PrestoCHILL

Best Operating Procedures (BOP) for special applications

MILESTONE
HELPING PATIENTS

Best Operating Procedures (BOP) for special applications

1. **BOP-101** How to identify frozen specimens
2. **BOP-102** How to freeze breast and fatty tissues
3. **BOP-103** How to simultaneously freeze small specimens
4. **BOP-104** How to freeze long-thin specimens
5. **BOP-105** How to freeze muscle tissues
6. **BOP-106** How to freeze thin specimens on edge
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8. **BOP-108** How to freeze skin for MOHS surgery
9. **BOP-109** How to freeze body fluids

Discover BOP video tutorials here

Developed by Milestone Application Team - BOP001-002

1. **BOP-101** How to identify frozen specimens

Problem

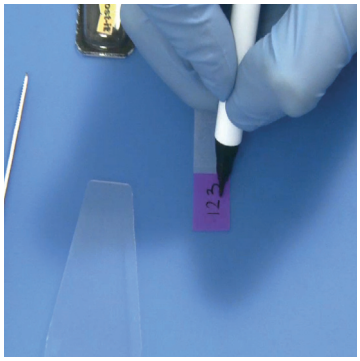
Proper identification of the frozen specimen is of the utmost importance. A method is needed to unequivocally match a case ID with a specific frozen specimen.

Solution

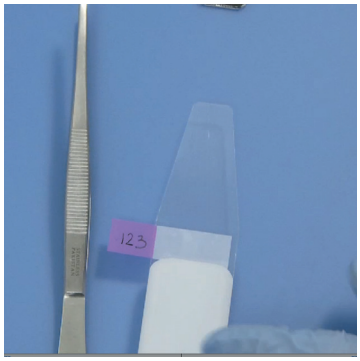
Milestone has developed a simple method to properly identify specimens by embedding a case ID label into the Milestone Cryo Compound (MCC) which contains the case specimen.

Milestone best operating procedure

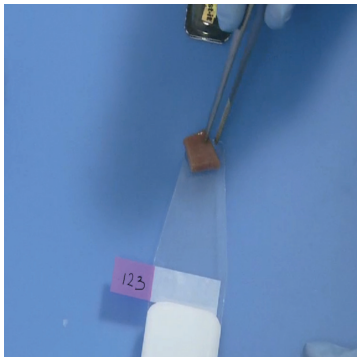
Material required: ID tags (e.g. Post-it®)



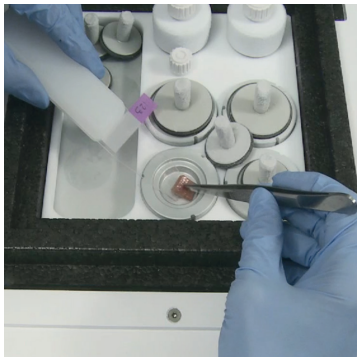
1. Write the specimen ID number onto a Post-it®.



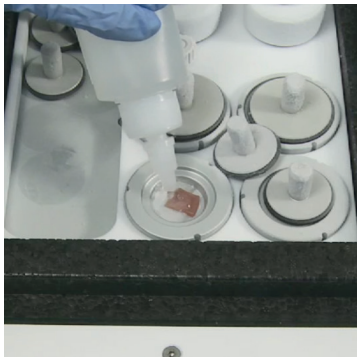
2. Stick the Post-it® onto a dispensing slide as shown.



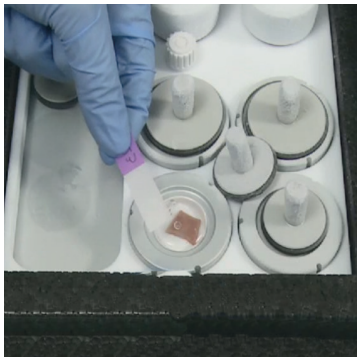
3. Squeeze a drop of embedding medium (MCC) on the tip of the dispensing slide and wet specimen surface.



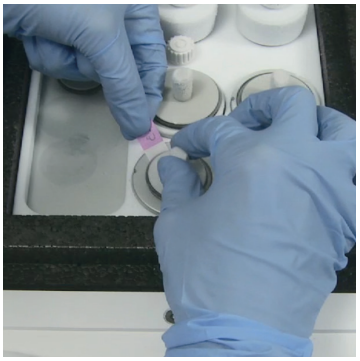
4. Slide the specimen to the bottom of the mould.



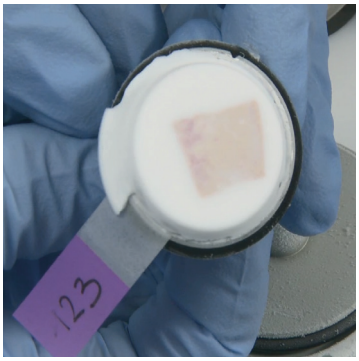
5. Fill the mould with embedding medium (MCC).



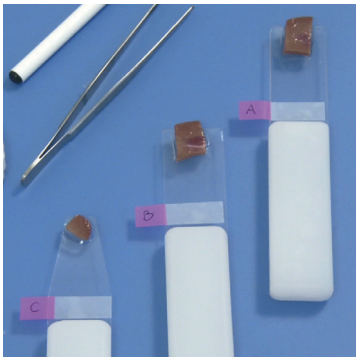
6. Turn the Post-it® upside down so that the writing is facing down.



7. Place the Post-it® into the mould (label facing out), contacting the embedding medium (MCC). Place the chuck and heat extractor on top of the mould.



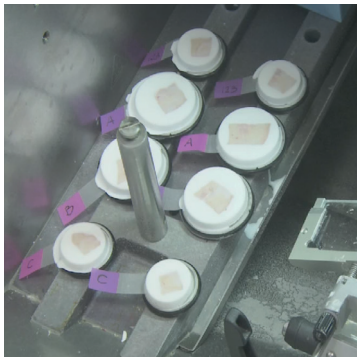
8. After 60 seconds the specimen is frozen and securely identified.



9. When several specimens arrive in a group, it is advisable to have several dispensing slides, each one identified with an individual specimen ID labels.

10. Several specimens can be simultaneously frozen with PrestoCHILL.

Results



Multiple specimens unequivocally identified.

2. **BOP-102** How to freeze breast and fatty tissues

Problem

Fatty tissues are known to be difficult to cut as they don't freeze at the usual temperature of the cryostat chamber.

Solution

Milestone has developed a 2-step simple method to easily freeze and cut fatty tissues or even pure fat.

Milestone best operating procedure

TEMPERATURE

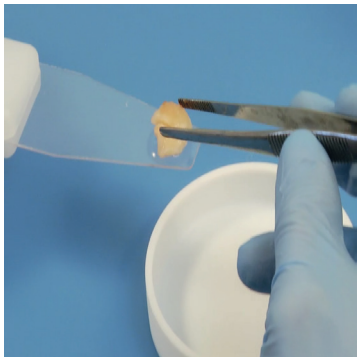
-40°C

TIME

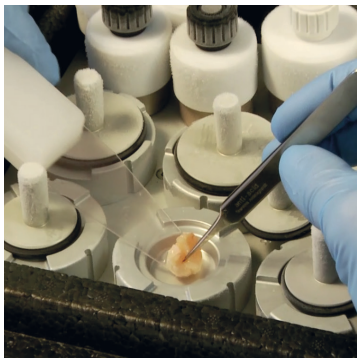
240"

1. Set temperature for the PrestoCHILL at -40°C. Set time at 240 seconds.

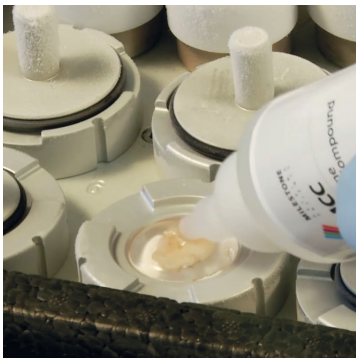
2. Set cryostat temperature at minimum of -20°C. The lower the temperature, the easier will be the sectioning of fat.



3. Place specimen on a drop of embedding medium (MCC) applied to the tip of the dispensing slide. If the specimen is very sticky, use the fine tipped forceps.

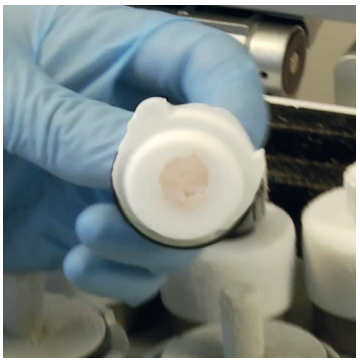


4. Slide the specimen into the mould according to PrestoCHILL Operator Manual.

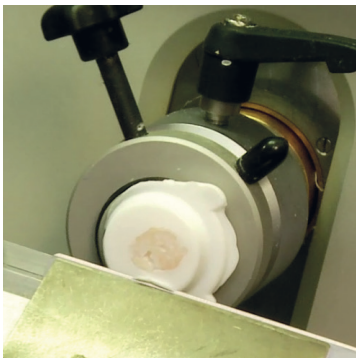


5. Add embedding medium (MCC) until mould is filled.

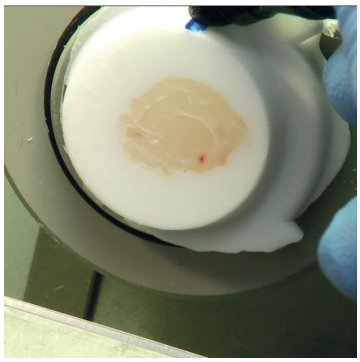
6. Place chuck on the mould. Set heat extractor on the mould and start timer.



7. After 240 seconds the specimen is frozen and perfectly planar.



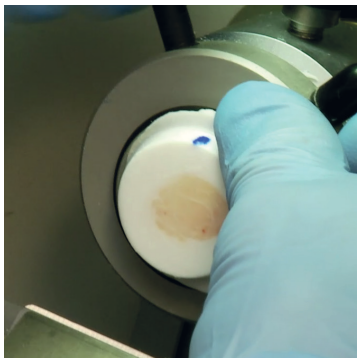
8. Transfer chuck as fast as possible to the cryostat. Orientate the tissue so that fat is the last part of the section to be cut. Start trimming.



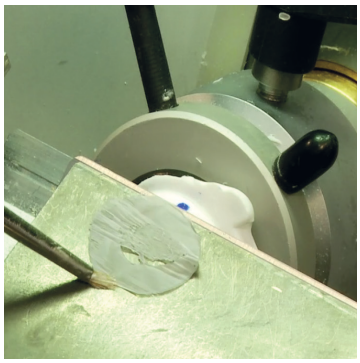
9. After trimming is completed, define univocally the position of the block in the cryostat with a felt marker.

10. Transfer back the trimmed block to the PrestoCHILL. Use a mould with larger diameter than the original one to compensate the reduction of the original thickness.

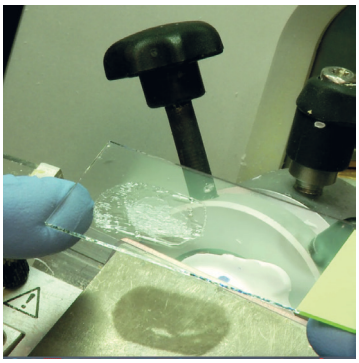
11. Close cover and start a second step of freezing for 60 seconds.



12. When time is expired, transfer as fast as possible the chuck with the block to the cryostat. Be sure to position it in the same position according to the preset mark.



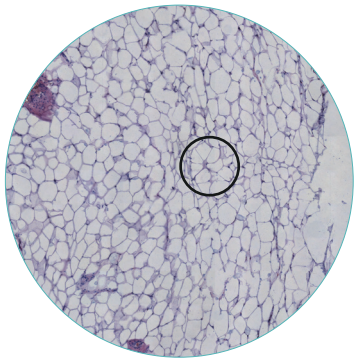
13. Start immediately cutting with a rapid movement of the cryostat wheel.

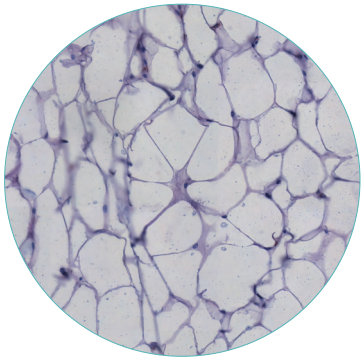


14. Retrieve tissues sections with a slide and process with PRESTO processor/stainer.

Results

Human breast H&E 40x





3. **BOP-103** How to simultaneously freeze small specimens

Problem

Proper preparation of multiple small specimens at the same cutting plane is very difficult with conventional freezing procedures.

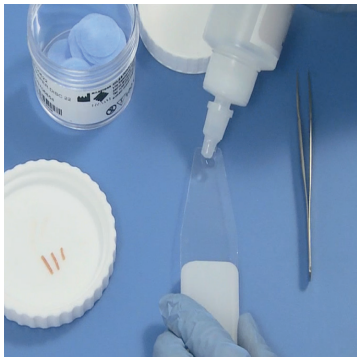
Solution

Milestone has developed a simple method to orient multiple specimens on a common cutting plane, enabling full samples of each specimen to be included in the same section.

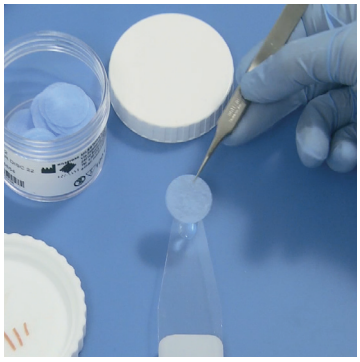
Milestone best operating procedure

Material required: Paper disks

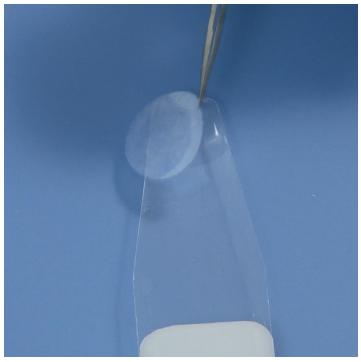
- Code 100623 - Box with 100 paper disks; **16** mm diameter.
- Code 100622 - Box with 100 paper disks; **22** mm diameter.
- Code 100615 - Box with 100 paper disks; **30** mm diameter.



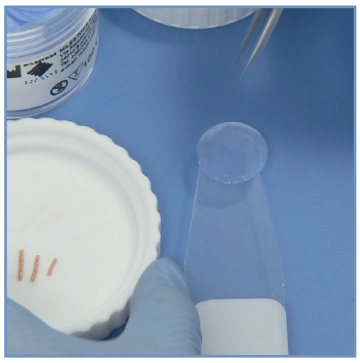
1. Apply a drop of embedding medium (MCC) to the tip of the dispensing slide.



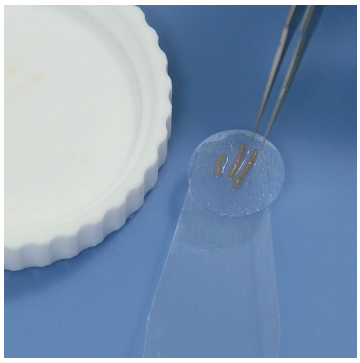
2. Place a paper disk of appropriate diameter on top of the embedding medium (MCC) drop.



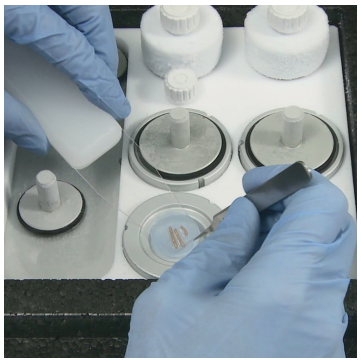
3. Be sure to wet the paper, leaving only a dry edge for better gripping.



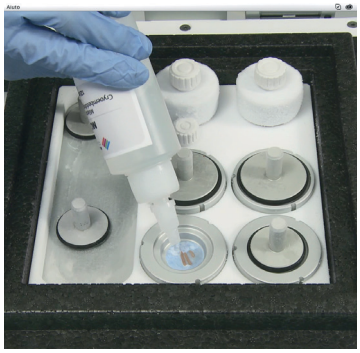
4. Flip the paper disk and make sure that this surface is also wetted.



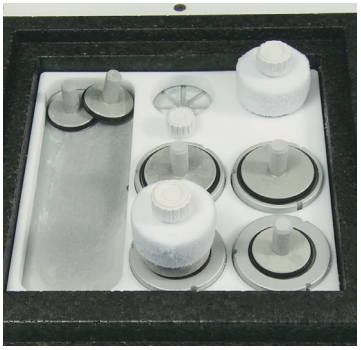
5. With the fine tip forceps orient the small specimens on the paper disk.



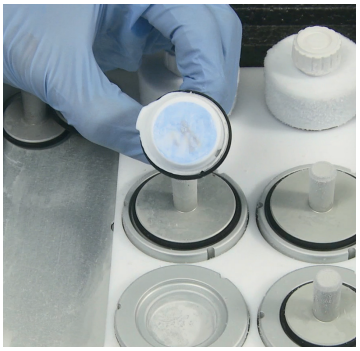
6. Slide the paper disk into the bottom of the mould.



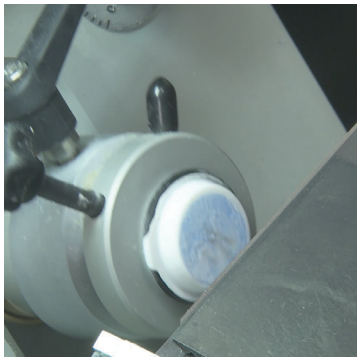
7. Add embedding medium (MCC) to fill the bottom part of the mould.



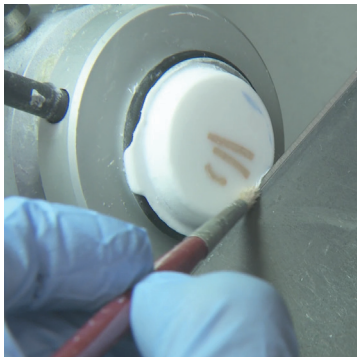
8. Place the chuck over the mould and the heat extractor on top of the chuck.



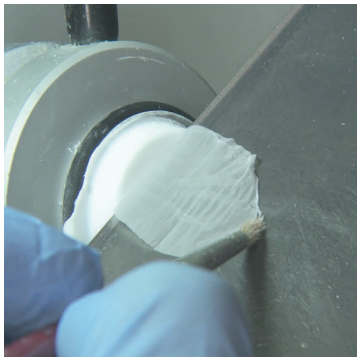
9. After 60 seconds, the specimens are frozen and covered by the paper disk.



10. Place the chuck in the cryostat and trim the block until the paper is cut away.

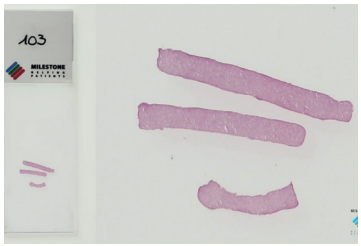


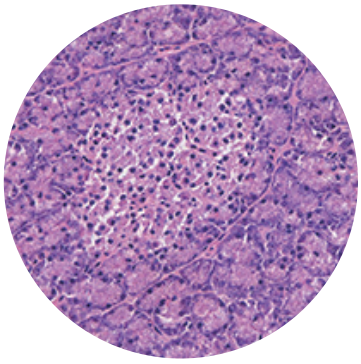
11. The specimens are now ready for cutting to obtain a perfect section, because of the planarity of the three specimens.



12. The slice is picked up by the slide and processed and stained with PRESTO.

Results





4. **BOP-104** How to freeze long-thin specimens

Problem

Proper preparation of long-thin specimens at the same level is very difficult with the conventional freezing procedures.

Solution

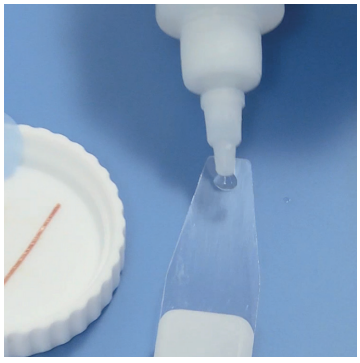
Milestone has developed a simple method to easily prepare long-thin specimens by using a paper disk.

Milestone best operating procedure

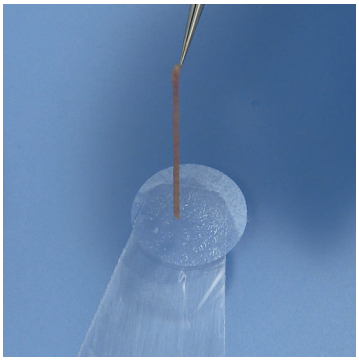
Material required: Paper disks

- Code 100623 - Box with 100 paper disks; **16** mm diameter.
- Code 100622 - Box with 100 paper disks; **22** mm diameter.
- Code 100615 - Box with 100 paper disks; **30** mm diameter.

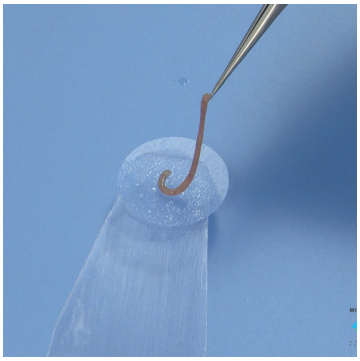
1. This method is suitable for specimens with a length of up to 6-8 cm (2-3 inches).



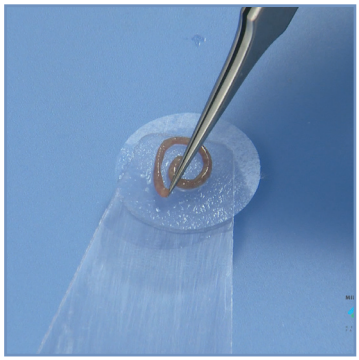
2. Squeeze a drop of embedding medium (MCC) on the tip of the dispensing slide.



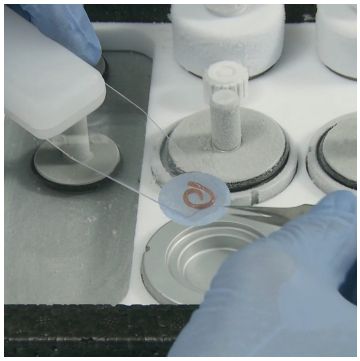
3. Place a paper disk of appropriate diameter on top of embedding medium (MCC) drop. Follow BOP 103 procedure.



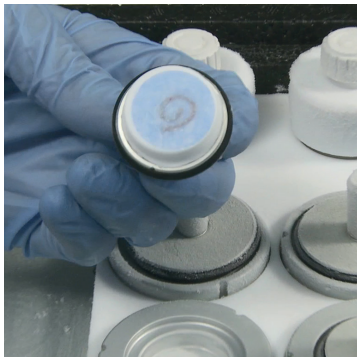
4. Orient the specimen, using a fine tip forceps, on the paper disk to create a spiral pattern.



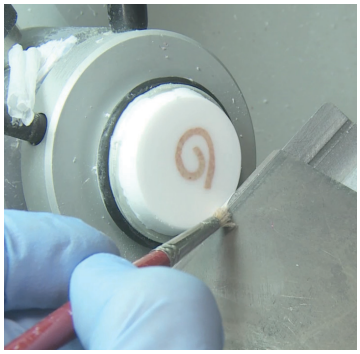
5. Make sure the entire length of the specimen is within the limit of the disk.



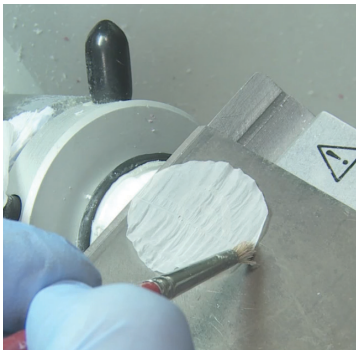
6. Slide the paper disk onto the bottom of the mould, fill mould with embedding medium (MCC) and place chuck on top, followed by the heat extractor.



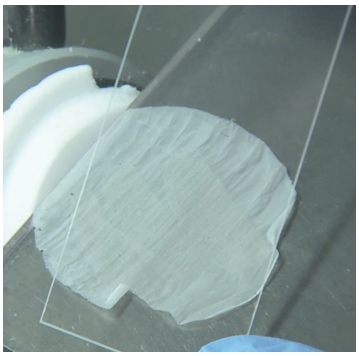
7. After 60 seconds the specimen chuck surface is ready and covered by the paper disk.



8. After trimming the paper away, the specimen is ready for cutting.

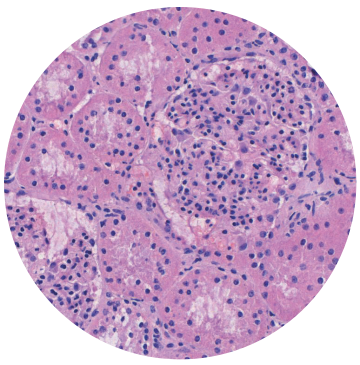


9. A section is prepared for picking up by slide.



10. Pick up the section with the slide, process and stain with PRESTO.

Results



5. **BOP-105** How to freeze muscle tissues

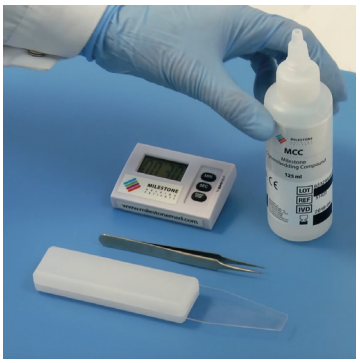
Problem

Obtaining high quality-frozen sections from muscle tissues is difficult because of the high water content, which generates large holes during the freezing step.

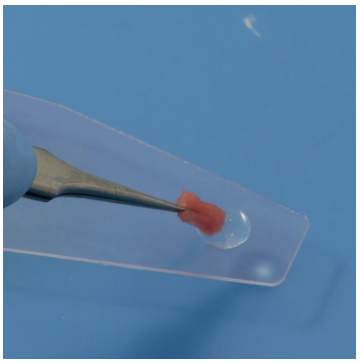
Solution

Milestone has developed a simple method to obtain high-quality muscle frozen sections by adopting a vertical orientation of fibers during the freezing step and an innovative thermal cycling of the unstained slides.

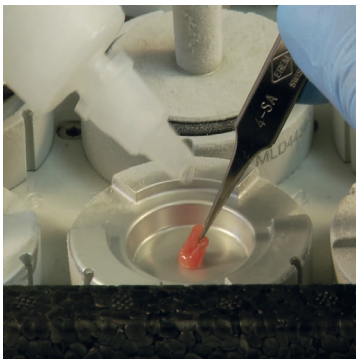
Milestone best operating procedure



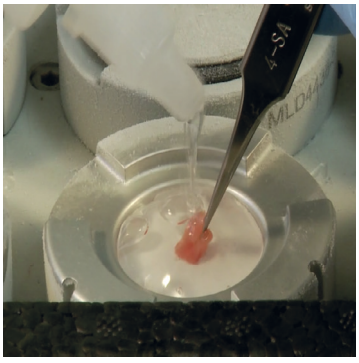
1. For the best morphological evaluation, it is important to have cross section of the muscular fibers.



2. Orientate the specimen with its fibers positioned vertically and wet the bottom part of the specimen with embedding medium (MCC).

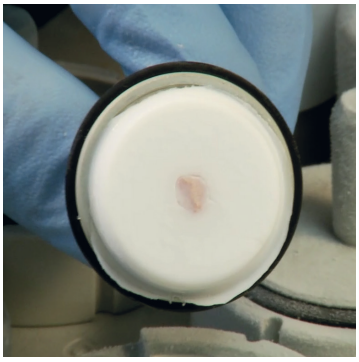


3. Freeze first the bottom part of the specimen and hold it stable in this position.

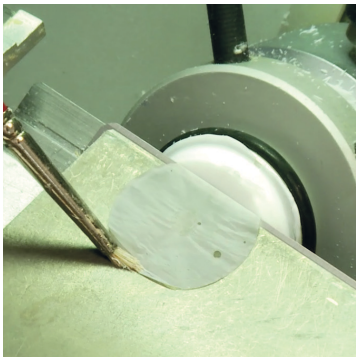


4. Fill the mould with the MCC medium while holding the specimen in the original vertical position.

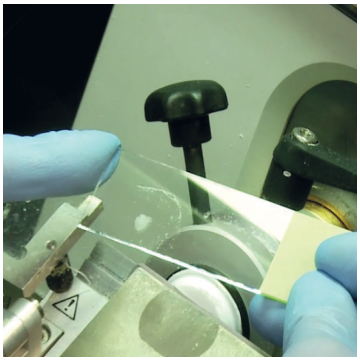
5. Place chuck and heat extractor according to the PrestoCHILL Operator Manual.



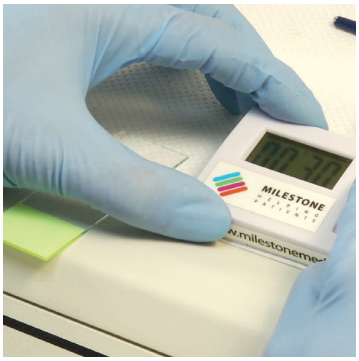
6. After 60 seconds, specimen is ready for trimming. If shuttering occurs during cutting, reduce freezing time to 30/35 seconds.



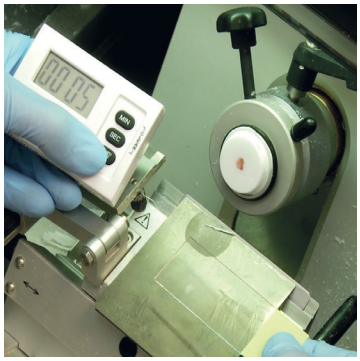
7. Cutting is made easier by the temperature homogeneity of both tissue and embedding medium.



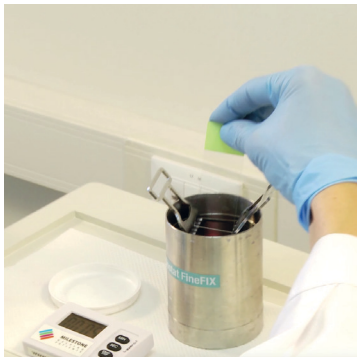
8. The section of tissue is picked up by the slide.



9. Place slide at room temperature on a bench and start a timer for 30 seconds.

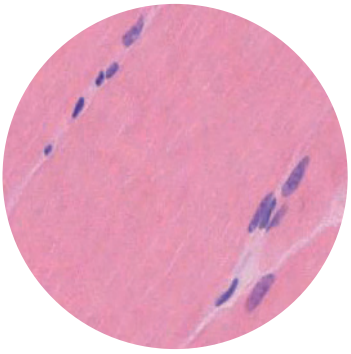


10. After the time expires, take the slide back to the cryostat and leave it for 5 seconds.



11. Place slide in FineFIX solution in the rack, process and stain with PRESTO.

Results



6. **BOP-106** How to freeze thin specimens on edge

Problem

Proper orientation of the frozen specimen is of the utmost importance.

A method is needed to obtain orientation of thin specimens on edge without collapsing during the embedding medium (MCC) freezing process.

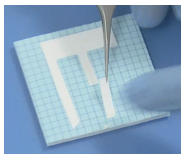
Solution

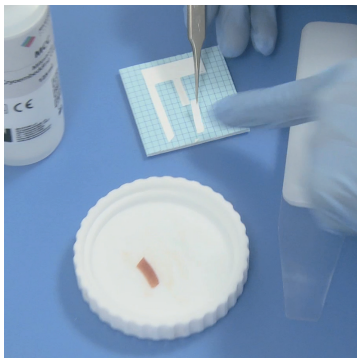
Milestone has developed a simple method to obtain thin specimens in the precise orientation required.

Milestone best operating procedure

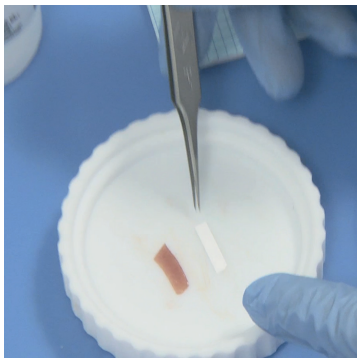
Material required:

Precut Nitrocellulose paper.

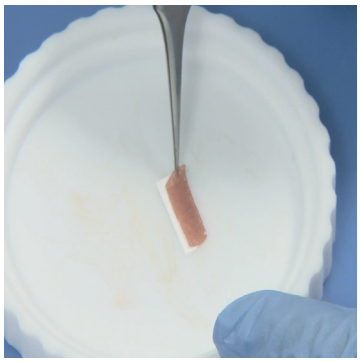




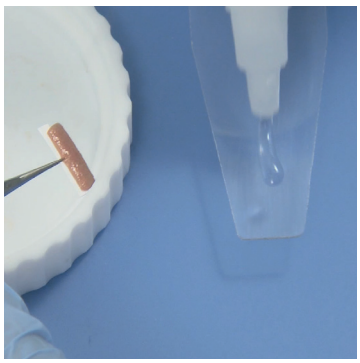
1. With a fine tip forceps pick up a stripe of nitrocellulose paper.



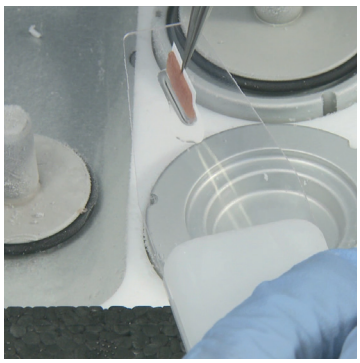
2. Place the strip of paper near the specimen to be frozen on edge.



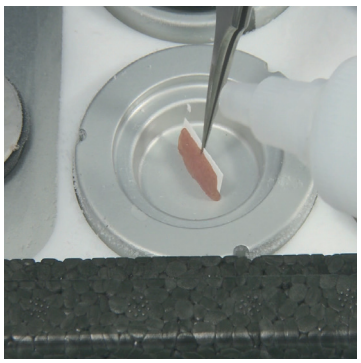
3. Orient the specimen on the paper so that the surface to be cut extends 2-3 mm from the edge, leaving empty space for the forceps to grip the strip of paper.



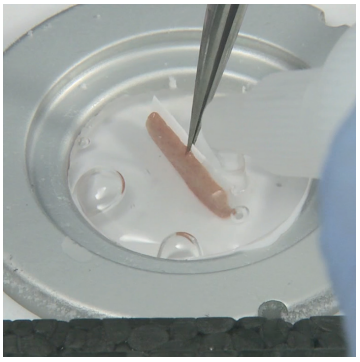
4. Squeeze a line of MCC medium onto the tip of the dispensing slide.



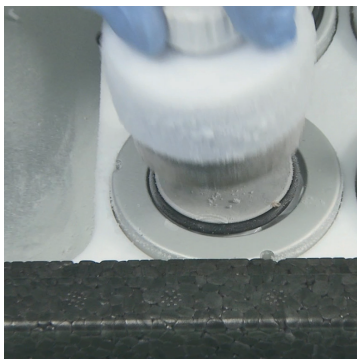
5. Wet the edge of the specimen with MCC.



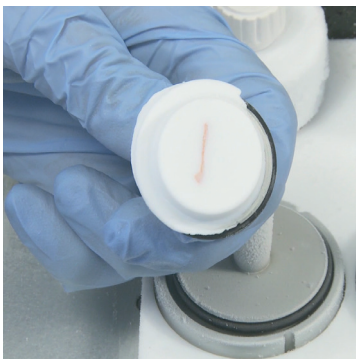
6. Make sure that only the tissue is in contact with the mould. The paper should be offset from the mold bottom.



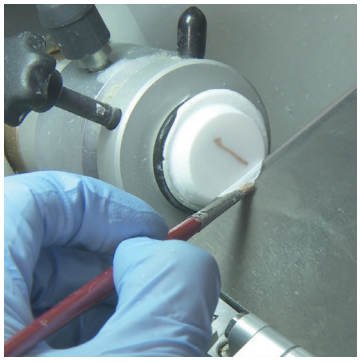
7. Fill the mould with embedding medium (MCC) maintaining the paper and specimen in the vertical position with the forceps until the mould is filled.



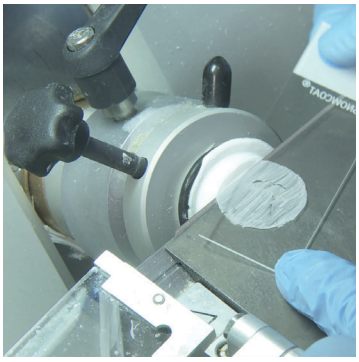
8. Take the forceps away with or without paper. Place chuck and the heat extractor on top of the mould.



9. After 60 seconds, the specimen is frozen on edge ready for trimming and perfectly planar.

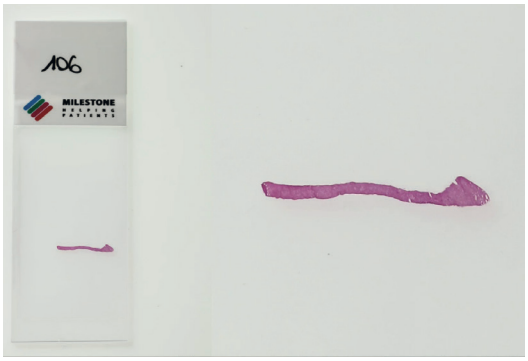


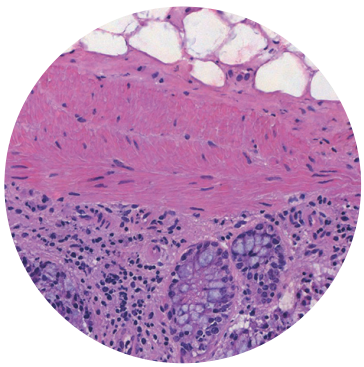
10. After trimming, the block is ready for cutting.



11. Pick up the section with the slide, process and stain with PRESTO.

Results





7. **BOP-107** How to freeze multiple thin specimens on edge

Problem

Freezing multiple thin specimens on edge is difficult using conventional freezing techniques.

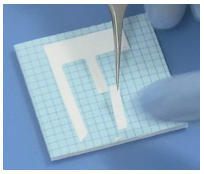
Solution

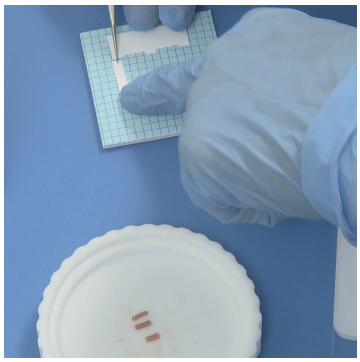
Milestone has developed a simple method to obtain thin specimens in the precise orientation required.

Milestone best operating procedure

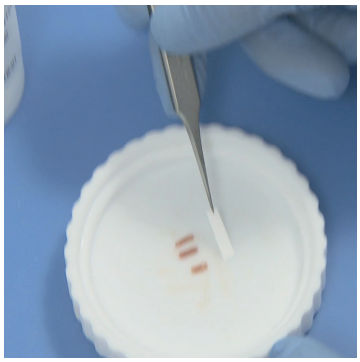
Material required:

Precut Nitrocellulose paper.

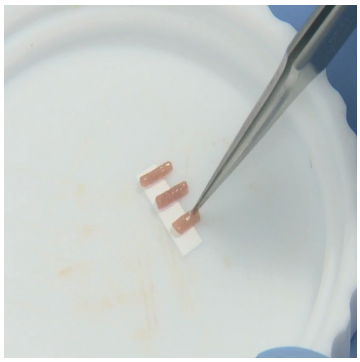




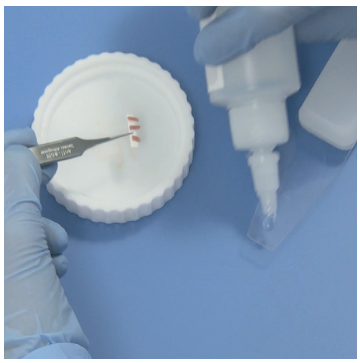
1. With fine tipped forceps pick up a strip of nitrocellulose paper.



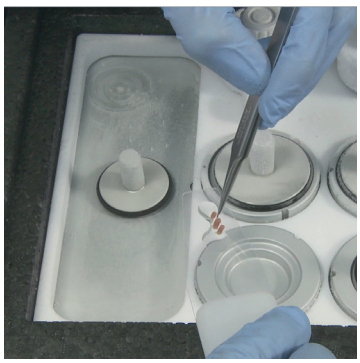
2. Place the strip of paper near the specimens to be frozen on edge.



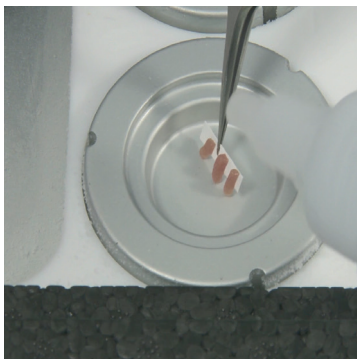
3. Orient the specimens on the paper so that the surfaces to be cut extend 2-3 mm from the edge, leaving empty space for the forceps to grip the strip of paper with the specimens.



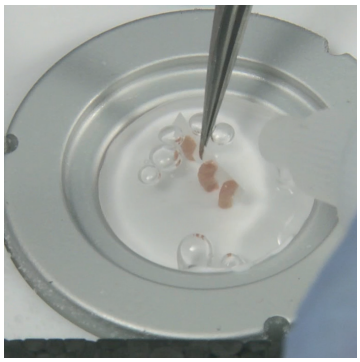
4. Squeeze a line of embedding medium (MCC) onto the tip of the dispensing slide.



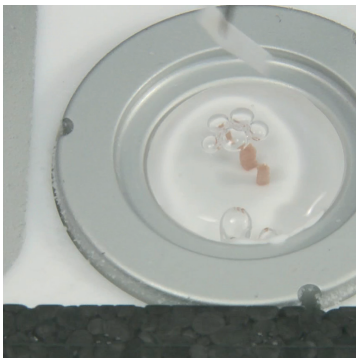
5. Wet the edge of the specimens on the strip of MCC.



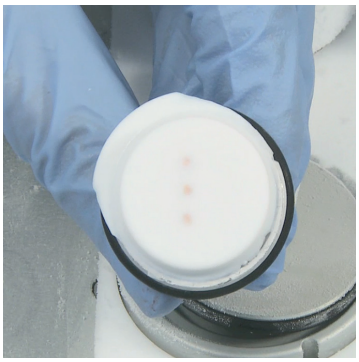
6. Gently place the specimens on the paper on edge in the mould. In this way, only the specimen surfaces to be cut are in contact with the bottom of the mould.



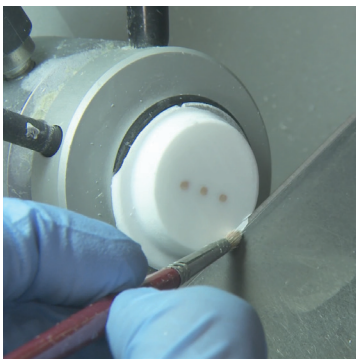
7. Fill the mould with embedding medium (MCC) maintaining the paper with the specimens in the vertical position with the forceps until the mould is filled.



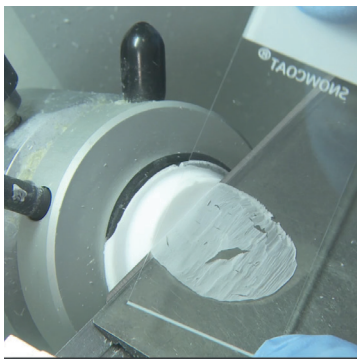
8. Take the forceps away with or without paper. Place chuck and the heat extractor on top of the mould.



9. After 60 seconds, the specimens are frozen on edge ready for trimming and perfectly planar.

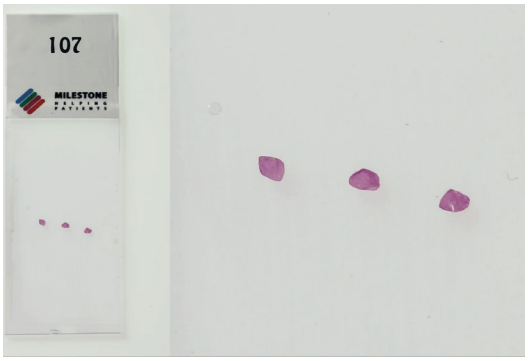


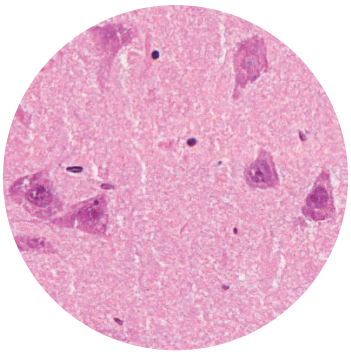
10. After trimming, the block is ready for sectioning.



11. Pick up the slice with the slide, process and stain with PRESTO.

Results





8. **BOP-108** How to freeze skin for MOHS surgery

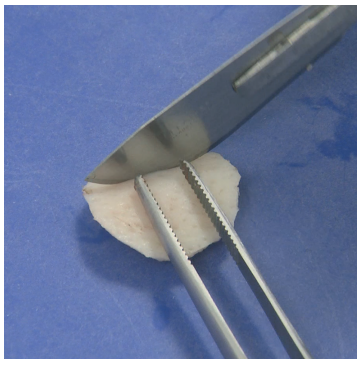
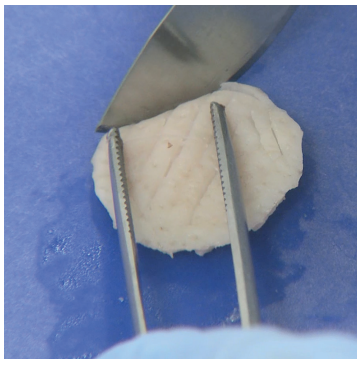
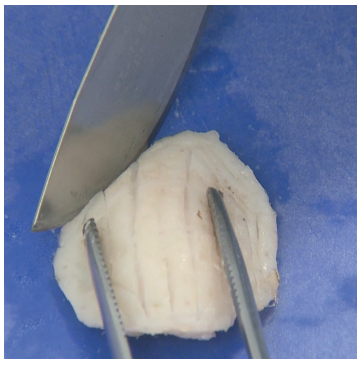
Problem

Proper preparation of skin specimen margins in MOHS surgery is of the utmost importance.
A method is needed to observe skin specimen margins, both lateral and basal, at the same level.

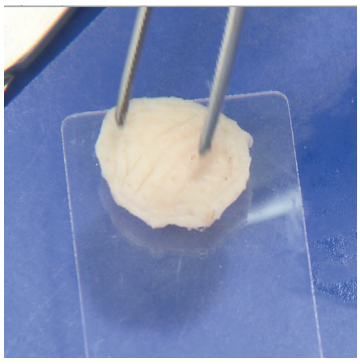
Solution

Milestone has developed a simple method to obtain skin specimen margins at the same level.

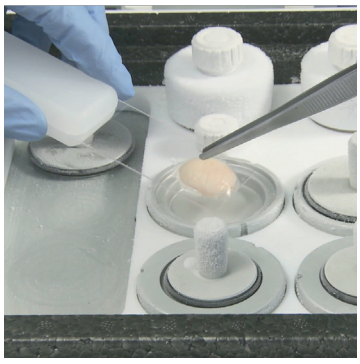
Milestone best operating procedure

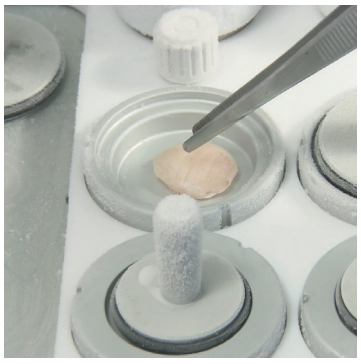
1. Relax the skin specimen making multiple incisions on the epidermis.



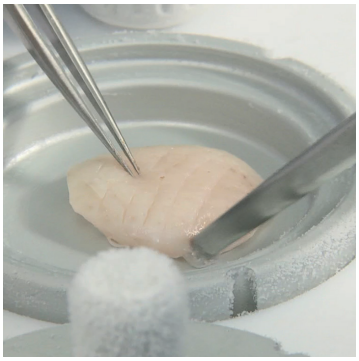
2. Squeeze a drop of embedding medium (MCC) onto the tip of the dispensing slide. Wet the specimen face-down with the embedding medium (MCC).



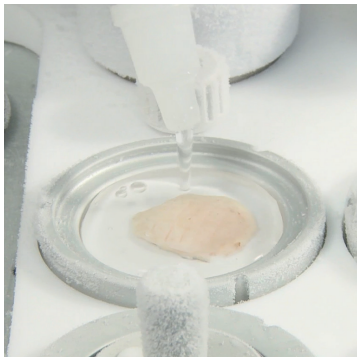
3. Slide the specimen into the bottom of the mould.



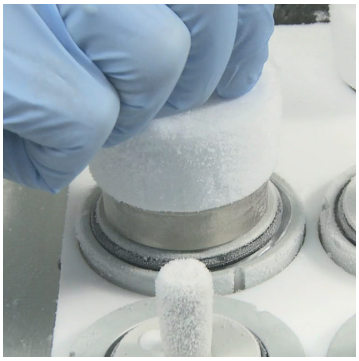
4. Using forceps press all the lateral margins to make them adhere to the flat mould bottom.



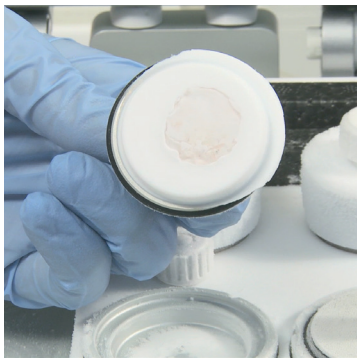
5. Using forceps press all the lateral margins to make them adhere to the flat mould bottom.



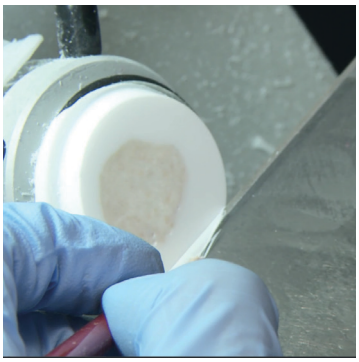
6. Add embedding medium (MCC).



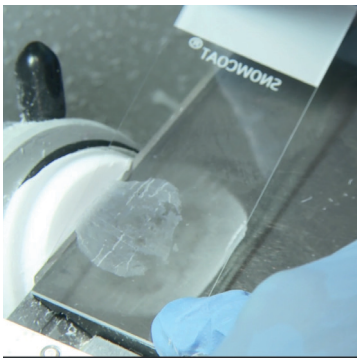
7. Place chuck and heat extractor on top of the mould.



8. After 60 seconds, the specimen is ready for trimming.

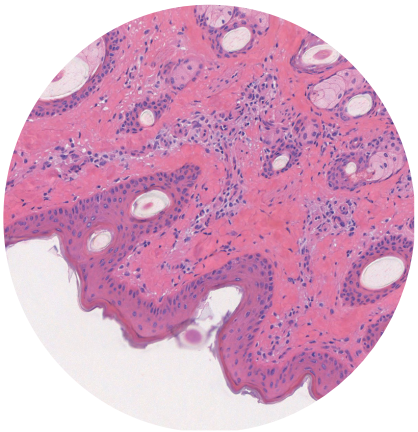


9. Cut tissue section from block.



10. Pick up the tissue section with the slide, process and stain with PRESTO.

Results



9. **BOP-109** How to freeze body fluids

Problem

Frozen sections of body fluids are difficult to prepare.

A method is needed to obtain diagnostic frozen specimens from fluids.

Solution

Milestone has developed a simple method to properly prepare fluid specimens.

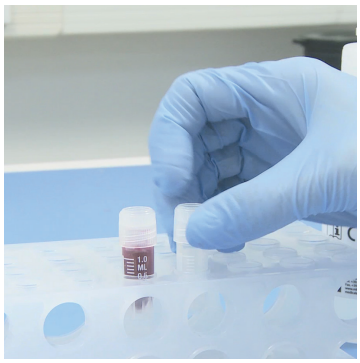
Milestone best operating procedure

Material required:

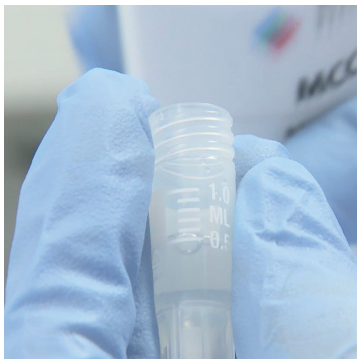
Pipette for mixing

Milestone Cryo Compound (MCC)

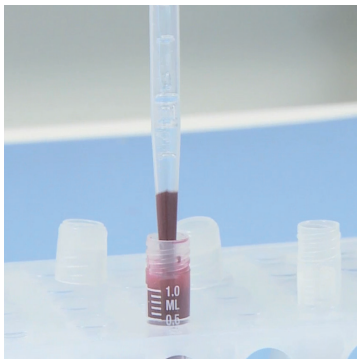
Vials



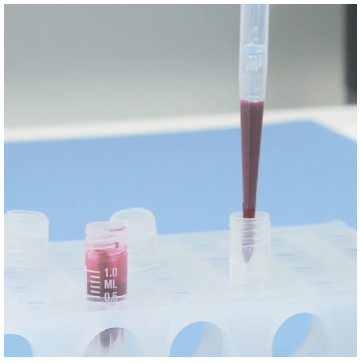
1. Prepare a vial with fluid sample and an empty vial.



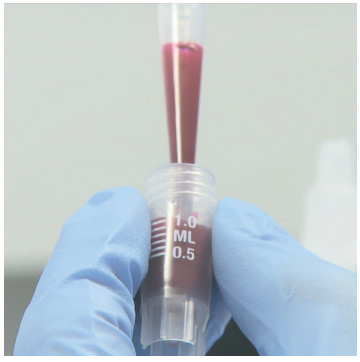
2. Add 0.5ml of embedding medium (MCC) into the 1 ml vial.



3. Aspirate 0.5 ml of sample with a pipette.



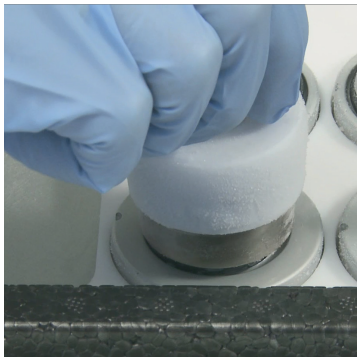
4. Add the 0.5 ml of fluid sample into the vial with embedding medium (MCC).



5. Agitate with pipette three times to assure a thorough mixing of sample with embedding medium (MCC).

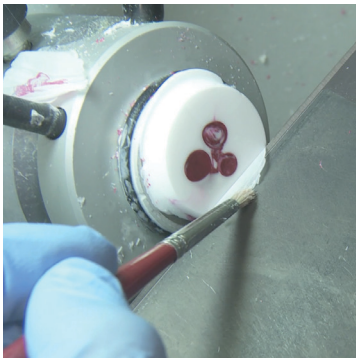
6. Transfer the mixed sample (embedding medium (MCC) + fluid) to the bottom of PrestoCHILL mould.

7. Add embedding medium (MCC) to fill the mould.

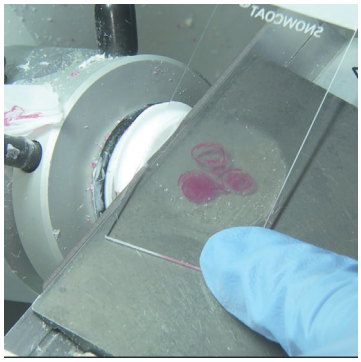


8. Place chuck and the heat extractor on top of the mould.

9. After 60 seconds, the sample is frozen ready for trimming.

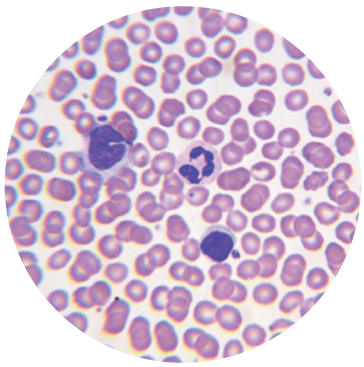


10. After trimming, the block is ready for sectioning.



11. Pick up the tissue section with the slide, process and stain with PRESTO.

Results



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