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Suggested Method of Applying HistoGel (Thermo Scientific) to Histology and Cytology Specimens.

Principle: HistoGel is an aqueous gel composition useful in processing very small **fixed** Histology and Cytology specimens.

Procedure:

Histology specimens:

Histology specimens including: tissue fragments, needle biopsies, lymph nodes, tissue aggregates, small arteries, nerves, and any other specimen types which require special handling during histological processing.

1. HistoGel is solid at room temperature. It must be liquefied for use by heating to $60^{\circ}\text{C} \pm 5^{\circ}$. This can be achieved by using one of the following:
 - A. Microwave on low for 5-15 seconds. Make sure to loosen the cap before heating a tube of HistoGel to prevent rupturing of the tube. Check frequently to see when liquefaction takes place.
 - B. Place HistoGel into a water bath for 3-10 minutes or until liquid.
2. After HistoGel is liquefied, the temperature may be brought down to $50^{\circ}\text{C} \pm 5^{\circ}$ and it will remain in the liquid state. A lower temperature will allow the gel to solidify more quickly after it is dispensed onto a specimen.
3. Depending upon your specimen type and personal preference, proceed as follows:

Specimen Removal Method With biopsy tissue cassette.

1. Prior to placement of specimen, position biopsy tissue cassette on top of a solid cool surface, such as a petri dish in an ice bucket.
2. Place **previously fixed** specimen directly inside of biopsy cassette with the desired orientation.
3. Dispense liquefied HistoGel with a pipette completely covering specimen and close the cassette lid.
4. Allow HistoGel to solidify ($<20^{\circ}\text{C}$). It takes 2-3 minutes if not aided by cooling.
5. Place cassette into formalin/paraformaldehyde or your fixative of choice for approximately 1 hour. Transfer to 70% ethanol and bring to Histology for processing.

Cytology Specimens:

Cytology specimens including: fine needle aspirates, tissue aggregates and any other specimen types resulting in a cell block.

1. HistoGel is solid at room temperature. It must be liquefied for use by heating to $60^{\circ}\text{C} \pm 5^{\circ}$. This can be achieved by using one of the following:
 - A. Microwave on low for 5-15 seconds. Make sure to loosen the cap before heating a tube of HistoGel to prevent rupturing of the tube. Check frequently to see when liquefaction takes place.
 - B. Place HistoGel into a water bath for 3-10 minutes.
2. After HistoGel is liquefied, the temperature may be lowered to $50^{\circ}\text{C} \pm 5^{\circ}$ and it will remain in the liquid state. A lower temperature will allow the gel to solidify more quickly after it is dispensed onto a specimen.
3. Centrifuge your ethanol/formalin/paraformaldehyde fixed processed cell suspension.
4. Remove the supernatants from the centrifuge tube.
5. Depending upon your specimen type and personal preference, proceed as follows:

Centrifuge Tube Method

1. Add 4-6 drops of liquefied HistoGel with a pipette to cell pellet at bottom of centrifuge tube.
2. Either vortex specimen for several seconds to adequately and thoroughly mix cells and HistoGel together, (if vortex is not available, carefully mix cells and HistoGel together by lightly shaking the tube in a swirling motion), OR allow HistoGel to settle to the bottom of tube.
3. Allow HistoGel to solidify by cooling to near room temperature ($<20^{\circ}\text{C}$). This can be achieved by use of a cooling plate, ice cubes, freeze pack, or allowing to cool naturally.
4. Remove HistoGel pellet containing the specimen and place inside a biopsy cassette.
5. Place cassette into formalin/paraformaldehyde/fixative of choice for approximately an hour. Transfer to 70% ethanol and bring to Histology for processing to paraffin.

