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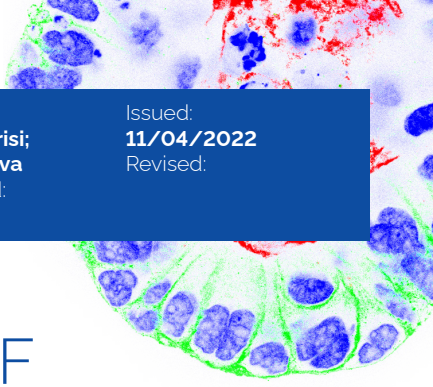

PROCESSING AND CONSERVATION OF HUMAN TUMOR TISSUE

Purpose

The purpose of SOP 1.0 is to describe how to collect, to process and to send tissue and haematological samples to the two project centres* for cultivation and subsequent long-term storage in a biobank of organoids, lymphocytes and monocytes from patients with cancer pathologies and other cells characterising the tumour microenvironment such as fibroblasts.

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Scope

SOP 1.0 is intended to cover all resources, personnel and equipment for collecting, processing and sending samples.

Introduction

Fresh material from surgically resected primary or metastatic tumors (both untreated and treated patients) collected during surgery is immediately transported to the Anatomy and Pathological Histology Department placed in the Hospital of Bolzano. The unit collects specimen to perform routine histopathological analysis of the tumor sample and then provides biologist with a piece (ca. 1cm3) of fresh tumor tissue for the cultivation of organoids. When possible, a skin sample tissue and a normal tissue were collected if available. Blood samples are collected (20 ml) 24 hours before surgery and approximately one month after surgery. Ascitic fluid and pleural effusion fluid were collected from patient with tumor at any time.

1. Cell culture media, reagents and solutions

- 1x PBS without calcium and magnesium (Sigma D1408)
- Cell culture freezing medium (Sigma S-002-10F)
- RPMI-1640 medium, HEPES modification, WI (Sigma R5886-500ML)
- L-Glutamine solution BIOXTRA, 200 MM, SO (Sigma G7513-100 ML)
- Antibiotic-antymicotic (100 x) (Sigma A5955)

- Ethylenediaminetetraacetico acid dipotassium magnesium salt for complexometry 98% 100 GR. (Fisher Scientific 33350)
- Ficoll -Paque PREMIUM 1.084 (Sigma cat. no.17-5446-02)
- DMSO (Sigma-Aldrich, cat. no. D8418)
- Fetal bovine serume (FBS) (Sigma F2442)
- Steril water (B. Braun 3521390)

2. Equipment

- Fridge +4°C (ACF refrigeration mod. EKV 160-TFT)
- Freezer -80°C (KW apparecchi scientifici, mod. KWK 568 PL)
- Refrigerated centrifuge (Eppendorf, mod. 5702 R)
- Sterile cell-culture cabin (STERIL-PBI, mod. Maxi Triofilter)
- Dry Ice
- Pipet volume 100-1000 µl (Gilson)
- Tips C/F (Gilson 50:1000 x96)
- Automatic pipet aid for large volume Levo plus (DLab)
- PS Sterile pipets C.S. ml 5
- PS sterile pipets C.S. ml 10
- Pasteur sterile pipets ml 3
- 500 ml. FBS (Sigma F2442)
- Petri dishes D.100 (Euroclone)
- Scalpels N.15 (CARBON S. xl00)
- Cryo-conservation vials; 2ml (VWR, cat. no. 479-0287)
- Conic tubes (Falcon, 50ml)
- Conic tubes (Falcon 1,5 ml)
- Blood collection tubes, Vacuette Premium, K3E K3EDTA 4 ml pink cap (Greiner Bio-One ref: 454099)

3. Reagent setup

- Human samples: Tissue samples for cancer research can be obtained by a trained surgeon. At least 1 cm3 of tissue was collected and processed within 3 hours. The tissue was kept cold at 4 °C until processing.
- Cell freezing solution: this solution is prepared by gradually adding 10% of DMSO to 90% of FBS.
- RPMI complete medium: this solution is prepared by adding 50ml FBS and 5ml antibiotic/antimycotic solution 445ml RPMI-1640 medium.
- Solution for ascitic or pleural effusion fluid: 10% solution of potassium EDTA in distilled water. Add 10 µl of this solution to every 1 ml of processed liquid.

4. Procedure

4.1 Human sample dissociation (normal, skin or tumor tissues)

- Tissue collection. After surgical excision, the tissue should be kept in a steril container on ice and processed within 3 hours.
- In a sterile cell culture hood, tissue is placed in a sterile 100-mm Petri dish. 2 ml cold FBS is added and tissues are mince it into small pieces using scalpels (See *Figures 1 and 2*).
- Processed material is transferred in cryotubes (in 800 µl aliquots).
- Add 200 µl ice cold freezing medium.
- After inverting tubes twice, the cryotubes are stored in a -80°C freezer until the shipping to research laboratories.
- Samples were collected and periodically shipped to Trieste partner

4.2 PBMCs isolation

- Collect 20 ml blood sample in 4 ml blood collection tubes containing EDTA
- Gentle invert the tubes
- Dilute blood 1:1 in complete RPMI complete medium (used for cell lines) in a 50ml sterile tube and resuspend gently.

- Layer cell suspension on Ficoll - Paque PREMIUM for lymphocyte preparations as follows:

    In a new 50ml tube put half a volume of Ficoll - Paque PREMIUM (compared to the volume of blood already diluted in RPMI complete medium);

    Slowly add the diluted blood on top of Ficoll - Paque PREMIUM, tilting the tube about 45° and leaning the pipet tip against the wall. At this stage it is important that the two solutions remain as separate phases.

- Centrifuge at 2000 rpm for 20 minutes at 25°C with an acceleration of 1 and deceleration of 0.
- At the end of the centrifugation, 4 phases should be distinguished (from bottom to top: red blood cells, Ficoll, a thin ring of mononuclear cells, serum) (See *Figure 3*)
- Proceed as follows:
- Remove the serum taking care not to aspirate the ring of white cells using a 5ml pipette.
- Using a p1000 pipette, aspirate the ring of cells by making gentle circular movements around the inner perimeter of the falcon (to aspirate the ring of cells evenly). Take care not to aspirate other components, especially avoid contamination with red blood cells located in the bottom layer;
- Transfer blood monocytes to a 15 ml Falcon tube and add prewarmed complete medium until reaching 12 ml. Resuspend gently.
- Centrifuge at 2000 rpm for 5 minutes at 25° C with acceleration equal to 6 and deceleration equal to 5.
- Carefully discard the supernatant be decanting
- Resuspend cell pellet carefully in 10 ml of prewarmed 1xPBS (Ca^{2+} and Mg^{2+} free). Centrifuge at 2000 rpm for 5 minutes at 25°C with acceleration equal to 6 and deceleration equal to 5.
- Discard supernatant and repeat wash of pellet with prewarmed 1xPBS.
- Proceed to freeze the PBMCs in cryovials by resuspending the pellet in 1.5ml of freezing medium using cryotubes.
- Store in -80°C freezer until the shipment to project partners.
- Ship periodically to Trieste partner

5. Shipment of biological material

5.1 Human tissues sample and Isolated PBMCs

- Contact Trieste partner for shipment
- Request dry ice from the Bolzano hospital workshop
- Put the samples on dry ice, close the package and hand over to the courier at the agreed time and day.

5.2 Ascitic or pleural effusion Fluids

- Contact Trieste partner for shipping
- If shipment is organized fort the same day like the collection of patient material, put the samples on refrigerated box (4°C) and hand over to the courier at the agreed time.
- If shipped the day after collection, add 10 µl of 10% of potassium EDTA solution to every ml of collected fluid (see point 6.0 of this SOP).
- Store at +4°C
- Put the samples on refrigerated box and hand over to the courier at the agreed time of the day after.

6. Figures

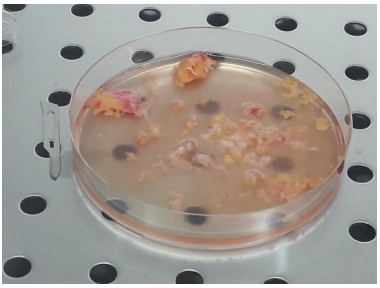


Figure 1. Representative image of dissected tumor sample

Figure 2. Representative image of the procedures of tumor sample dissection

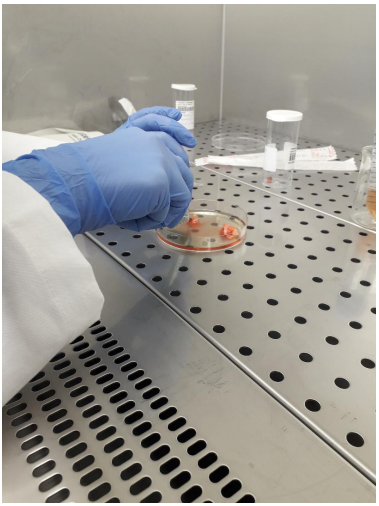
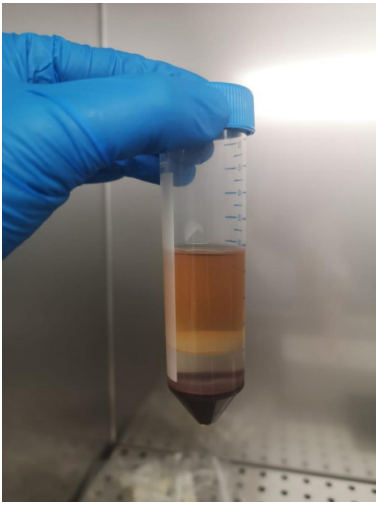


Figure 3. Representative image of fase separation during PBMCs extraction from whole blood



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