"Sampling and storage of biological material for collaborative studies"

General introduction

These Guidelines describe the different necessary steps to ensure the collection, storage, shipment and tracking of the human biological samples, as well as the phases from samples collection to delivery of the material in order to:

1) ensure adequate packaging, storage, and dispatch of the biological material;

2) ensure appropriate management and timely treatment of the biological samples;

3) ensure correct management of the documentation that highlights the process and ensures traceability;

SAMPLING FOR TRANSLATIONAL RESEARCH ACTIVITIES

Incisional biopsy is not mandatory but allows to collect more pathological tissue than CT-guided needle biopsy, for the purpose of a correct histological analysis and to acquire tissue for the tissue Biobanks and for the Research Laboratories, in order to carry out scientific studies to understand sarcomatous pathogenesis and to identify new therapeutic targets and personalized therapeutic strategies.

On the other hand, surgical specimens collected after chemotherapy frequently show high percentage of necrotic tissue.

SHIPPING SPECIMENS FROM SURGERY ROOM TO PATHOLOGY LABORATORY

1 FORMALIN-FIXED TISSUE: specimens can be conserved in neutral 10% formalin; this method is not the gold standard for translational research

2 FRESH TUMOR TISSUE: this is the favorite method for tissue sampling, since it allows to preserve; see following instructions

a) the biological material must be inserted inside a vacuum packaging bag of suitable size, at controlled temperature (4° C).

b) each vacuum packaging bag must be labeled and sent to the laboratory of reference as soon as possible; (within 48 hours from the surgical explant)

3 FRESH TUMOR TISSUE WITHOUT VACUUM : the sampling, must be sent immediatly to laboratory in order to preserve and conserve in a properly method

FORMALIN-FIXED, PARAFFIN-EMBEDDED (FFPE) TISSUE SAMPLES

Formalin-fixed, paraffin-embedded (FFPE) tissue is suitable for genetic analysis. Compared to frozen tissue, working with FFPE may offer some advantages:

1) In order to obtain fresh tissue to be frozen and stored in Biobanks, biopsies and surgical specimens must arrive in ~~the~~ Pathology Dept. laboratories without formalin Conversely, FFPE blocks are obtained also from material that arrives to the Pathology Dept. laboratories fixed in formalin, as happen in many laboratories.
2) FFPE blocks are routinely stored and kept in the Pathology Dept. laboratories for many years, thus they can generally be easily retrieved. FFPE blocks should be kept at a temperature between 19°C and 27°C, humidity between 30% and 70%, and with control on parasites and flooding; there is no need of -80°C freezers.
3) FFPE blocks can easily sent by normal mail, by using a box that prevent the blocks from being crushed. The material can travel at normal temperature, only avoiding the exposure to high temperature that can melt the paraffin.

On the other hand, the big disadvantage in using FFPE tissue instead of fresh tissue is the lower quality of the material obtained for the genetic analyses.

The most important problem when using FFPE material from bone tumors is the possible decalcification of the tissue, since the use of decalcifying solutions containing strong acids (i.e. nitric, hydrochloric) generally destroys DNA and RNA, thus precluding the possibility to perform genetic analysis. In order to avoid this problem, decalcifying solutions containing weak acids (that preserve the possibility to perform genetic analysis in a good percentage of cases Anyhow, during the gross sampling procedures, pathologists should spare, when possible, the softest parts of the tumor from being decalcified.

PREPARATION OF SPECIMENS FOR BIOMOLECULAR AND GENETIC ANALYSES FROM ARCHIVED FFPE BLOCKS

1) Collect 3-5 slices from each FFPE block with a thickness of 10 µm and place them in a 1.5 ml labeled eppendorf tube. The FFPE block selected for the preparation of the slices must be chosen by an expert pathologist, who should also label on the slides the representative tumoral viable areas.

2) Eppendorf tubes containing FFPE tissue slices should be stored at room temperature until use or shipment.

VITAL FREEZING AND STORAGE OF TUMOR TISSUE SAMPLES

The whole procedure must be performed inside a class II biosafety cabinet using sterile and aseptic reagents.

- Put tissue sample(s) in cryovial(s) and add in each tube with 0.5 ml of pre-chilled fetal bovine serum (FBS)

- Cool the vial(s) in ice for 10 minutes

- Add SLOWLY (drop by drop) 0.5ml of pre-chilled freezing medium (80% FBS + 20% DMSO) to the cryovial(s) and mix gently (final mix: FBS + 10% DMSO).

- Place immediately the cryovials at -80 °C inside a polystyrene box

- After 12-72 hours, transfer the cryovial(s) to liquid nitrogen.

- All processed samples must be sent in dry ice.

INSTRUCTIONS FOR BLOOD SAMPLES COLLECTION

Reagents and solutions required

- FICOLL-HYPAQUE (Lymphoprep)
- Dimethyl sulfoxide (DMSO)
- RPMI-1640
- Fetal bovine serum (FBS)
- Trypan blue (for cell count)
- Freezing medium: 80% FBS + 20% DMSO (e.g. 0.8 ml FBS + 0.2 ml DMSO)

Material and instrumentation required

- Class II biosecurity hood
- Benchtop centrifuge for 15 ml tubes
- Microcentrifuge
- 15 ml tubes
- Cryovials (for PBMC)
- Eppendorf 1.5ml or 2ml (for FFPE tissue slices)
- Optical microscope for cell counting (or alternatively automatic cell counter)
- Refrigerator + 4 ° C
- Container for temporary storage of cryovials at -80 ° C
- Freezer -80 ° C
- Liquid nitrogen (LN2) in suitable cryogenic containers

Whole blood should be collected in blood-dedicated tubes.

Once received, tubes of blood samples (5-10 ml for each tube) must be checked for integrity and labeling. The following samples must be rejected:

- Clotted samples;

- Hemolyzed samples;

- Leaking Samples (evaluate whether the sample is usable or not on the basis of the remaining volume).

- Samples unlabeled or incorrectly labeled.

ISOLATION AND STORAGE OF SERUM FROM BLOOD SAMPLES

- After collection of the whole blood, allow the blood to clot by leaving it upright and undisturbed at room temperature for 30-45 min (max. 60 minutes)

- Separate the clot from the serum by centrifuging at 2,000g for 10 minutes at room temperature

- Immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette

- The serum should be apportioned into 1 mL aliquots, stored at -20°C or colder until ready for the shipment

- Serum samples must be shipped in dry ice

ISOLATION AND STORAGE OF PLASMA FROM BLOOD SAMPLES

- Samples must be processed inside a class II biosafety cabinet using sterile and aseptic reagents.

- Centrifuge the tubes with blood at 800 g (or RCF) for 10 minutes at 18-25 ° C.

- After centrifugation, aspirate the plasma taking care not to disturb the lower cell layer.

- Taking the volume into account, transfer the plasma into a single 15 mL conical tube.

- Complete the plasma processing by centrifuging the collected plasma at 800g for 10 minutes. This second centrifugation will remove any cell debris.

- Aliquot 1 ml of plasma into 1 pre-labeled cryovial

- Store the plasma at -80 ° C.

- Plasma samples must be shipped in dry ice

ISOLATION AND STORAGE OF LYMPHOCYTES AND PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) FROM BLOOD SAMPLES

To isolate lymphocytes and peripheral blood mononuclear cells (PBMC), blood tube(s) will be processed according to the Ficoll Paque method (the procedure will take about 2-3 hours).

- Samples must be processed inside a class II biosafety cabinet using sterile and aseptic reagents.

- Estimate the volume of blood / RPMI mix in each tube.

- Add to each new tube a volume of FICOLL-HYPAQUE equal to the estimated volume of blood / RPMI mix for each collection (ficoll-hypaque ratio: blood mix = 1: 1). *For example, for an estimated 6 mL of blood / RPMI mix, put 6 mL of ficoll-hypaque into the tube.*

- Slowly (drop by drop along the edge) add the blood / RPMI mix to the ficoll-hypaque. Be very careful not to mix blood and ficoll-hypaque.

- Immediately centrifuge 800g for 10 minutes without the brake, at 18-20 ° C.

- Use a 2ml sterile pipette to aspirate the white PBMC rings, combine them and transfer them to a new 15ml tube. Fill the tube with RPMI.

- The lymphocytes/PBMC layer will be centrifuged/washed in RPMI, and the pellets stored as described below.

FREEZING AND STORAGE OF LYMPHOCYTES/PBMC PELLETS AT -80°C

- Check that the cells have been pelleted, otherwise repeat the centrifugation.

- Discard the supernatant and resuspend the pellet in 1 ml of RPMI.

- Transfer the PBMCs resuspended in RPMI to a 1.5 ml cryovial and centrifuge in a microcentrifuge for a maximum of 2 minutes at 18-25 ° C.

- Check that the cells have been pelleted, otherwise repeat the centrifugation.

- Discard supernatant and store pellets at -80°C

- All processed samples must be sent in dry ice.

VITAL FREEZING AND STORAGE OF LYMPHOCYTES/PBMCS

The whole procedure must be performed inside a class II biosafety cabinet using sterile and aseptic reagents.

- Centrifuge tubes with the PBMCs for vital storage for 10 minutes at 250g at 18-20°C.

- Completely discard the supernatant.

- Resuspend the cells in each tube with 0.5 ml of pre-chilled FBS and transfer them to pre-labeled cryovials

- Cool the cells in FBS at 4 ° C (refrigerator) for 30 minutes.

- Then add SLOWLY (drop by drop) 0.5ml of pre-chilled freezing medium (80% FBS + 20% DMSO) to the cryovials and mix gently (final mix: FBS + 10% DMSO).

- Place immediately the cryovials at -80 °C inside a polystyrene box.

- After 12-72 hours, transfer the cells to liquid nitrogen.

- All processed samples must be sent in dry ice.

INSTRUCTIONS FOR PACKAGING AND SHIPMENT OF FROZEN MATERIAL

Frozen tissue, blood and blood-derivates samples must be sent frozen, by following these shipping rules:

- Place the cryogenic tubes in an absorbent rack, then in the sample shipping pouch. Seal the envelope.

- Place the sample shipping bag in the bottom section of the frozen shipping package. Do not use bubble envelopes used for shipping material at room temperature.

- Place approximately 3.5 kg of dry ice directly on top of the sample mailing bag. For bulk shipping, you can place more than one sample shipping bag or cryogenic container (if equipped) in the same shipping box.

- Place the polystyrene lid on top of the thermal insulation package for shipping frozen material.

- Indicate the amount of dry ice and package information for shipping on the white and black outer shipping package label.

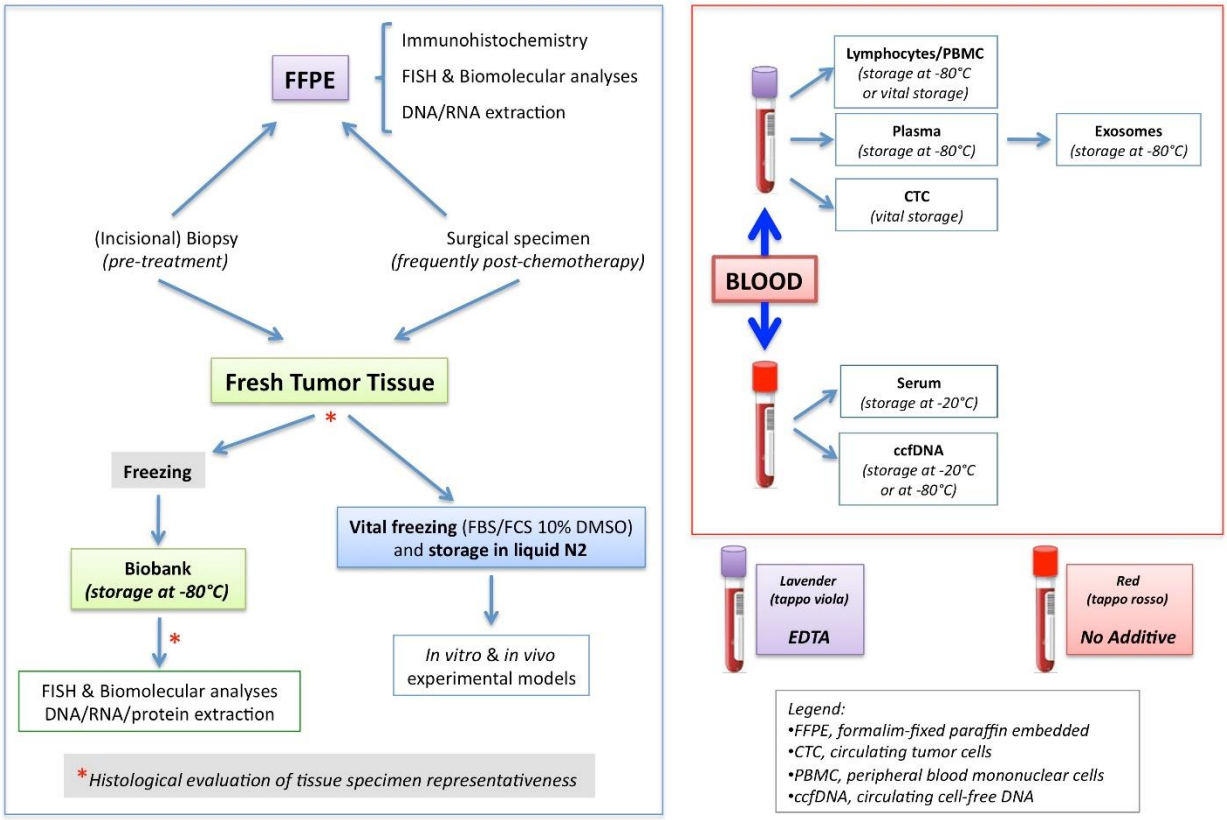
ETHICAL ISSUES: Ethic Committee approval and signed informed consent must be available when necessary. Signed Material Transfer Agreement (MTA) and rules for protections of data must be defined between the provider and the recipient. This item should be discussed inside the working group, in order to try to define a unique MTA format.

Bibliography

-Linee Guida per la Tracciabilità, Raccolta, Conservazione e Archiviazione di cellule e tessuti per indagini diagnostiche di Anatomia Patologica (Ministero della salute, Consiglio Superiore di sanità, 2015)

FIGURA RAPPRESENTATIVA DEI DIVERSI PROCESSI

Sampling and treatment of biological material for collaborative studies inside the Working Group Musculoskeletal Tumors



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Vers 1.0 20Apr2022 redatta da:

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