Shared health
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Document History:

Title: Frozen Sections **Site(s): Shared Health Diagnostic Services HSC, SBH, WL, MHC, GGH**

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Section:	**Pathology**	**Subsection:**	**Histology**

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Details of Recent Revision

Tracking of FS TAT is no longer a CAP requirement and considered optional

1.0 PURPOSE

1.1 To provide instructions on use of a cryostat to produce a rapid H&E stained tissue section from a fresh (unfixed) specimen.

2.0 RESPONSIBILITY

2.1 It is the responsibility of the Charge Technologist or designate to ensure that staff are properly trained in this technique.

2.2 Pathologist's direct responsibilities are outlined in SOP 170-130-12.

3.0 POLICY

3.1 Cryostat temperatures should be monitored daily and logged into the temperature control log in SOP #100-10-22A.

3.2 All frozen section cassettes should be indicated as "FS". In Block Detail (CoPath) enter FS for each frozen section block.

3.3 Frozen sections may be audited for turnaround time. We aim to complete 90% of single case frozen sections within 20 minutes with exceptions for cases with multiple blocks or other unforeseen difficulties.

3.4 Frozen sections with a single block which are not completed within 20 minutes can have the reason for delay documented on the yellow sticker. (this optional but some sites like to keep the sticker for other visual ques).

3.5 Frozen section slides are permanently stained, mounted and labeled. These slides are retained with the rest of the slides from the case. If levels are cut, the slide level designator number needs to be added by hand using **an insoluble** marker/chemical resistant felt pen (Leica or similar).

3.6 Tissue used for frozen section is removed from the chuck and placed in formalin. When the specimen is grossed, the specimen used for frozen section is identified and blocked. The block is processed into paraffin and a slide prepared and examined for comparison with the frozen section interpretation.

The paraffin sections are reviewed by a second pathologist who did not read the initial frozen sections.

3.7 Verbal reports are given to the surgeon. The time the verbal report is given is written on the frozen section sticker which is applied to the requisition.

3.8 The pathologist confirms the patient's identification prior to giving the verbal report.

3.9 The results of intra-operative consultations are verbally provided to the surgeon.

3.9.1 The pathologist calling in the report will ask to speak to the surgeon.

3.9.2 If the surgeon is not available, the pathologist will ask the name of the individual to whom he or she is speaking. If the individual is not the surgeon, he or she will be asked to read back the report once it has been given.

3.9.3 The pathologist will document in the OR consultation box the name of the individual who was given the report.

3.10 The intraoperative consultation is signed by the pathologist who made the diagnosis.

4.0 SAFETY

4.1 Follow appropriate routine laboratory safety practices for personal protective equipment and microtome safety procedures.

4.2 Specimens may be potentially infectious. When handling fresh lung specimens (which may contain H1N1, COVID virus or TB) use an N95 mask for cutting tissues if a biosafety cabinet (BSC) is not available.

5.0 MATERIAL AND EQUIPMENT

5.1 Appropriate personal protective equipment; gloves, lab coat, etc.

5.2 Tissue Support Medium: cryostat mounting media; OCT compound (Optimum Cutting Temperature), or other.

5.3 Glass slides-Charged, Silanized, or similar

5.4 Coplin jars

5.5 Chemical resistant felt pen for labeling cassettes and slides

5.6 Cryostat chucks

5.7 Disposable microtome blade

5.8 Pre-filled formalin container of appropriate size

5.9 Ethanol-95%, 99%

5.10 Xylene

5.11 Stains-Hematoxylin, Eosin (alcohol based)

5.12 Water

5.13 Ammonia water

5.14 Lens paper

5.15 Small ruler

5.16 Forceps

5.17 Scissors

5.18 Safety pins or colored pins to denote margins

5.19 Tissue marking solution

5.20 Pencil or permanent cassette labeling pen

5.21 Scalpel holder for size #21

5.22 #21 size scalpel blades

5.23 Permount or micromount

5.24 Litmus paper or other pH indicator strips

5.25 Tuberculocidal decon solution: Cavicide, or similar (facility approved)

5.26 5% bleach solution (site specific)

5.27 Virocidal disinfectant: Accel Intervention or other similar (facility approved only)

5.28 Frozen section labels (optional)
EXAMPLE OF FS LABEL:

Date/Time Received_____ Tech initials _____
Time Surgeon contacted with results_____
If time exceeds 20 minutes indicate reasons for delay_____
_____
Corrective Actions (if applicable):_____
_____

6.0 PROCEDURE

Note: Frozen sections are typically indicated on the operating room (OR) slate.

6.1 The operating room will contact the pathology department to request a technologist and a Pathologist for a frozen section. HSC communication process SOP 170-140-20

6.2 When a frozen section arrives in the laboratory or frozen section room, immediately label the requisition with a (yellow) frozen section label. (See sample 5.27).

6.3 Write or time stamp the time received on the frozen section label.

6.4 Ensure cryostat is operating at the appropriate temperature. The external display should read (-35^0 C $\pm$ 5 ^{0}C). This temperature is recorded as per temperature monitoring SOP #100-10-22.

6.5 The thermometer should be placed on the cryobar in the left-hand corner of the cryostat.

Note: The Sakura Tissue Tek Cryo3D cryostat temperature display on the outside of the cryostat will indicate (-35^0 C $\pm$ 5 ^{0}C) due to the location of the temperature sensing probe. The **true** cutting temperature is -25° C.
The temperature readings have been tested to comprehend the discrepancy between the equipment readout and the actual operating temperature.

6.6 Ensure there is a sharp disposable knife blade in holder.

6.7 Remove lids from manual staining station and ensure solutions are fresh and at the appropriate fill levels.

6.8 When specimen received, apply specimen acceptance policy to ensure sample and requisition match.

6.9 When the tissue is received from the Pathologist, place tissue support medium on the chuck and place onto the cryobar to begin freezing the support medium (this will ensure the specimen will stick to the chuck).

6.10 Each slide and container used to submit residual tissue for routine processing is labeled with two identifiers.

NOTE: Acceptable patient identifiers include name, date of birth, medical record, PHIN and accession number.

6.11 Place specimen on the partially-frozen tissue support medium and orientate as necessary. Add extra tissue support medium if necessary.

6.12 When the tissue support medium begins to turn opaque or white, place the heat extractor (optional) firmly and directly on top of the tissue. When heat extractor can be easily lifted off of tissue, place the chuck in the chuck holder.
The heat extractor also provides a flat surface for cutting.

Note: If frozen section specimens are sticking to the heat extractor surface, wipe the surface with a small amount of microtome oil before freezing the tissue.

6.13 In the cryostat, unlock turning wheel and bring chuck to the edge of the knife using the coarse advance.

6.14 Trim gently (turn wheel clockwise) until the full face of the tissue is exposed.

6.15 Cut at 5 or 6 microns and use a brush to gently pull the sections past the blade.

6.16 When there is a full-face section on the knife (no creases or lines through tissue), pick up the section on a glass slide (room temperature- charged slide).

Note: Be certain to check the slide against the tissue in the chuck to ensure the section is complete before staining.

6.17 Immediately place in 95% alcohol to fix for 1 minute.

6.18 Stain immediately as outlined in Appendix 1-4 (site specific staining procedures) or as per SOP 170-70-29 if using the automated FS stainer.

6.19 Hand coverslip the slide and remove excess mounting media.

Note: Staining solutions are changed as required at all sites and tracked using Appendix 5. Site specific staining procedures are listed in Appendices 1-4.

6.20 Ensure slide is appropriately labeled with two identifiers. See 5.38

6.21 Give the slide(s) to Pathologist to read.

6.22 Write the time the frozen section is verbally reported to the surgeon on the requisition frozen section label. If the pathologist does not fill this in, the tech is responsible to ensure this detail is entered.

6.22.1 If the time exceeds 20 minutes from receipt to reporting for a single FS, indicate the reasoning on the frozen section label. (Example: tissue fell off of the slide, cryostat skipped and tissue fell of the chuck, multiple frozen sections, pathologists consulting with other pathologists, etc.) Requisitions will be randomly audited for TAT completion.

6.23 When Pathologist indicates the frozen section procedure is completed, paint the tissue cut side with marking solution, gently remove from chuck (option: use warm water if necessary or dip the chuck into formalin to loosen the tissue), wrap small pieces in lens paper in a cassette labeled with the patients' name and place in formalin.

6.24 Clean the cryostat by carefully removing any shavings that are in the bottom Place all used chucks in disinfecting solution (PerDiem, Virox or similar-using the appropriate contact time according to the vendor's instructions), rinse in hot running water and allow to dry completely. Cryostat maintenance is performed according to SOP #170-10-04.

Critical Control 1:

If frozen sections are submitted in saline, check with the pathologists and ensure the entire specimen is placed into formalin when the frozen section is complete.

7.0 HANDLING

7.1 Pathologists covering frozen sections will macroscopically examine the specimen and note any gross abnormalities, and document the specimen dimensions on the requisition. Pathologists' Assistants (PAs) perform this function at Westman Lab and Grace General Hospital in some cases. See SOP 170-80-10 for PA CoPath processes; section 15.

7.2 Imprints may be performed (if required).

Note: Imprints**:** Prepare imprints prior to cutting frozen sections.

As per the lymphoma protocol, it is not recommended to perform frozen sections on suspect lymphoma cases (SOP# 170-10-11).

7.3 Once sections are cut and stained, the pathologists will read the slides and provide a verbal diagnosis to the surgeon or a resident in the operating room.

Note: The patient's identification should be verified prior to delivering the verbal report.

7.4 Frozen section findings are written into the "*intraoperative consultation*" box on the pathology services laboratory requisition. This requisition must be scanned into the case to keep all details of the FS report electronically. (site specific process for who does the scanning). Scanning personnel need to ensure they check both sides of the requisition in case the FS report extends to the back of the requisition. Both sides would need to be scanned into PicsPlus in CoPath.

7.5 Pathologists, PAs or technologists will fill in the appropriate frozen section tracking book to document the following**:**

7.5.1 Date
7.5.2 Name of patient
7.5.3 Name of surgeon
7.5.4 Initials of pathologist
7.5.5 Initials of technologist

7.6 Pathologists will initial the FS tracking book.

7.7 At Westman lab, PAs will dictate the number of frozen section (FS) blocks & slides into the LIS and log into their workload book.

7.8 Pathologists may choose to contact another pathologist to consult on the case.

7.9 All frozen sections are reviewed by a second pathologist, by assigning the frozen section case to a different pathologist when the paraffin sections/surgical case is complete. FS QA review is entered as per SOP #170-20-21.

7.10 Frozen sections are reviewed through the quality assurance standards process as outlined in SOP #170-20-02.

8.0 REFERENCES

7.1 Sakura Tissue- Tek Cryo 3D operating manual 5801, 5802

Appendix 1: Frozen Section Staining Procedure

Health Sciences Centre/ / Misericordia Health Centre

If using automated FS stainer, see SOP 170-70-29

Solution	Time	Comments
95% alcohol	1 minute	
Wash in water	10 seconds	With agitation
Rinse in Distilled or deionized water	6-10 dips	Optional
Hematoxylin: Richard Allen	45 s -1 minute	
Wash in water	10 seconds	With agitation
*Ammonia Water	6-8 dips	Until sections appear blue macroscopically
Wash in water	10 seconds	With agitation
95% alcohol	10 seconds	
Eosin	20 seconds	
95% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
95% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
100% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
100% alcohol/xylene	~5 seconds	2-3 dips until xylene runs cleanly on slide
Xylene	~5 seconds	2-3 dips until xylene runs cleanly on slide
Xylene	~5 seconds	This xylene step is optional 2-3 dips until xylene runs cleanly on slide
Mounting medium (permount or similar)		Hand coverslip

***Test the pH of the ammonia water with litmus or pH indicator strips each time it is freshly prepared.**

Ammonia water: the pH range should be close to 10

Note:

Do not agitate in ammonia water as this can increase frozen section loss during staining.

Wash thoroughly after bluing as the increased pH can adversely affect the Eosin staining.

Appendix 2: Frozen Section Staining Procedure

St. Boniface

If using automated FS stainer, see SOP 170-70-29

Solution	Time	Comments
95% alcohol	1 minute	
Wash in water	10 seconds	With agitation
Hematoxylin – Richard Allen	1 minute	
Wash in water	10 seconds	With agitation
*Ammonia Water	6-8 dips	Until sections appear blue macroscopically Ammonia water is made by adding 8 drops of ammonium hydroxide to 50 ml of tap water.
Wash in water	10 seconds	With agitation
95% alcohol	10 seconds	
Eosin	20 seconds	
100% alcohol	~5 seconds	4-5 dips until alcohol runs cleanly on slide
100% alcohol	~5 seconds	4-5 dips until alcohol runs cleanly on slide
100% alcohol	~5 seconds	4-5 dips until alcohol runs cleanly on slide
Xylene	~5 seconds	4-5 dips until xylene runs cleanly on slide
Xylene	~5 seconds	4-5 dips until xylene runs cleanly on slide
Xylene	~5 seconds	This xylene step is optional 2-3 dips until xylene runs cleanly on slide

***Test the pH of the ammonia water with litmus or pH indicator strips each time it is freshly prepared.**
Ammonia water: the pH range should be close to 10
Note:
Do not agitate in ammonia water as this can increase frozen section loss during staining.
Wash thoroughly after bluing as the increased pH can adversely affect the Eosin staining.

Appendix 3: Frozen Section Staining Procedure

Grace General Hospital

Solution	Time	Comments
95% alcohol	1 minute	**Imprints** place into fresh 95% alcohol for 5 minutes prior to staining
Wash in water	10 seconds	With agitation
Rinse in Distilled or deionized	6-10 dips	Optional
Hematoxylin-Prokop's	3 minutes	
Wash in water	10 seconds	With agitation
*Bluing Solution	6-8 dips	Until sections appear blue macroscopically
Wash in water	10 seconds	With agitation
70% alcohol	10 seconds	
Eosin	30 seconds	
100% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
100% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
100% alcohol	~5 seconds	2-3 dips until alcohol runs cleanly on slide
Xylene	~5 seconds	2-3 dips until xylene runs cleanly on slide
Xylene	~5 seconds	2-3 dips until xylene runs cleanly on slide
Xylene	~5 seconds	This xylene step is optional 2-3 dips until xylene runs cleanly on slide
Mounting medium (permount or similar)		Hand coverslip

***Test the pH of the bluing solution with litmus or pH indicator strips each time it is freshly prepared.**

Bluing solution: the pH range should be close to 8

Note:

Do not agitate in bluing solution as this can increase frozen section loss during staining.

Wash thoroughly after bluing. The increased pH can adversely affect the Eosin staining.

Appendix 4: Frozen Section Staining Procedure

Frozen Section Staining Procedure- Westman Laboratory

Solution	Time	Comments
95% alcohol	1 minute	
70% alcohol	6-10 dips	With agitation
Rinse in Distilled or deionized	6-10 dips	Optional
Hematoxylin-Surgipath	1 minute	
Wash in water	6-10 dips	With agitation
*Ammonia Water	10 sec.	Until sections appear blue macroscopically
Wash in water	10 seconds	With agitation
Eosin 0.8%	2-3 dips	
70% alcohol	6-10 dips	2-3 dips until alcohol runs cleanly on slide
100% alcohol	6-10 dips	2-3 dips until alcohol runs cleanly on slide
100% alcohol	6-10 dips	2-3 dips until alcohol runs cleanly on slide
Xylene	6-10 dips	2-3 dips until xylene runs cleanly on slide
Xylene	6-10 dips	2-3 dips until xylene runs cleanly on slide
Xylene	~5 seconds	This xylene step is optional 2-3 dips until xylene runs cleanly on slide
Mounting medium (permount or similar)		Hand coverslip

***Test the pH of the ammonia water with litmus or pH indicator strips each time it is freshly prepared.**

Ammonia water: the pH range should be close to 10

Note:

Do not agitate in ammonia water as this can increase frozen section loss during staining.

Wash thoroughly after bluing as the increased pH can adversely affect the Eosin staining.

Appendix 5: Frozen Section Stain Quality Maintenance

Winnipeg Laboratories

Solution	Date/Initial	Date/Initial	Date/Initial	Date/Initial	Date/Initial	Date/Initial
95% alcohol-*fixative*						
Hematoxylin						
Ammonia Water or Bluing Solution (indicate pH)						
Eosin						
95% alcohol						
95% alcohol						
100% alcohol						
100% alcohol						
Xylene						
Xylene						
Xylene						
Mounting medium						
Comments						

Charge technologist or designate monthly sign off _____ Date_____

Appendix 5A: Frozen Section Stain Quality Maintenance Westman Laboratory

Solution	Date/Initial	Date/Initial	Date/Initial	Date/Initial	Date/Initial	Date/Initial
95% Alcohol						
70% Alcohol						
Distilled H20						
Hematoxylin						
Distilled H20						
Ammonia Water						
Eosin						
70% alcohol						
100% alcohol						
100% alcohol						
Xylol						
Xylol						
Mounting Medium						
Comments						

Charge technologist or designate monthly sign off _____ Date _____

Appendix 6- Frozen Section Turn Around Time Report

Date: _____

Time period reviewed (month): _____

Total number of frozen sections in the time period reviewed: _____

Number of single frozen sections performed: _____

Number of FS cases exceeding 20-minutes: _____

Number of Sentinel Lymph Nodes performed: _____

Number of SLN exceeding 30 minutes: _____

List of all cases over target TAT:

Accession Number	Diagnosis	TAT	Pathologist	Cryostat (Optional)	Comments

Conclusion/recommendations:

Action taken:

Follow up required:
Charge Technologist:_____ Date:_____