Breast
General
Frozen section procedure

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PubMed Search: Frozen section methodology

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Definition / general

- A frozen section (cryosection) is a pathological laboratory technique used for rapid microscopic analysis / diagnosis of a specimen / disease
- Usually used with oncologic surgery
- Rapid diagnosis can guide intra-operative patient management

Indications

- Use frozen section to
  - Provide rapid gross or microscopic diagnosis to identify an unknown pathologic process, identify extent of disease / evaluate margins, identify metastases or simply identify a tissue
  - Process tissue to provide appropriate and accurate diagnosis, prognosis and to adhere to research and special study protocols
  - Confirm that pathological tissue is present for diagnosis on permanent sections
- Do not use if
  - Frozen section diagnosis has no immediate implications for decision making
  - Tissue is needed for permanent processing (is unique or small or requires extensive study for diagnosis)
- Consider not freezing tissue if
  - Frozen section is known to produce severe artifacts that hinder proper interpretation
  - Tissue is heavily ossified / calcified
  - Risk of serious infection (HIV, TB, hepatitis B or C)

- Tissue is fatty

Tissue type

- Tissue should be received fresh, otherwise it will not stay on slide
- At time of receipt of tissue, decide whether to obtain smears or touch preps and whether to freeze all or part of it
  - Touch preps and smears are often performed on lymph nodes suspicious for lymphoma
  - Some primary small lesions should not be entirely submitted for frozen section
  - There is debate on whether sentinel nodes should be entirely or representatively submitted for frozen section
- Fixed tissue:
  - There are special slides to keep tissue affixed to slide
  - To freeze fixed tissue, make sure it has been preserved in formalin and not alcoholic fixatives like Carnoy's, because tissue fixed in alcohol is harder to freeze
  - Avoid freezing tissue fixed with heavy metal salts such as B5 and Helly's (Zenker’s formal solution), which can denature proteins and shrink the tissue
  - Avoid hard tissues like bone and cartilage that require decalcification
  - Avoid tissues with a lot of fat
  - Avoid tissues from patients with known TB or other infection (if absolutely necessary, wear appropriate protection)
  - Avoid freezing tissue that will be needed to make a permanent diagnosis

Freezing tissue

- OCT (optimal cutting temperature) or similar embedding media like TBS or Cryogel should be placed on an appropriate sized chuck that has been precooled in a cryostat
- The chuck should be clean
- A toothbrush is useful to remove tissue and OCT
- Dipping the chuck in methanol removes ice crystals
- Place the chuck into a -20 to -15 degree (optimal) cryostat; note that the OCT media should not be frozen completely
- It is better to have a semisolid consistency; this will alleviate tissue artifact
- Tissue size should be no greater than 3mm - 5mm in greatest dimension (thinner specimens have shorter freezing time and minimal ice crystal artifact formation)
- The smaller the tissue, the more even and thorough the freeze
- Place the tissue on the semisolid chuck and add more media rapidly over the tissue, covering it entirely but avoiding overflow
- Place chuck quickly back into the cryostat
- Apply heat sink or CO_2 aerosol (optional) to rapidly freeze or use "quick freeze" option on cryostat
  - Histobath: being phased out
  - Cryowells: useful in keeping all tissue on an even plane; also helpful in eliminating loss of smaller tissues that are frozen with larger ones, although recommended to not freeze different sizes together
  - Aerosol sprays: often canned CO_2 (but may aerosolize infectious diseases)
  - Liquid nitrogen
  - Isopentane based workflow (Virchows Arch 2008;452:305)

Cryosectioning

1. Once the chuck is in position, there should be a manual or an automatic advance option to move the block close to the cutting blade

Tissue embedded within OCT

2. Fully face the tissue by using a trim setting on your cryostat; if you do not have this setting, then an advance button should be available, which should be pressed each time before one full revolution of the instrument's wheel
3. If wells are used to freeze the blocks, then the tissue should be on an even plane and the tissue will be faced faster
4. To polish the tissue, avoid advancing the cryostat or deselect the trim setting on the cryostat and turn 10 - 15 times
5. As you cut the tissue, anchor the tissue to prevent folding or curling; this can be done with an anti roll bar (a plastic plate attached to cryostat) or by using a precooled paintbrush with stiff bristles and a wide gripping surface
6. The brush should be held like a pen with your left hand at an angle
7. You can rest your fifth finger on the stage for stabilization
8. Cutting the brushes' bristles at an angle can aid in the brush meeting the tissue flat over its length because you will hold it at an angle
9. Turn the wheel with your right hand in a continuous motion without stopping; avoid speeding up or slowing down
0. Avoid stopping the wheel at the beginning of the section, slowly grabbing the tissue and then resuming wheel revolutions; this can cause artifacts such as variation in section thickness and tissue folding

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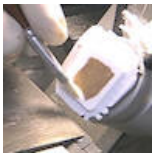
1 Move the brush as the chuck moves towards the blade; your brush should move down in pace with the chuck



Riding the block: as the block descends toward the brush, the brush keeps pace with the block by gently resting on the bottom 2 - 3 mm of the block

2. You can rest your brush softly on the very bottom of your chuck avoiding tissue contact
3. Pull the brush away easily as the chuck meets the blade

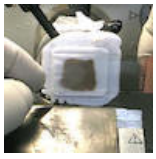

Catching the curl: as the block meets the blade and the section begins its curl, the brush leaves the block while catching the curling edge of the section; then the brush jumps off the block with the curl

4. The downward motion of the brush allows you to keep a continuous motion as you take your section

Pull over the blanket: the brush holding the curl pulls the section horizontally over the stage, like pulling the blanket over yourself, without pressing the tissue to the stage

5. A glass slide is gently laid upon the tissue section





Gently touch the section to the slide; avoid stretching or folding the section by keeping a steady hand, and keep the transverse axis of the slide parallel to the section

6. The tissue section should melt onto the slide
7. Prepared slides should immediately go into formal alcohol, 95% alcohol (methanol/ethanol) or formalin while awaiting the stain line; if you delay this step, drying artifact will occur
8. You can take a deeper level after approximately 20 turns (multiple levels may be needed for breast or prostate biopsies)
9. Optimal cutting thickness is 4 - 7 microns for sectioning and 20 - 40 microns for trimming

Staining slides

- Keep all stains and solutions fresh and well maintained
- Dip slide in reagents in this order for H&E staining:
  - After obtaining frozen section, IMMEDIATELY fix in 95% ethanol (even 15 seconds of delay can cause significant artifact)
  - Formal alcohol, formalin or 95% alcohol: 45 - 60 seconds
  - Water: 5 - 7 seconds
  - Hematoxylin: 60 seconds
  - Lithium carbonate or 0.2 % aqueous ammonia (Bluing): 15 - 20 seconds
  - Eosin: 20 - 60 seconds
  - 95% alcohol: 10 seconds
  - 100% alcohol: 10 seconds
  - Xylene, toluene, limonene derivatives and Clearite: 10 seconds

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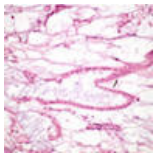
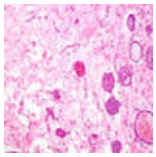
- Then add mounting media for cover slipping

Troubleshooting

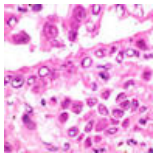
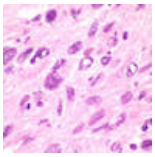
- **Ice crystal artifacts**
  - Due to slow freezing of tissue
  - **Solution**: Freeze fast (flash / snap); the faster the freeze, the smaller the ice crystals, the less tissue damage (best freezing method is arguably liquid nitrogen)
  - Smaller tissues yield less artifact - optimally tissue should be 0.5 x 0.5 x 0.3 cm or less
  - Never freeze fragments larger than the diameter of the chuck
  - Avoid freezing fat around tissue
  - Blot the outer surface of the tissue dry with gauze before making your block
- **Knife artifact**
  - A nicked cutting blade will produce a split / tear in your section
  - **Solution**: change your blade every few cases; some institutions use a new blade for each case
- **Overfreezing**
  - Can cause section to have holes
  - **Solution**: polish block with a couple extra turns of the blade to create friction and warm up block by pressing on it with your finger (5 - 10 seconds)
- **Underfreezing**
  - Underfreezing can be troublesome for fatty tissue
  - **Solution**: add heat sink to block or select rapid freeze setting on your cryostat (if available)
- **Staining issues**
  - Dirty "stain line" can cause floaters (extraneous foreign tissue) to adhere to slides; overly diluted stains and alcohols can diminish slide quality
  - Poor staining hinders frozen section diagnoses, as nuclear detail is compromised
  - **Solutions**: (a) maintain a clean stain line by frequent solution changes; (b) follow recommended staining times; (c) don't rush
  - Note: brain tissue may stain best in eosin for 60+ seconds
  - Water: should be changed after each frozen section
  - Alcohols and stains: change at least weekly, alcohols may need to be changed more frequently depending on work load
- **Fatty tissue**
  - Includes lymph nodes, breast, skin; may be too soft to cut
  - **Solution**: maintain an extremely cold cutting temperature (-20C)
  - Firm lymph nodes, spleen, brain and liver cut better at -10C; tissue may shatter if sectioning is performed at lower temps
- **Air bubbles**
  - May be trapped under cover slips, which can cause the underlying tissue to dry out
  - **Solution**: make sure an appropriate amount of resin (2 drops) is applied; gently move air bubbles off the slide with finger or tweezers; do not press on the slide too hard or it will break
- **Overly thick sections**
  - May cause tissue to fall off slide
  - **Solution**: reduce the cryostat's sectioning thickness

Microscopic (histologic) images

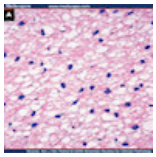
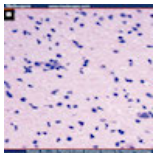
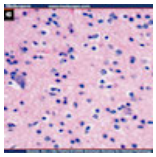
***Images hosted on other servers*:**

Left: ice crystals in edematous stroma by frozen section, right: H&E

Nuclear ice crystals (particularly a problem with thinner sections): left - lung adenocarcinoma, right - uterine sarcoma

Glioma

Videos

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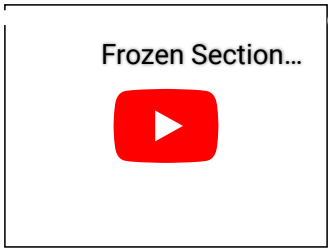
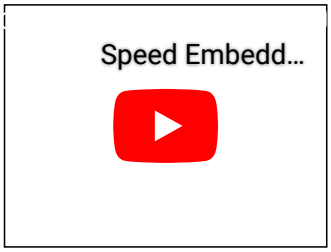


Brush technique

Embedding small specimens



Speed embedding

Additional references

- Pathology Innovations [Accessed 3 January 2019], IHC World [Accessed 3 January 2019]