
LBDV	Lewy body disease stage 5
LBDVI	Lewy body disease stage 6
Amyg-LBD	Amygdala-predominnat Lewy body disease
Atyp-LBD	Atypical Lewy body disease
fPD	Familial Parkinson disease ***including PD without Lewy bodies***
MSA	Multiple system atrophy
	Prionopathies
CJD	Creutzfeldt-Jakob disease
vCJD	Variant Creutzfeldt-Jakob disease
fCJD	Familial Creutzfeldt-Jakob disease (genetic CJD)
FFI	Fatal familial insomia
GSS	Gerstmann-Sträussler-Scheinker syndrome
HD	**Huntington disease**
DEG oth	**Other degenerative diseases of the basal ganglia**
SCA	**Spinocerebellar ataxias**
SCA1	Autosomal dominant spinocerebellar atrophy 1
SCA2	Autosomal dominnat spinocerebellar atrophy 2
SCA3	Autosomal dominant spinocerebellar atrophy 3
SCA oth	Other autosomal dominant spinocerebellar atrophies
DRPLA	Dentatorubropallidoluysian atrophy
FRI	Friedreich ataxia
ATAX oth	Other autosomal recessive and sporadic ataxias
	Motor neuron diseases
sALS	Sporadic amyotrophic lateral sclerosis
fALS	Familial ALS
LMND	Lower motor neuron diseases
UMND	Upper motor neuron diseases

	Primary axonal diseases with spheroids
H-S	Hallervorden-Spatz disease
Neuro-ax	Neuroaxonal dystrophies
TDP-43	**TDP-43 pathology** (other than disease-specific: FTLD-TDP-43+, ALS)
	B. Cerebrovascular and circulatory diseases
gISCH	Global ischemia
fISCH	Focal ischemia: unique infarction
LAC	Lacunae
SCRIB	Status cribosus
MINF	Multi-infarct encephalopathy
AT	Atherosclerosis
SVD	Small vessel disease
BINS	Binswanger encephalopathy
HAEMd	Haemorrhage extradural and subdural
CAD	Cadasil
HAEMarac	Haemorrhage subarachnoidal
HAEMp	Haemorrhage intraparenchymal
An-VM	Aneurisms and vascular malformations
Vitis	Vasculitis
Deg Vas Dis	Degenerative vascular diseases (other than amyloid angiopathy, AT and SVD)
G Emb	Gas and fat embolism
	C. Nutritional and toxic diseases
Wern	Wernicke encephalopathy
Pel	Pellagra
B12-F	Vitamin B12 and folate deficiency
OH	Late effects of alcohol in adults
FAS	Fetal alcohol syndrome
M-B	Marchiafava-Bignami
Cen P	Central pontine and extrapontine myelinolysis
Hep E	Hepatic encephalopathy
Tox	Other toxics
	D. Metabolic diseases
Lip	Lipidoses (excepting Krabbe and metachromatic leukodystrophy)
NCL	Neuronal ceroid lipofuscinoses
Mucos	Mucopolysaccharidoses
Polygl	Polyglucosan diseases
Gly	Glycogenoses

Mucol	Mucolipidoses
Leuk	Leukodystrophies
ADLS-per	Adrenoleukodystrophy and other peroxisomal diseases
Amino	Disorders of amino acids
Mit	Mitochondrial disorders
	E. Inflammatory diseases
MS	Multiple sclerosis
ADE	Acute disseminated encephalomyelitis
GB	Guillain-Barre syndrome
	F. Viral infectious diseases
HV	Herpes virus infection
CYT	Cytomegalovirus infection
PME	Progressive multifocal encephalitis
HIV	Retrovirus infection: HIV
Other VIR	Other viral diseases
	G. Non-viral infections
MENIN	Bacterial meningitis
ABS	Septic encephalitis and abscess
WP	Whipple disease
TB	Mycobacterial diseases: tuberculosis
NOACT	Nocardiosis, actinomycosis
SYPH	Neurosyphilis
Other BACT	Other bacterial diseases
TOX	Toxoplasmosis
Other PROT	Other protozoal infections
ARTH	Arthropod infections
METINF	Metazoal infections
FUNG	Fungal infections
MALF PRIM	**H. Primary brain and spinal cord malformations including disorders of cell migration**
MALF SEC	**I. Secondary malformations and destructive pathologies in the perinatal period**
TRAU	**J. Trauma**
	K. Tumors
TUM1	Primary tumors of the nervous system
TUM2	Secondary malignancies
PARANEO	**L. Paraneoplasic diseases**
MUSC	**M. Muscular diseases**

PNS	**N. Diseases of the peripheral nerves**
	O. Undetermined and non-specific lesions
HIPSCL	Hippocampal sclerosis
SUBGLIO	Subcortical gliosis of unknown origin
LEUKOARA	Demyelination of the white matter of the centrum semi-ovale
CAL	Calcifications
NOT CAT	**P. Disorders or lesions not categorized in the previous paragraphs**

3. SNOMED CODES

No neuropathological lesions	70671000135107
A. Degenerative diseases of the central nervous system	DA-20000
Alzheimer disease and amyloid angiopathies	70701000135106
Alzheimer disease stage I/II = NFT Braak stage I-II	
Alzheimer disease stage III/IV = NFT Braak stage III-IV	
Alzheimer disease stage V/VI = NFT Braak stage V/VI	
Alzheimer disease β-amyloid: stages A, B, C	
Alzheimer disease only amyloid plaques	71001000135104
Amyloid angiopathies non-alzheimer	71031000135108
Familial Alzheimer disease	71061000135100
Down syndrome	D4-02214
Tauopathies	71121000135105
Pick disease	DA-20021
Corticobasal degeneration	DA-21200
Progressive supranuclear palsy	DA-21055
Argyrophilic grain disease	DA-20017
Myotonic dystrophy type I	D4-00621
Frontotemporal lobar degeneration tau+	71151000135102
Familial frontotemporal lobar degeneration tau+	71181000135106
Other tauopathies including atypical tauopathies (tauopathy with globular inclusions in oligodendrocytes)	71211000135107
Frontotemporal lobar degeneration tau-	71241000135108
Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43+	71271000135104
Frontotemporal lobar degeneration tau-, ubiquitin-ir, TDP-43-, FUS+	71301000135101
Frontotemporal lobar degeneration with motor neuron disease	71331000135105
Frontotemporal lobar degeneration with no inclusions	71361000135102
NIFID	71391000135106
BIBD (basophilic inclusion body disease)	71421000135102
Alpha-synucleinopathies	71451000135105
Lewy body disease stage 1/2	
Lewy body disease stage 3/4	

Lewy body disease stage 5/6	
Amygdala-predominnat Lewy body disease	71661000135106
Atypical Lewy body disease	71691000135102
Familial Parkinson disease ***including PD without Lewy bodies***	71721000135109
Multiple system atrophy	DA-2105A
Prionopathies	71751000135101
Creutzfeldt-Jakob disease	DE-3B020
Variant Creutzfeldt-Jakob disease	DE-3B021
Familial Creutzfeldt-Jakob disease: genetic Creutzfeldt-Jakob disease	71781000135105
Fatal familial insomia	DE-3B040
Gerstmann-Sträussler-Scheinker syndrome	DE-3B030
Huntington disease	DA-21120
Other degenerative diseases of the basal ganglia	71811000135108
Spinocerebellar ataxias	DA-22005
Autosomal dominant spinocerebellar atrophy 1 (SCA1)	71841000135109
Autosomal dominnat spinocerebellar atrophy 2 (SCA2)	71871000135100
Autosomal dominant spinocerebellar atrophy 3 (SCA3)	71901000135100
Other autosomal dominant spinocerebellar atrophies	71931000135109
Dentatorubropallidoluysian atrophy (DRPLA)	DA-21220
Friedreich ataxia	DA-22010
Other autosomal recessive and sporadic ataxias	71961000135101
Motor neuron diseases	DA-23000
Sporadic amyotrophic lateral sclerosis (ALS)	71991000135105
Familial ALS	72021000135101
Lower motor neuron diseases	DA-23040
Upper motor neuron diseases	DA-23003
Primary axonal diseases with spheroids	
Hallervorden-Spatz disease	DA-21052
Neuroaxonal dystrophies	DA-20019
TDP-43 pathology (other than disease-specific: FTLD-TDP-43+, ALS)	72051000135109

B. Cerebrovascular and circulatory diseases	D3-89000
Global ischemia	72081000135100
Focal ischemia: unique infarction	72111000135106
Lacunae	72141000135107
Status cribosus	M-50551
Multi-infarct encephalopathy	72171000135103
Atherosclerosis (AT)	M-52110
Small vessel disease (SVD)	72201000135102
Binswanger encephalopathy	DA-25050
CADASIL	D3-89055
Haemorrhage extradural and subdural	72231000135106
Haemorrhage subarachnoidal	D3-89110
Haemorrhage intraparenchymal	72261000135103
Aneurisms and vascular malformations	72291000135107
Vasculitis	D3-80650
Degenerative vascular diseases (other than amyloid angiopathy, AT and SVD)	72321000135104
Gas and fat embolism	72351000135107
C. Nutritional and toxic diseases	
Wernicke encephalopathy	D6-C1150
Pellagra	D6-C1005
Vitamin B12 and folate deficiency	72381000135103
Late effects of alcohol in adults	72411000135101
Fetal alcohol syndrome	D4-000E2
Marchiafava-Bignami	DA-00070
Central pontine and extrapontine myelinolysis	72441000135100
Hepatic encephalopathy	D5-81110
Other toxics	72471000135109
D. Metabolic diseases	D6-00000
Lipidoses (excepting Krabbe and metachromatic leukodystrophy)	D6-6000F
Neuronal ceroid lipofuscinoses	D6-77100
Mucopolysaccharidoses	D6-70100
Polyglucosan diseases	72501000135104

Glycogenoses	D6-50200
Mucolipidoses	D6-70010
Leukodystrophies	DF-008EF
Adrenoleukodystrophy and other peroxisomal diseases	DB-70660
Disorders of amino acids	D6-A0000
Mitochondrial disorders	M-62635
E. Inflammatory diseases	DF-00A00
Multiple sclerosis	DA-25010
Acute disseminated encephalomyelitis	DA-10131
Guillain-Barre syndrome	DE-A1300
F. Viral infectious diseases	DE-30000
Herpes virus infection	DA-00081
Cytomegalovirus infection	DE-32610
Progressive multifocal encephalitis	DE-32A51
Retrovirus infection: HIV	DE-36300
Other viral diseases	72531000135108
G. Non-viral infections	
Bacterial meningitis	DE-10170
Septic encephalitis and abscess	DA-00034
Whipple disease	DE-19820
Mycobacterial diseases: tuberculosis	DE-14867
Nocardiosis, actinomycosis	DA-1040D
Neurosyphilis	DE-14450
Other bacterial diseases	72561000135100
Toxoplasmosis	DE-512FA
Other protozoal infections	72591000135109
Arthropod infections	DE-70000
Metazoal infections	DE-01762
Fungal infections	DE-40000
H. Primary brain and spinal cord malformations including disorders of cell migration	72621000135107

I. Secondary malformations and destructive pathologies in the perinatal period	72651000135104
J. Trauma	DF-00777
K. Tumors	M-8FFFF
Primary tumors of the nervous system	DA-F0003
Secondary malignancies	M-80006
L. Paraneoplastic diseases	72681000135108
M. Muscular diseases	D1-50008
N. Diseases of peripheral nerves	DA-40000
J. Undetermined and non-specific lesions	72711000135107
Hippocampal sclerosis	72741000135108
Subcortical gliosis of unknown origin	DE-3B050
Demyelination of the white matter of the centrum semi-ovale	72771000135104
Calcifications	DA-13071
K. Disorders or lesions not categorized in the previous paragraphs	72801000135101

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