A Simple Tool For Securing Tissue Orientation and Standardizing Tissue Location: From Grossing Through Review

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Introduction

One of the challenges that Histologists face is orienting tissues and locking the orientation of small biopsies and core tissue punches during manual embedding. If not orientated correctly, the subsequent sections are not adequate for the Pathologist to diagnose and the tissue must be re-oriented and re-embedded. Sakura has developed five (5) types of Tissue-Tek® Paraform® Orientation Gels which allow establishing orientation at the time of grossing thus eliminating the need to orient the processed specimens during embedding downstream. The grossed biopsy or specimen is placed in the easy-to-use, precast gels in the desired final orientation, the cassette lid is closed/locked and the cassette processed and embedded. The Orientation Gels are dyed green to provide contrast with the specimen at grossing, facilitating visualization of the tissue and ensuring correct placement and orientation.

Materials and Methods

Tissue Processing and Embedding

The gastrointestinal (GI) and skin specimens (Proteogenex, Culver City, CA) were fixed and grossed. Tissue-Tek Paraform biopsy, 2-lane or 3mm Punch Orientation Gels (Sakura Finetek, Torrance, CA) were chosen for best fit, either used as supplied or customized to fit the tissue specimens. The Orientation Gels containing specimens were placed into Tissue-Tek Paraform biopsy cassettes, preprinted using Tissue-Tek AutoWrite® Cassette Printer (Sakura Finetek, Torrance, CA) and loaded into Tissue-Tek VIP® 6 Processor (Sakura Finetek, Torrance, CA) or Tissue-Tek Xpress® x120 Rapid Tissue Processor (extended cycle protocol) for tissue processing. The specimens in biopsy cassettes were embedded using Tissue-Tek AutoTEC® a120 Automated Embedder (Sakura Finetek, Torrance, CA) and sectioned using Tissue-Tek AutoSection® Fully Automated Microtome (Sakura Finetek, Torrance, CA).

Tissue Staining

Tissue sections were Hematoxylin and Eosin (H&E) stained using Tissue-Tek Prisma® Automated Slide Stainer (Sakura Finetek, Torrance, CA) and the slides were coverslipped using Tissue-Tek Film® Coverslipper (Sakura Finetek, Torrance, CA). Images were taken using Visiontek® Digital Microscope (Sakura Finetek, Torrance, CA).

Tissue sections were special stained (Iron, Trichrome, Grocott's Methenamine Silver (GMS), Acid-Fast Bacilli (AFB), and Periodic Acid Schiff (PAS)) using manufacturers' suggested protocols (American MasterTech Scientific, Lodi, CA). The slides were coverslipped and images taken.

Tissue sections were immunohistochemically (IHC) stained using antibodies to cytokeratin (AE1/AE3) with a hematoxylin counterstain. The slides were dehydrated and coverslipped and images taken.

Results

Figure 1. Tissue-Tek Paraform Orientation Gels in Tissue-Tek Paraform biopsy cassettes.

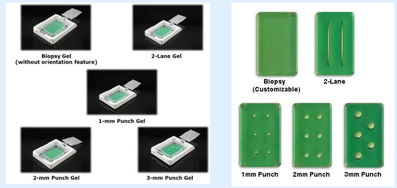


Figure 2. Orientation Gels locking orientation at grossing

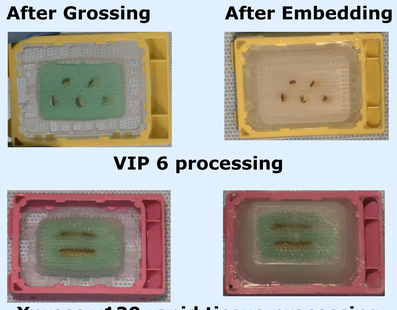


Figure 3. Weak cross-staining of Orientation Gels' matrix by H&E or AFB.
No cross-staining of tissue section by Orientation Gels' coloring.

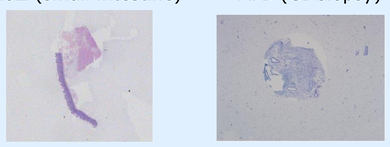


Figure 4. No cross-staining of Orientation Gels' matrix by Iron special stain.
No cross-staining of tissue section by Orientation Gels' coloring.

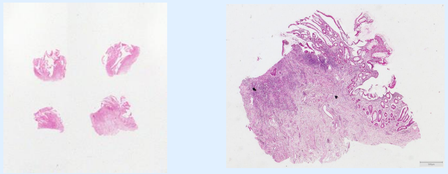


Figure 5. No cross-staining of Orientation Gels' matrix by Trichrome stain or GMS.
No cross-staining of tissue section by Orientation Gels' coloring.

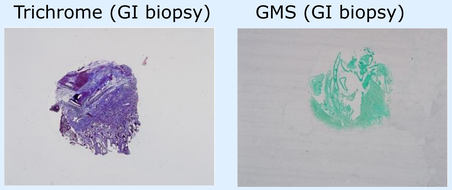


Figure 6. Intense Staining of Orientation Gels' matrix (A) using PAS due to Orientation Gels' carbohydrate matrix.

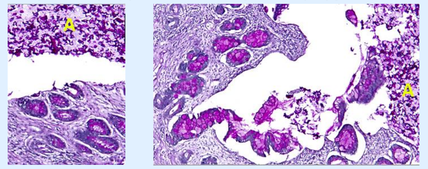
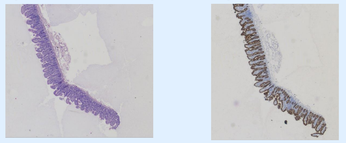


Figure 7. No cross-staining of Orientation Gels' matrix by IHC using antibodies to cytokeratin.
No cross-staining of tissue section by Orientation Gels' coloring.



Conclusions

- Tissue-Tek Paraform Orientation Gels enables flexible and customizable tissue orientation and location at the time of grossing and accommodates multiple tissue types (e.g. core biopsies, punch biopsies, shaves, arteries, nerves).
- Orientation Gels lock orientation, eliminates mis-orientation and eliminates the need of Histologist to handle tissue specimens during downstream embedding.
- Orientation Gels are available in multiple, precasted formats: without orientation features, 2-lane, 1mm, 2mm and 3mm punches.
- There is no or faint cross-staining of the Orientation Gels' matrix using the following stains: H&E, Iron, Trichrome, GMS, AFB, and IHC.
- There was no cross-staining of tissue specimens by the Orientation Gels' coloring.
- Even though PAS staining for carbohydrates did stain the Orientation Gels' matrix, this does not impact the tissue staining nor hinder review of the tissue.
- Orientation Gels are a valuable tool for Pathologists and Pathology Assistants to lock desired orientation of specimens during grossing and to standardize specimen location within a tissue block and on the slides.

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