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	Title: **Pleural Fluid, Ascitic Fluid, Bone Marrow Fluid, Cerebrospinal Fluid Specimen Collection and Processing Process**	Version/Total Pages: No. 1.1 / 5 pages
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1. Purpose

"National Biobank Consortium of Taiwan" (hereinafter referred to as the "Integration Platform") primarily integrates domestic human biobanks — their data, biospecimens, and related information (hereinafter referred to as "Integration Platform biospecimen data") — through a cloud-based system, making them available for application and use by medical, clinical, industry, and related biomedical research investigators nationwide. To achieve cross-institutional collaboration, it is necessary to establish consistent quality standards for dispensed biospecimens and consistent clinical data content. To maintain good specimen quality and to ensure consistency in the collection and processing procedures for various bodily fluid specimens, such as pleural fluid, ascitic fluid, bone marrow fluid, and cerebrospinal fluid specimens, so as to facilitate future related research, this Standard Operating Procedure is hereby established.

2. Scope of Application

This SOP applies to the collection and processing procedures for pleural fluid, ascitic fluid, bone marrow fluid, cerebrospinal fluid, and other specimens.

3. Responsibilities

All human biobanks that have joined the Integration Platform, when dispensing pleural fluid, ascitic fluid, bone marrow fluid, cerebrospinal fluid, and other specimens, are recommended to follow this Standard Operating Procedure.

4. Description

This SOP covers the collection and processing of pleural fluid, ascitic fluid, bone marrow fluid, cerebrospinal fluid, and other specimens; these specimens are collected primarily from tumor patients. The processing procedures differ slightly for each.

5. Perating Procedures

5.1.1 Collection and Processing of Pleural Fluid or Ascitic Fluid from Tumor Patients Procedure:

1. After obtaining case consent, first prepare two 50ml conical centrifuge tubes.Collect at least 50cc of pleural fluid
2. or ascitic fluid, aliquoted into the two 50ml conical centrifuge tubes.
3. Centrifuge at 1,250 rpm for 10 minutes; if the cell content is high, centrifuge at 1,500 rpm for 20 minutes instead.
4. Using a plastic dropper, transfer the supernatant to another 50ml conical centrifuge tube. Retain the sedimented cell layer below.
5. The supernatant has little research value and may be destroyed and discarded.
6. For the two tubes containing sedimented cell layers below, remove the cell layer below in one of the tubes using a pipette, aliquot it into 1 ml microcentrifuge tubes, and store frozen at -20°C (or -80°C) freezer; this may be used to extract DNA in the future.
7. The cell layer below in the other tube is reserved for cytospin (cytospin), and must first be placed on crushed ice in a bucket
8. After cytospin (cytospin) has been performed, the remaining cell layer below in this tube may also first be frozen at -20°C. Once the cytospin slide has been confirmed to have a sufficient quantity of tumor cells (>50%), it too may be removed using a pipette, aliquoted into 1 ml microcentrifuge tubes, and stored frozen at -20°C (or -80°C) freezer, for future use in extracting DNA freezer; this may be used to extractDNA.
9. If a wax block is to be prepared, an additional 50ml of specimen must be collected separately.

Cytospin (Cytospin) (requires a **cytocentrifuge**):

1. Add 1ml of PBS to one tube of centrifuged, sedimented cell layer below cells (the tube must first be placed on crushed ice in a bucket.)
2. Adjust the PBS dilution ratio according to the quantity of the cell layer below, for example 1:10, or 1:100.
3. Using the cytocentrifuge (Cytospin), centrifuge at 1,500 rpm for 2 minutes, depositing the cells onto a coating slide.
4. Deposit 50 ul onto each slide, preparing 4 slides, with one stained with Liu's stain and the other slides reserved for future immunostaining.
5. For the slide stained with Liu's stain, **observe** the number of tumor cells under a microscope.
6. As needed, steps 2 and 3 may be repeated, depositing cells repeatedly at the same spot, **to increase** the number of cells on the smear.

Liu's stain:

1. Drop 0.8ml of Liu's A buffer onto the slide, and let stand for approximately 3-5 seconds
2. Drop 1.5ml of Liu's B buffer onto the slide, mix solutions A and B, and let stand for approximately 30-60 seconds.
3. Rinse under running tap water for 5 minutes.
4. Once air-dried, the slide is ready for reading.

5.1.2 Process for Preparing Wax Blocks from Centrifuged Sediment Cells of Pleural or Ascitic Fluid

Equipment:

Tissue processor, embedding machine

Consumables:

10% neutral buffered formalin, absolute ethanol, paraffin wax pellets

Procedure:

1. Collect at least 50cc of pleural fluid or ascitic fluid into one 50ml conical centrifuge tube.
2. Centrifuge the pleural or ascitic fluid specimen at 1,500 rpm for 20 minutes.
3. Draw off the upper layer of tissue fluid, then add 15ml PBS buffer, gently tapping side to side to mix thoroughly with the cells.
4. Once more, centrifuge at 1,500 rpm for 20 minutes; draw off the upper layer of tissue fluid, then add 15ml PBS buffer, gently tapping side to side to mix thoroughly with the cells.
5. Centrifuge at 2,500 rpm for 20 minutes; draw off the upper layer of PBS, then add 15ml of 10% neutral buffered formalin and mix thoroughly; let stand at room temperature for 40 minutes, to allow the settled cells to fix and form a pellet.
6. Once more, centrifuge at 2,500 rpm for 20 minutes; draw off the supernatant (10% neutral buffered formalin), then add 15ml 95% ethanol without shaking to mix, place in the 4°C freezer for at least two hours, to fix and dehydrate the cell layer.7. Remove the conical centrifuge tube from the freezer; the cell layer will have formed a pellet by this point, and the cell pellet can be transferred into the dedicated embedding cassette and sent for dehydration and embedding to produce a wax block.

Precautions:

Tissue dehydration and embedding to produce wax blocks requires specialized equipment and trained technical personnel; it is recommended to simply request assistance from a hospital pathology laboratory.

5.1.3 Process for Extracting DNA from the Centrifuged Sediment Cell Layer Below of Pleural or Ascitic Fluid: This process may follow the same method used to extract DNA from the buffy coat layer; please refer to Integration Platform SOP-007 v1.1 NBCT Blood Specimen DNA Extraction Process.

5.2 Bone Marrow Fluid Collection and Processing

Procedure:

1. After obtaining case consent, first prepare a 10ml EDTA anticoagulant vacuum blood collection tube (EDTA K2 10ml) (abbreviated as “CBC tube”) for the bone marrow fluid specimen to be preserved, affix a pre-numbered freeze-resistant label, have medical staff draw 2 ml of the case's bone marrow fluid specimen and place it into the CBC tube; after mixing thoroughly, aliquot it into 1 ml microcentrifuge tubes.Store
2. frozen in the -80°C freezer. This may be provided to applicants for DNA extraction in the future.
3. Based on the bone marrow biopsy report, record the percentage of bone marrow tumor cells.

5.3 Cerebrospinal Fluid Collection and Processing

Procedure:

1. After obtaining case consent, first prepare a 10ml EDTA anticoagulant vacuum blood collection tube (EDTA K2 10ml) (abbreviated as “CBC tube”) for the cerebrospinal fluid specimen to be preserved, affix a pre-numbered freeze-resistant label, have medical staff draw 2-5 ml of the case's cerebrospinal fluid specimen and place it into the CBC tube; after mixing thoroughly, aliquot it into 1 ml microcentrifuge tubes.Store
2. frozen in the -80°C freezer. This may be provided to applicants for DNA extraction in the future.
3. Based on the cerebrospinal fluid cytology report, record the percentage of cerebrospinal fluid tumor cells.

6. Quality Standards for Dispensing

1. For cell blocks prepared from centrifuged cell pellets obtained from the pleural effusion or ascitic fluid of patients with tumors, a pathologist shall first confirm that the corresponding tissue section contains a sufficient number of tumor cells. The tumor cell percentage should preferably be greater than 50%.
2. For DNA extracted from centrifuged cell pellets obtained from the pleural effusion or ascitic fluid of patients with tumors, the tumor cell percentage should preferably be greater than 50%.
3. For bone marrow aspirate specimens, the percentage of tumor cells in the bone marrow shall be provided based on the corresponding bone marrow pathology report.
4. For cerebrospinal fluid specimens, the percentage of tumor cells shall be provided based on the corresponding cerebrospinal fluid cytology report.
5. For DNA extracted from centrifuged cell pellets obtained from pleural effusion or ascitic fluid, the optical density (O.D.) ratios shall meet the following criteria:

- The A260/A280 ratio should be between 1.6 and 2.2.
- The A260/A230 ratio should be between 1.6 and 2.5.

Notes:

1. Some specimens are difficult to obtain or are available only in limited quantities, resulting in a low DNA yield. In such cases, the matter may be discussed with the applicant. If the applicant agrees to accept the specimens, they may be released even when the O.D. ratios do not meet the recommended criteria. However, the deviation shall be clearly documented, and the applicant shall be fully informed before the specimens are released.
2. The A260/A280 and A260/A230 ratios should preferably be greater than 1.8 to confirm acceptable DNA purity and the absence of substantial protein or other chemical contaminants.