Case Processing SOP (Spleen) V.3

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

The purpose of this Standard Operating Procedure is to outline procedures for processing and storing spleen received for HuBMAP consortium assay and analysis.

ATTACHMENTS

Appendix for SOP.docx

GUIDELINES

This SOP will be applied to all human donor tissues received for use in HuBMAP applications.
Responsibilities: Managers and supervisors are responsible for assuring that technicians are properly trained and equipment and facility are maintained in proper working order. Laboratory personnel are responsible for reading and understanding this SOP and its related documents and to perform these tasks in accordance with the SOP. They are also responsible for following individual laboratory best practices and safeguards.

MATERIALS TEXT

1. Sterile dissecting instruments (forceps, scissors, and scalpels)
2. Dissection boards with grids, sterile gauze sponges/paper towels
3. Ruler in millimeters
4. Dissection matrix 1cm X 1cm (Electron Microscopy Sciences Cat. No. 69010)
5. Centrifuge tubes (50 ml)
6. Dulbecco's Phosphate Buffered Saline (D-PBS), Mg2+ Ca2+ free (Invitrogen, Cat. No. 10010- 023), store at 4°C
7. Complete culture media (DMEM/F12 50/50 with L-Glutamine (Corning Cat. No. 10-090-CV + 10% Fetal Bovine Serum (Corning, Cat. No. MT35016CV) + 1x Antimycotic/Antibiotic
8. Uni-cassettes (Tissue-Tek®)
9.Macro Cassettes (ThermoFisher)
10. 10% neutral buffered formalin (NBF) in specimen container
11. O.C.T. ™ compound (Tissue-Tek®) and cryomolds, aluminum foil
12. Pipettes and sterile filter tips (200 µl, 1000 µl)
13. Serological pipets and pipet controller
14. 4% PFA (26ml d water + 4ml 10X PBS pH 7.4 without Mg or Ca + 10ml 16% paraformaldehyde EMS cat# 15710 for every 40ml of total volume needed)
15. Dry ice and ice bucket, 2-Methylbutane with dry ice in ice bucket and long forceps
16. Liquid Nitrogen in dewar, freezer gloves
17. Permanent markers, pencils
18. Foil squares (5cm)
19. 70% ethanol
20. 30 % Clorox bleach (6% Sodium hypochlorite)
21. Tissue waste container for formalin
22. Sharps container

SAFETY WARNINGS

1. Use universal safety precautions when handling human samples and employ personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
2. Perform dissection steps in a bio safety hood.

3. Follow chemical safety procedures and dispose of waste tissues in accordance with specific jurisdictional guidelines.
4. Handle sharps (e.g., scalpels, blades, glass) carefully and dispose of appropriately.
5. Dry ice and liquid nitrogen can cause freeze burns, handle carefully and use appropriate gloves.

BEFORE STARTING

Prepare 4% Paraformaldehyde in 1xPBS, place 350ml in a lidded speciman container.
Place 350ml NBF in a lidded specimen container.
Have liquid nitrogen and dry ice/methylbutane slurry on hand.
Assemble prefered dissection tools, gauze wipes and prelabeled cassettes and molds in biosafety hood.
Nest a metal pan half filled with D-PBS in wet ice to contain the organ until dissection begins

1 The tissues received will be identified as follows: Spleen (SP)

2 Sample prepared from tissues will be identified using the following nomenclature, abbreviations, and formats: Formalin Fixed Paraffin Embedded---FFPE (paraffin block)
Formalin Fixed Frozen Embedded---Fixed OCT Block
Fresh Frozen Embedded---Fresh OCT Block
Fresh Tissue piece cut to size---Tissue (in 4% PFA or complete medium in 50cc conical)

3 Case Number Assignment: HuBMAP donor tissues will be assigned case numbers adhering to the following template: two digit year identifier, hyphen, sequential 3 digit number beginning with 001.

4 Labeling Samples:
a. Cassettes for paraffin embedding and Fixed OCT block fixation step
i. Generate label using cassette printer or print legibly in pencil
ii. Line #1: Case ID + Sample type abbreviation
iii. Line #2: Block number. Where applicable, block # is determined by common coordinate region followed by position moving lateral (hilar capsule) to medial (central)
b. Cryomolds for fixed or fresh frozen OCT blocks
i. Print on mold legibly using permanent ink, apply printed label to bottom
ii. Line #1 as for cassettes
iii. Line #2 as for cassettes + FX if prepared to receive fixed frozen tissue
c. Dishes, foil wraps and containers (culture, petri or conical)
i. Print legibly using permanent ink or apply printed label
ii. Case ID + Sample type abbreviation (Table 1) + common coordinate location if relevant

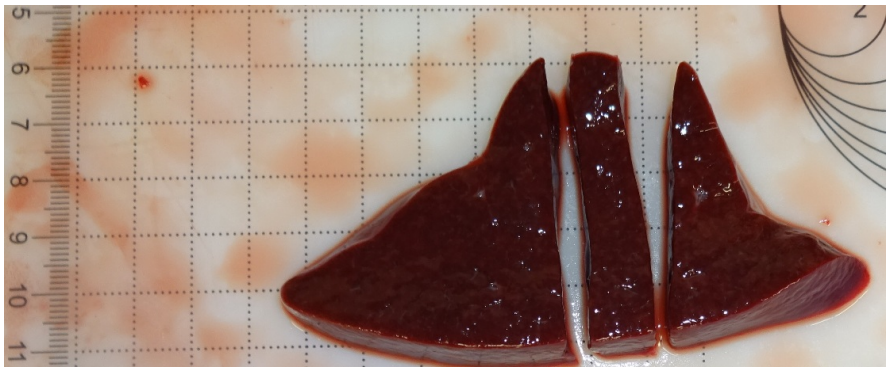
5 Processing Records:
a. Tissue handling data will be recorded on the Case Worksheet form.
b. Required fields during case processing include case identification, date received, any shipment or packaging.anomalies, processing date and time (start and end), staff, tissues received, aliquot types and numbers, and tissue quality notations.
c. Photo documentation should be employed to verify harvests are in keeping with common coordinate guidelines.

6 Tissue Dissection:
a. Photo document intact organ from all angles. Orient with hilum to left, inferior end at top.

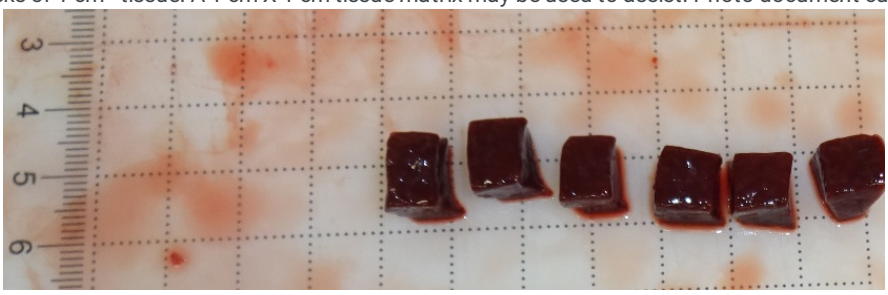
b. Place a 0.5 cm cube of tissue for RNA QC purposes into a sterile vial and flash freeze in liquid nitrogen. Use RNAse free conditions and collect from an interior, non-Common Coordinate site.

c. Harvest Common Coordinate tissue regions (Appendix). Cut 1 cm thick cross sectional slabs and photo document location of origin by sliding slabs slightly to right of residual organ. Use ruler or grid to map locations.

d. Place slabs with superior pole surface facing up and rotate until hilum is located at 12 o'clock. Cut a 1 cm wide strip through center of each slab starting at middle of hilum facing side. Photo document position prior to harvesting strip.



e. Cut strips into individual blocks of 1 cm^3 tissue. A 1 cm X 1 cm tissue matrix may be used to assist. Photo document cuts. Maintain orientation and order.



f. Place the Worksheet (Appendix) designated 1cm^3 , bisected block into the two cassettes labeled for NBF (p), maintaining orientation. Immerse in NBF, room temperature with mild agitation, at a volume of at least 20 mls per cassette added. *cassettes are moved to 70% ethanol at 20-24 hours of fixation, and will be processed within 3 days.

g. Place the Diagram Worksheet (Appendix) designated 1cm^3 cut block into the cassettes labeled for PFA (p) maintaining orientation. Immerse in 4% PAF at a volume of at least 20 mls per cassette added. *cassettes are moved to 70% ethanol at 20-24 hours of fixation, and will be processed within 3 days.

h. PPlace the Diagram Worksheet (Appendix) designated 1 cm^3 cut block into the cassette labeled for FX frozen OCT, maintaining orientation. Immerse in 4% PFA at a volume of at least 20 mls per cassette added. Complete processing and embedding following SOP:
https://dx.doi.org/10.17504/protocols.io.basniede

i. Place the Diagram Worksheet (Appendix) designated 1 cm^3 cut block for Fresh OCT into a labeled, OCT filled cryomold maintaining orientation. Complete processing and embedding following SOP:
https://dx.doi.org/10.17504/protocols.io.bcwsixee Hold and transport prepared blocks on dry ice.

j. Place the Diagram Worksheet (Appendix) designated 1cm^3 block of tissue into 4% PFA for CLARITY analysis. Use a ratio of at least 20 volumes of fixative to volume of tissue. Fix tissue for 20-24 hours at room temperature with gentle agitation. * Tissue is then transferred into hydrogel solution and incubated 5 days at 4C.

k. Prepare the Diagram Worksheet (Appendix) designated 1 cm^3 cut block of spleen tissue to be used for 10X analysis.
i. Place the tissue into a 50 ml tubes containing 25ml complete DMEM/F12 media. Store in the refrigerator until released for further isolation.

i. If the tissue is selected to be used for collaboration projects identify protocol B on the work sheet and follow protocols as provided by the requesting lab(s).

7 Sample Archiving:

a. All materials obtained for this program will be inventoried by the University of Florida Tissue Mapping Center (UF TMC) and archived in the Organ Processing and Pathology Core (OPPC) until use.

b. Samples will be released for use or shipped to collaborator(s) upon request by the Principle Investigator.

8 Worksheet /Appendix for Spleen Processing