SERVICE PORTFOLIO
NAVARRABIOMED
BIOBANK
2024

INTRODUCTION

Navarrabiomed Biobank belongs to the Miguel Servet Foundation. We offer human biological samples for biomedical research purposes as a service platform, providing scientific and technical support to the scientific community through various services. Our Biobank carries out its activity following the UNE-EN-ISO 9001:2008 quality standard, having obtained this certification in June 2012 and renewing it annually.

A detailed description for each service is provided further below.

CONTACT

To request Navarrabiomed Biobank´s services, call +34 848 4222673 (Opening hours: Monday to Friday from 8:00 a.m. to 3:00 p.m. Central European Time) or send an email to the following address: biobanco.navarrabiomed@navarra.es.

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SAMPLE DISTRIBUTION

The Navarrabiomed Biobank has active sample collection and distribution circuits for the following collections:

- Tumours
- Neurological tissues
- Type 1 diabetes mellitus
- Heart failure
- Cerebrospinal fluid
- Nephrology
- Oncology
- Asthma
- Acute pancreatitis
- Chronic pancreatitis
- Control: healthy donors
- Thrombus library
- Biliopancreatic tumours

In addition, we have the capacity to initiate new prospective sample collections and begin collecting the corresponding samples, depending on the client's needs.

To request samples, the researcher must send us the application form according to the standard model accompanied by a project report, the CV of the Principal Investigator, the favourable report from the Research Ethics Committee for the project and the funding entity of the project, if available. The application, together with the documentation, will be evaluated by the scientific and ethical committees to which the Biobank is attached.

Once the application has been approved, a sample transfer agreement will be signed in accordance with current legislation.

COLLECTION MANAGEMENT

Collection, processing and preservation of samples

We offer sample collection and processing services. The different types of processing carried out appear in the “Sample processing” section. Once processed, the biobank has the capacity to store the samples at room temperature, 4ºC, -20ºC, -80ºC and in liquid nitrogen, keeping track of the temperature record.

Custody of samples

The Navarrabiomed Biobank offers sample storage services, with the capacity to store samples at room temperature, 4ºC, -20ºC, -80ºC and in liquid nitrogen, keeping track of the temperature record.

Custody of data

The Navarrabiomed Biobank safeguards not only samples, but also data associated with these samples, or data from other samples not kept in the biobank, using specific software.

Preparing samples for shipping

The Navarrabiomed Biobank offers the service of preparing samples for shipment, with the possibility of carrying out this preparation based on the client's needs.

SCIENTIFIC-TECHNICAL ADVICE

The Navarrabiomed Biobank offers different options for scientific and technical advice. All this advice is provided with the ultimate goal of achieving the highest quality of samples and, therefore, facilitating research with quality results.

Preparation and review of sample collection and processing protocols

The Navarrabiomed Biobank prepares and reviews the protocols for sample collection and processing, taking into account the conditions required by the type of sample, coordination with the collection points, and the stages needed based on the required technical characteristics.

Participation, advice and support in research projects

The Navarrabiomed Biobank offers the possibility of participating in research projects, through support and/or advice on these projects.

Advice on the design of experiments with human samples

Thanks to the experience that the Navarrabiomed Biobank has in working with different types of human samples, we can advise on the experimental design of a project in which human samples are required, taking into account the number of cases, the number of samples and the type of samples required.

Advice on the design and interpretation of immunohistochemical studies

We offer advice on the experimental design of immunohistochemical studies, as well as the interpretation of the results obtained in these studies.

Advice on setting up techniques and launching collections

We provide advice on the development of techniques and offers the possibility of carrying out this development. Taking advantage of our knowledge in managing different collections, the biobank also advises on the launch of new collections.

Staff training

We offer staff training in all the activities it carries out, which are included in its portfolio of services. It also collaborates with various centres and universities by hosting staff for internships.

ETHICAL-LEGAL ADVICE

Review of research projects using samples

The Navarrabiomed Biobank provides advice on the review of research projects carried out using human biological samples. The advice includes the scientific-technological aspects of the collection, processing and conservation of said samples and/or the ethical-legal aspects of the collection, in order to guarantee that the essential ethical principles of patients and donors are respected.

Preparation and review of sample transfer agreements

We may prepare and review sample transfer agreements, in accordance with current legislation, based on the guidelines established by the Biobank Platform, applicable not only to biobanks but also to other institutions.

Preparation and review of information sheet and informed consent templates

We may offer the preparation and review of information sheet and informed consent forms, in order to convey to the donor in an understandable manner, the usefulness and destination of their samples and associated data.

SAMPLE PROCESSING

CELLULAR TECHNIQUES

Isolation of peripheral blood mononuclear cells (PBMC)

We isolate peripheral blood mononuclear cells (PBMC) from fresh whole blood using a Ficoll gradient. This technique allows the separation of blood components such as erythrocytes and leukocytes. After centrifugation, the blood components are separated from top to bottom as follows: plasma, PBMC layer, Ficoll-Paque and erythrocyte and granulocyte layer.

General characteristics of the service:

1- **Sample of origin:** Whole blood in a tube with anticoagulant.
2- **Necessary information of the sample required from the applicant:** Samples must be correctly identified and must contain the date and time of extraction.
3- **Required sample volume:** 10-15 mL of blood, preferably 12 mL.
4- **Shipping Conditions to the biobank:** The applicant must send the samples at room temperature in case the processing in the biobank is carried out before 4 hours after the sample extraction. Otherwise, keep samples at 4ºC until processing.
5- **Sample processing:** Blood must be processed before 12 hours after extraction. The processing is performed under sterile conditions.
6- **Quality control:** Quantification of the number of cells and viability tests (Tryptan blue). The resulting cell concentration is 10-15 million cells per millilitre.
7- **Sample preservation:** Cells are preserved in liquid nitrogen.
8- **Sample delivery:** Cells delivered with dry ice (freezing medium: 7.5-10% DMSO + FBS.

The number of cells obtained and their viability depend on many factors, such as the conditions of storage and transport of the samples and the time elapsed between blood extraction and the performance of the test. To obtain a considerable number of cells (10-15 million cells per millilitre), we recommend that the time between extraction and receipt of samples in the biobank does not exceed 12 hours.

MOLECULAR TECHNIQUES

Quantification of nucleic acids using Nanodrop

Nanodrop 1000 facilitates the quantification of DNA and RNA samples taking into account the absorbances at 260 and 280 nm. The main advantage is that a small volume (1-2 µL) of sample is required to perform the measurement. Thanks to the surface tension, the sample can pipetted directly onto the surface of the pedestal without the need to use any type of cuvette. This allows quantification of highly concentrated samples without the need to be diluted, accepting samples 50 times more concentrated than those required by standard methods with cuvettes.

General characteristics of the service:

1. **Sample of origin:** DNA and/or RNA.
2. **Necessary sample information required from the applicant:** Samples must be correctly identified.
3. **Required sample volume:** 2 µL of sample for each measurement. An aliquot of the diluting medium will also be requested.
4. **Shipping Conditions to the biobank:** The applicant must send the DNA samples at 4ºC or -70ºC. RNA samples must be sent in dry ice.
5. **Sample processing:** Measurement ranges for the DNA samples are between 2 and 3700 ng/µL, while for RNA samples they are between 2 and 3000 ng/µL.
6. **Quality control:** The following ratios are taken into account:
    - Ratio 260/280: optimal range 1.8-2.0.
    - Ratio 260/230: secondary indicator of the purity of nucleic acids.

DNA extraction (from whole blood, Buffy coat and PBMC)

Navarrabiomed Biobank offers the service of DNA extraction and quantification from whole blood, Buffy coat and PBMC. Extraction is carried out with the ArchivePure purification kit (5 Prime) and quantified with Nanodrop 1000. To do this, after the cell lysis and protein precipitation, isopropanol is added to achieve DNA precipitation. Finally, the DNA is hydrated and quantified.

General characteristics of the service:

1. **Sample of origin:** Whole blood in a tube with anticoagulant (preferably EDTA) / Buffy coat / PBMC.
2. **Necessary information about the sample required from the applicant:** The samples must be correctly identified and accompanied by the date and time of extraction.
3. **Required sample volume:**
    - Blood: 5 mL
    - Buffy coat: 500 µL
    - PBMC: Between 100 and 100 million cells. It is necessary to inform the biobank of the exact amount of PBMC.
4. **Shipping conditions to the biobank:** Whole blood samples can be sent at room Tª if the procedure is carried out no later than 4 hours after extraction. Otherwise, store them at 4ºC. Buffy coat and PBMC samples are sent frozen.
5. **Sample processing:** DNA extraction is achieved by manual alcohol precipitation.
6. **Sample standardization:** According to the applicant's requirements.
7. **Quality control:**
    - Quantification by Nanodrop 1000 (DNA range: 2-3700 ng/µL)
    - Absorbance ratio 260/280
8. **Sample storage:** Stored at -80ºC.
9. **Sample delivery:** Delivered at 4ºC or frozen in dry ice, as required by the applicant.

After DNA extraction, the concentration and the ratio between the absorbance at 260nm and that obtained at 280nm (ratio 260/280) will be measured by spectrophotometry with Nanodrop 1000. With this technology, we can obtain important information from each sample considering its concentration. Our quality control considers an optimal range of the absorbance 260/280 ratio between 1.8 and 2.0, and an acceptable range from 1.7 to 2.0.

ISOLATION OF BLOOD COMPONENTS

For serum, plasma, and Buffy coat isolation there are parameters such as the time and speed of centrifugation that may vary depending on the type of sample or the protocol followed. Navarrabiomed Biobank adapts these parameters to the protocol established by the client.

Plasma isolation

Plasma isolation is carried out by centrifuging blood samples collected in tubes with anticoagulant, either EDTA, ACD or heparin.

General characteristics of the service:

1. **Sample of origin:** Whole blood in a tube with anticoagulant.
2. **Necessary information of the sample required from the applicant:** Samples must be correctly identified and accompanied by the date and time of extraction.
3. **Shipping Conditions to the biobank:** The applicant must send the samples at room Tª or at 4ºC for periods longer than 4 hours between the extraction and the processing of samples.
4. **Sample processing**: It is recommended to process the sample within the first 2 hours from the extraction.
5. **Sample storage:** The plasma is stored at -80ºC.
6. **Sample delivery**: Samples are delivered frozen in dry ice.

Serum isolation

Serum isolation is carried out by centrifuging blood samples collected in tubes containing coagulation-activating particles. This is done in a centrifuge refrigerated between 15 ºC and 24ºC.

General characteristics of the service:

1. **Sample of origin:** Whole blood in a tube without anticoagulant.
2. **Necessary information of the sample required from the applicant:** Samples must be correctly identified and accompanied by the date and time of extraction.
3. **Shipping Conditions to the biobank:** The applicant must send the samples at room Tª or at 4ºC for periods longer than 4 hours between the extraction and processing of the samples.
4. **Sample processing:** It is recommended to process the sample between 30 minutes and 2 hours after the extraction, the optimal time being 1.5h ± 30min.
5. **Sample storage:** Samples are stored at -80ºC.
6. **Sample delivery:** Samples are delivered frozen in dry ice.

Buffy coat isolation

Buffy coat isolation is carried out by centrifuging blood samples collected in tubes containing anticoagulant, EDTA, ACD or heparin.

General characteristics of the service:

1. **Sample of origin:** Whole blood in a tube containing anticoagulant.
2. **Necessary information of the sample required from the applicant:** Samples must be correctly identified and accompanied by date and time of extraction.
3. **Shipping Conditions to the biobank:** The applicant must send the samples at room Tª or at 4ºC for periods longer than 4 hours between the extraction and processing of the samples.
4. **Sample processing:** It is recommended to process samples within the first 2 hours from the extraction.
5. **Sample storage:** Buffy coat is stored at -80ºC.
6. **Sample delivery:** Samples are delivered frozen in dry ice.

COLLECTION, PROCESSING AND STORAGE OF TISSUE SAMPLES

Tissue freezing in cryomold/OCT

We offer the possibility of preserving tissue samples in cryomolds at -80ºC using OCT. OCT is an embedding medium that protects the tissue during the freezing process and guarantees the optimal temperature for making sections of tissue in a cryostat.

General characteristics of the service:

1. **Sample of origin:** Fresh tissue.
2. **Necessary information about the sample required from the applicant:** Samples must be correctly identified and accompanied by date and time of extraction.
3. **Shipping Conditions to the biobank:** Specific conditions for each tissue.
4. **Sample processing:** Ideally, the sample should be frozen no later than 30 minutes after the tissue has been extracted.
5. **Sample storage:** Samples are stored at -80ºC.
6. **Sample delivery:** Samples are delivered frozen in dry ice.

If required, haematoxylin/eosin and PAS stains can be performed as quality control of tissue freezing in the cryomold.

Freezing of fresh tissue

Fresh tissues are quickly frozen by immersion in liquid nitrogen to prevent the formation of crystals and the restructuring of the tissue. Samples are then stored at -80ºC.

General characteristics of the service:

1. **Sample of origin:** Fresh tissue.
2. **Necessary information about the sample required from the applicant:** Samples must be correctly identified and accompanied by the date and time of extraction.
3. **Shipping Conditions to the biobank:** Specific conditions for each tissue.
4. **Sample processing:** Ideally, samples should be frozen no later than 30 minutes after the tissue has been extracted.
5. **Sample storage:** Samples are stored at -80ºC.
6. **Sample delivery:** Samples are delivered frozen in dry ice.

Tissue processing and paraffin block production

We offer the service of processing tissues from extraction to paraffin blocks, including tissue fixation and dehydration. Any of these three processes (fixation, dehydration and production of the blocks) can be ordered independently.

Regarding fixation, the stage that allows tissues to be preserved, we use formalin and/or formaldehyde as a fixative. The tissue is fixed immediately after extraction; the time elapsed being normally less than 30 minutes.

After fixation, tissues are dehydrated to finally produce the paraffin blocks. Once solidified, histological sections are made with a manual or automated microtome.

General characteristics of the service:

1. **Sample of origin**:
    - Fresh tissue to carry out the three stages.
    - Tissue already fixed for dehydration and obtaining blocks.
2. **Necessary information of the sample required from the applicant**: Samples must be correctly identified and accompanied by the date and time of extraction.
3. **Shipping Conditions to the biobank**: Specific conditions for each tissue.
4. **Sample processing**: Fixation of the samples no later than 30 minutes after the extraction of the tissue.
5. **Sample storage:** Paraffin blocks at room temperature.
6. **Sample delivery**: Samples delivered at room temperature.

Haematoxylin/eosin and PAS stains are also performed to carry out quality control of tissue fixation and paraffination.

HISTOLOGY: SAMPLE PROCESSING AND STAINING

Histological imaging

We offer the service of capturing images of histological preparations with optical microscopy. This allows the detection of areas of interest. For this purpose, an Olympus BX1TF triocular optical microscope is used, as well as an Olympus DP73 digital photomicrography system with the image capture analysis software cellSens Entry.

General characteristics of the service:

1. **Sample of origin:** Sections of the sample on slides with the desired staining.
2. **Necessary information about the sample required by the applicant:** Samples must be correctly identified.
3. **Shipping Conditions to the biobank:** Slides are sent at room Tª.
4. **Sample storage:** Slides are stored at room Tª.
5. **Sample delivery:** Slides and digital files of the images obtained.

Histological sections from samples embedded in OCT

Once the sample has been frozen in OCT, we offer the service of making histological sections of these samples using the cryostat. These sections will have different thicknesses depending on the subsequent use of said sections, either for histochemical, immunohistochemical/immunofluorescence techniques, or for DNA and RNA extraction.

General characteristics of the service:

1- **Sample of origin:** Frozen tissue.
2- **Necessary information of the sample required from the applicant:** Samples must be correctly identified.
3- **Shipping Conditions to the biobank:** Samples must be sent frozen.
4- **Sample storage:** Stored at -80ºC until delivery.
5- **Sample delivery:** Delivered in dry ice.

Haematoxylin/eosin and PAS are performed for quality control of the histological sections.

Histological sections from paraffin-embedded samples

Once the paraffin blocks have been made, we offer the service of making histological sections of these samples using a Leica RM2255 microtome, either for histology purposes or to obtain DNA and RNA samples. These sections can range from 2 to 15 µm, with 3 µm being the most common for samples from histological techniques.

General characteristics of the service:

1- Sample of origin: Tissue in paraffin blocks.

2- Necessary information of the sample required from the applicant: Samples must be correctly identified.

3- Shipping Conditions to the biobank: Samples are sent at room Tª.

4- **Sample storage**: Storage at room temperature until delivery.

5- Sample delivery: Samples are delivered at room Tª.

Haematoxylin/eosin and PAS stains are also performed to carry out quality control of the histological sections.

Sample staining

Different types of staining are performed on both paraffin blocks and frozen OCT samples. For paraffin block samples, prior treatment is required, consisting on the removal of the paraffin with an organic solvent and hydration with an increasing series of alcohols. However, OCT samples do not require any pre-treatment. Some staining performed may be:

- **Haematoxylin-eosin:** to stain nuclei in blue and cytoplasm in pink.
- **Luxol:** stains myelin blue.
- **Gallyas (silver stain):** allows detection of small amounts of proteins and nucleic acids.
- **Congo red:** allows protein staining, staining amyloid with an intense red colour.
- **PAS:** allows magenta red staining of carbohydrates and glycosylated proteins.
- **Perls' Prussian blue:** stain that allows detection of ferric ion (Fe3+) in tissue preparations, blood smears or bone marrow smears.

General characteristics of the service:

1. **Sample of origin:** Sample sections on a slide.
2. **Necessary information of the sample required from the applicant:** The applicant must specify: type of tissue, type of preservation, sample identification, type of staining to be performed, number of sections to be stained and their thickness.
3. **Shipping Conditions to the biobank:**
    - Paraffin samples: room Tª.
    - OCT samples: dry ice.
4. **Sample processing:**
    - Paraffin samples: deparaffinization and hydration of the samples before staining.
    - OCT samples: do not require any treatment prior to staining.

5. **Sample storage:** Room Tª.
6. **Delivery of the samples:** Samples are delivered at room Tª.

We will send the stained slides, a technical report with images, the methodology used and the results of samples. We also offer the possibility of adapting each of the techniques to the needs of each client as well as performing other types of staining.

Immunohistochemistry

We have the capacity to carry out both immunohistochemical determinations and the fine-tuning of these techniques. To do this, we use an automated system that allows the staining of immunohistochemical tests. Typically, a secondary antibody, marked with a visible substance, recognises the primary antibody that binds to the antigen of interest.

This process is automated using the Leica Bond-Max immunostainer. The Leica Bond kit provides the polymers that act as the secondary antibody. Navarrabiomed Biobank has the capacity to fine-tune the technique with any primary antibody that does not generate interactions, in addition to offering positive and negative controls for each specific antibody.

General characteristics of the service:

1- **Sample of origin**: Sample sections on a slide.
2- **Necessary information about the sample required by the applicant:** Samples must be correctly identified.
3- Shipping Conditions to the biobank:
   - Paraffin samples: room Tª.
   - OCT samples: -80ºC.
4- Sample storage: Room Tª.
5- **Sample delivery:** Room Tª.

Immunofluorescence

We also have the capacity to carry out both immunofluorescence determinations and the fine-tuning of these techniques. To do this, a manual procedure is carried out, either directly, with an antibody marked with a fluorophore which binds to the antigen of interest, or indirectly, where a secondary antibody marked with a fluorophore recognises the primary antibody that binds to the antigen of interest. This technique can be performed using different types of antibodies and fluorophores.

General characteristics of the service:

1- **Sample of origin:** Sample sections on slides.
2- **Necessary information about the sample required by the applicant:** Samples must be correctly identified.
3- Shipping Conditions to the biobank:
   - Paraffin samples: room Tª.

- OCT samples: -80ºC.

4- **Sample storage:** At room Tª.
5- **Sample delivery:** At room Tª and in dark conditions.

OBTAINING, PROCESSING AND PRESERVING NEUROLOGICAL TISSUE

We offer the service of obtaining, processing, and preserving post-mortem neurological tissue. Within this group of services, several actions are carried out, which are detailed below.

Capturing microscopic and macroscopic images

Macroscopic images of neurological tissues are taken when the tissue is received and during the sectioning and preparation of these tissues for freezing or inclusion. These macroscopic images are taken with the Nikon D5300 digital camera, which is fitted with a 40mm DX-format Nikkor AF-S lens. The aim is to spot potential anomalies in the tissue, which can help the client in their study.

Microscopic images of the histological sections of these neurological tissues are also captured, as detailed in the section “Capturing histological images”.

Prion screening by immunohistochemistry

We offer as a service prion screening through immunohistochemistry. This is done in order to detect whether the sample being treated comes from a patient with prion disease or not. To do this, the process explained in “Immunohistochemistry” is followed, always taking the appropriate biosecurity measures.

Histochemistry and immunohistochemistry of neurological tissue

We offer the service of histochemistry and immunohistochemistry of neurological tissue to perform neuropathological diagnosis. Paraffin blocks of the required anatomical regions are made following the procedure explained in the section “Tissue processing and preparation of paraffin blocks”. Histochemical and immunohistochemical stains are then performed as described in the sections “Sample staining” and “Immunohistochemistry”, respectively.

Cerebrospinal fluid (CSF) processing

We separate cerebrospinal fluid (CSF) from the rest of the cells contained in the fluid extracted from the brain, such as erythrocytes and leukocytes. To do this, the sample is centrifuged, the speed and temperature of which will depend on the client's request and the origin of the CSF. Once centrifuged, the CSF is quickly frozen at -80ºC.

General characteristics of the service:

1- **Sample of origin:** CSF sample.
2- **Necessary information of the sample required from the applicant:** The samples must be correctly identified, and accompanied by the date and time of extraction.
3- **Shipping Conditions to the biobank:** Send the CSF no later than 2 hours after extraction.
4- **Sample storage:** Stored at -80ºC.
5- **Sample delivery:** Sample is delivered frozen in dry ice.

DNA extraction from CSF

The CSF is processed as explained in section "CSF processing". For DNA extraction and quantification, the procedure is described in the "DNA Extraction" section.

General characteristics of the service:

1- **Sample of origin:** CSF or cell pellet obtained from CSF.
2- **Necessary information of the sample required from the applicant:** Samples must be correctly identified and accompanied by the date and time of extraction.
3- **Shipping Conditions to the biobank:**
    - Cells: frozen at -80ºC.
    - Liquid samples: They must not be sent later than 2 hours after extraction.
4- **Quality control:**
    - Quantification by Nanodrop 1000 (DNA range: 2-3700 ng/µL)
    - Abs ratio 260/280
5- **Sample storage:** Stored at -80ºC.
6- **Delivery of samples:** Samples delivered at 4ºC or frozen in dry ice as required by the applicant.

After DNA extraction, the concentration and the 260/280 ratio will be measured by spectrophotometry using the Nanodrop 1000.

OTHER SERVICES: upon request

For other similar services that do not appear in this portfolio of services, the Navarrabiomed Biobank will study each case in particular and assess its viability. To do so, the service request must be sent to the following email address: biobanco.navarrabiomed@navarra.es