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We use this protocol and it's working

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UPitt TriState SenNet TMC FFPE Specimen Section

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ABSTRACT

To perform imaging analysis of the FFPE blocks were submitted to hematoxylin and eosin stain. Initially FFPE blocks were prepare by the Research Histology Pitt Biospecimen Core and kept at room temperature (preserved from light) until sectioning.

GUIDELINES

- Sectioning is generally improved when the specimen and the wax are well matched in hardness. It is for this reason that most paraffin blocks must be cold

when sections are cut.

- The choice of slide and adhesive will be influenced by the staining methods to be subsequently applied.
- It is important to remove accumulated tissue debris and wax after use. Regular preventative maintenance is important

SAFETY WARNINGS

- Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
- You must be familiar with the safety features of the microtome you are using and observe some basic safety rules when cutting sections.

BEFORE START INSTRUCTIONS

- Monitor the water bath temperature carefully. The temperature will need to be 5 - 9 ˚C below the melting point of the wax. Make sure the water is clean and free of bubbles and section waste (to avoid cross-contamination).
- Make sure the appropriate slides are ready.

Section Collection

1h 30m

1 Using a Histo-Quill Pen, hand label all slides with project number and block ID (add type of stain if needed) .

2 Ensure paraffin block is at room temperature.

3 Place block into holder of microtome.

4 Use clean, sharp microtome knife or disposable blade.

4.1 Use forceps or brush instead of your fingers to pick up sections or wax fragments from blade or block face.

4.2 The knife or blade should be removed from the microtome when the instrument is left unattended or when cleaning the instrument. It can then be grasped with forceps (not fingers) or picked up with the magnet at the end of a brush and safely removed. Used blades should be disposed of appropriately in a “sharps” container.

4.3 Set blade clearance to the optimal clearance angle & orient the specimen in the appropriate position.

5 Cut section to a 5 µm thickness. 5 µm sections

5.1 *Note: Dip block face into molten paraffin to seal the tissue and allow to cool before storage.*

6 Float paraffin ribbons on deionized H_2O (or RNase-free H_2O), heated to just below the melting point of the paraffin being used (usually 42 °C).

7 Mount sections on slide by slowly bringing the flat side of slide up from underneath the floating tissue section, so that the tissue section is centered in the membrane window.

8 Place slide at an angle to allow water to drain and incubate sample at room temperature for 01:30:00 . 1h 30m

9 Slides can now be stored at room temperature for future use.

10 Proceed with staining protocol (if needed).