Pathology Specimens SOP for processing of FFPE tissue for downstream molecular/cellular profiling - U01 MCL PCA Supplemental Project

Version 1-Final 5/31/2018

Fixative

The sample must be fixed in a formalin based fixative (neutral buffered 4% formalin (NBF)-sometimes referred to as 10% see note, is recommended) Note: NBF is typically made by diluting aqeuous 37-40% formalin in H_2O + Na_2HPO_4/ $Na_2H_2PO_4$ such that the final concentration is really 4% even though it is often called 10%.

1. Record fixation time (approximate if needed)
2. Use adequate volume of formalin for fixation (ideal is 15:1 formalin: tissue)
3. Use formalin within its expiration date

Tissue Block Age

Keep the tissue block age less than 1 year old when possible; Always record the tissue block age.

Tissue Processing

1. Use high quality tissue processing reagents (replace regularly per mfg guidelines)
2. Record tissue processing parameters
    a. For automated processors, the manufacturer and protocols and:
        i. Microwave processing?
        ii. Total time on processor
        iii. Type, temperature and length of formalin, ethanol, paraffin
3. Embedding:
    a. Record type of paraffin (e.g. manufacturer and catalog numbers and melting point)

FFPE Tissue Sectioning for IHC and IF

1. Cut fresh sections
2. Use disposable blades to avoid cross-contamination (change blades between cases)
3. Sectioning (all onto charged slides unless otherwise indicated): ***Do not bake slides.***
    SEE TABLE (choose if block is ample: 17-20 sections or scant: 10 sections)
    Label slides according to protocol syntax (below).
    a. 1 x 4 µm section for H&E
    b. 3 x 7 µm section on membrane slides for Capture
    c. Additional 4 µm sections for multiplex IF/Opal
    d. Extras sections x 4 µm
    e. Deep end 1 x 4 µm section for H&E

4. **FFPE Tissue Sectioning for Stanford (Rob West Lab) Protocol for single Cell RNAseq (this is being worked on separately)**
5. **FFPE Tissue Sectioning for Broad Protocol for single nuclei RNAseq (this is not known yet)**
6. Number slides consecutively
7. Place slides in order in a plastic ziplock bag; include desiccant in bag

    Note: Time from sectioning to freezing should be minimized, but within the day is sufficient. Try not to allow slides to remain at room temperature for several days.
8. Store at -20 °C (1,2) unbaked

Shipping: See Shipping Protocol

SOP for Slide Labeling and Shipping Protocol for FFPE slides for U01 MCL PCA Project
A.M. De Marzo, Last Updated 5/25/2018

Slide Labeling:

- Do not use any patient identifiers that could id the patients outside your lab
- Label Unstained slides as follows: MCL-PCGA1-XYX-Specimenid-Slide#,
    - where "XYZ" is your 3 letter institution designation (e.g. JHU for Johns Hopkins, UCD for University of California at Davis)
    - Where "Specimeid" is your lab's unique identifier for the corresponding paraffin block which you can use to identify the specimen/patient in your lab if needed
    - Where "Slide#" is the consecutive slide that was cut as outlined in the slide cutting SOP

Slide Shipping:

- Contents of package may be categorized as being non infectious (FFPE sections).
- Put slides in a plastic slide carrier
  An example is here: http://www.tedpella.com/histo_html/slidebox.htm#_2119
- Make sure the slides are tightly packed in the slide mailer so they cannot wiggle around at all (pack the slide mailer with kimwipes on top if needed) and the slide mailer is taped shut
- Tape bubble wrap around the slide mailers
- Place packaged slides inside a padded envelope and seal using tape
- Place in a mailing container with a refrigerant gel pack for protection against melting
- Place a list of the slides with specimen ids in the package
- Ship Monday- Thursday, overnight to avoid weekend deliveries
- Ship sealed shipping pack to:

Alexander Borowsky
UC Davis Pathology
PATH Buldg.
4400 V Street
Sacramento, CA 95817
Attn: Ed Hubbard
916-734-2525

References:

1. Baena-Del Valle JA, Zheng Q, Hicks JL, Fedor H, Trock BJ, Morrissey C, et al. Rapid Loss of RNA Detection by In Situ Hybridization in Stored Tissue Blocks and Preservation by Cold Storage of Unstained Slides. Am J Clin Pathol. 2017;148:398–415.

2. Andeen NK, Bowman R, Baullinger T, Brooks JM, Tretiakova MS. Epitope Preservation Methods for Tissue Microarrays: Longitudinal Prospective Study. Am J Clin Pathol. 2017;148:380–9.