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| **LEICA 1850 Cryostat** | | | | | | | | |
| **Purpose** | To provide working guidelines in the operation of the Leica 1850 cryostat. The cryostat is designed for the rapid freezing and sectioning of tissue samples. | | | | | | | |
| **Scope** | Histology staff; Pathology Assistant; Pathologist | | | | | | | |
| **Materials** | **Reagents** | | **Supplies** | | | **Equipment** | | |
|  | * 80 % Alcohol * CAVICIDEor Sani-Cloths | | * OCT media * Superfrost plus slides | | | * Leica 1850 cryostat * Cryostat specimen chucks * Disposable microtome blades * Brushes * Biohazard Sharps * Gloves,PPE | | |
| **Specimen** | Fresh, unfixed tissue | | | | | | | |
| Related Policies and Procedures | HI 1.12 Frozen Sections.doc  HI 1.17 Handling & Disposal of Infectious Tissue and Chemical Reagent Waste.doc  HI.103 Quality Control in Anatomic Pathology.doc | | | | | | | |
| **Special Safety Precautions** | ALL components of the cryostat chamber MUST be below -10° C before attempting sectioning.  Use appropriate Personal Protective Equipment during frozen sections.  Lock the handwheel while clamping the specimen chuck, changing microtome blade and while not in use.  Dispose of used microtome blades and other sharps in appropriate Biohazard sharps containers.  The cryostat chamber is not appropriate for storing tissue specimens overnight due to automatic defrosting. Frozen tissue specimens may be placed in the Histology freezer or the -70° C Freezer in the Main Lab. | | | | | | | |
| **Quality Control** | Tissue freezing, sectioning and slide staining quality are performed by visual examination of slides by Histology staff, Pathology Assistant and Pathologist.  Temperature monitoring and daily preventative maintenance is recorded in Histology.  Yearly preventative maintenance and/or additional service or repairs are performed and documented by BioMed and North Central Instruments. | | | | | | | |
| **Procedure** |  | | | | | | | |
|  | **Step** | **Action** | | | | | **Related Document** | |
|  | 1 | Place OCT compound onto a specimen chuck and place on peltier to freeze and form a base to orient the tissue specimen. | | | | | HI 1.12 Frozen Sections.doc | |
|  | 2 | Give Pathologist the prepared chuck to orient the tissue on, place in cryostat and cover well with OCT.Place heat extractor gently on tissue to freeze. | | | | |  | |
|  | 3 | Place chuck into the chuck holder and secure firmly. Press the coarse feed (double arrow button) to “horizontal fast”. Press the "up" arrow to move the chuck holder to the “home position”. | | | | |  | |
|  | 4 | Press the double arrow button to “horizontal slow” and bring the chuck with the specimen on it back towards the microtome blade by pushing the “down arrow” button. Turning the handwheel, carefully face off the OCT until the specimen is full face. | | | | |  | |
|  | 5 | Cut 3 separate sections of the specimen, beginning with the first level just full face. Do 2-3 rotations and take each additional section. | | | | |  | |
|  | 6 | As the first section is cut, hold it down with a brush close to the blade plate. Take the first labelled slide and touch it to the section beginning at on edge until it has made contact and is adhered to the slide. The slide is now ready to be stained. Please refer to the Frozen Section procedure and/or the posted Bench Reference for staining guidelines. | | | | | HI 1.12 Frozen Sections.doc | |
| **Routine cleaning** |  | Cleaning of the interior of the chamber is accomplished by wiping surfaces with gauze soaked with 80% alcohol. Trimmings of tissue that accumulate in the chamber are removed and disposed of in Red Biohazard trash after each case. Documentation is recorded on the Daily PM form in Histology. | | | | |  | |
| **Cutting Infectious Tissue (Includes HIV/AIDS and TB):** | |  | | | | | | |
| **Protective Clothing:** |  | Wear double gloves, N95 mask, safety eyewear and gown. | | | | | | |
|  | 1. | Prepare the specimen chuck as previously directed. Freeze the specimen with heat extractor - DO NOT use Cytocool on these cases. | | | | | | |
|  | 2. | After sectioning, place slides into 95% Alcohol for fixation. | | | | | | |
|  | 3. | Remove outer layer of double gloves, withdraw slide from fixative with clean  forceps, and proceed with staining procedure (please refer to the Staining  Manual and/or posted bench reference). | | | | | | |
| **Cleaning after cutting infectious cases** | 4. | When technical procedure is completed, put on clean double gloves and place residual  Tissue directly into 10% formalin to fix thoroughly before it is handled again. | | | | | | |
|  | 5. | When all cutting is completed, remove disposable blade and discard in appropriate  BIOHAZARD SHARPS container. With protective clothing in place, remove the knife holder from the microtome. Then wipe clean with two changes of Sani-cloths, Cavicide and 100% alcohol. Retrieve all tissue shavings from the waste tray. Dispose directly into a Red Biohazard Trash. Remove outer layer of double gloves, also disposing into the same bag. Then close the bag securely and dispose into the Red Biohazard trash bin. Wipe all interior surfaces of the chamber with alcohol and dry well. | | | | | | |
|  | 7. | \*\*If complete decontamination is desired, the cryostat must be shut off long enough for the microtome area to reach ambient temperature. Wipe all exposed surfaces inside and outside the cryostat chamber with gauze soaked with Cavicide disinfectant or a Sani- Cloth wipes. Then wipe all surfaces with a towel or sponge that has been soaked in 100% alcohol. Let the surface area air dry with the cover open to dissipate any fumes. **Do NOT use Bleach in the cryostat or on any of its components.** (Bleach will damage the stainless steel). | | | | | | |
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|  | 9. | Turn the cryostat back on and allow to cool completely before using (-18 to -25 C). | | | | | | |
| **Troubleshooting** |  | | | | | | | |
| **Error messages** | The CM 1850 has a self-diagnosis system. Should error messages appear, please refer to the Leica 1850 Cryostat User Guide for specifics. BioMed or North Central Instruments should be notified immediately. | | | | | | | |
| **Repairs** | Replacement of the lamp (light bulb) fuses and/or other repairs should be performed by the BioMed department or by North Central Instruments. | | | | | | | |
| **Thin/thick sections** | Make sure that chuck holder is all the way back in cryostat. Check and readjust clearance angles, clamping of the chuck holder, and the clamp of the blade holder. | | | | | | | |
| **Compressed sections** | The specimen/ OCT may be too warm  Microtome blade may be dull. Replace.  Clearance angle should be set at approximately 5; check and adjust as necessary | | | | | | | |
| **Brittle sections** | Specimen too cold. Warm slightly by wiping surface with thumb. | | | | | | | |
| **Frost on chamber walls or microtome** | Window sash left open. Humidity may cause components to "stick" and other problems; exposure to air currents (open doors and air conditioning) will also cause the chamber temperature to lower and cause frost buildup. | | | | | | | |
| **References** | Leica 1850 Cryostat User Guide \*\*NOTE: Decontamination of the interior of cryostats may be accomplished with 80% ethanol.  Trimmings and sections of tissue that accumulate inside the cryostat should be removed during decontamination. In addition to this decontamination process, the cryostat should be defrosted and decontaminated with a tuberculocidal disinfectant at an interval appropriate for the institution; this should be weekly for instruments used daily. | | | | | | | |
| **Authorization** |  | | | | **Signature** | | | **Date** |
| **Medical Director** | | | | Dennis Drehner DO | | | 6/5/09 |
| **Revised by** | | | | Melissa Turner PA | | | 7/15/10 |
| **Approved by** | | | | Melissa Turner PA | | | 7/15/10 |
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|  | **Medical Director** | | | | Megan K. Dishop MD | | | 7/7/15 |
|  |  | | | | Megan K. Dishop, MD | | | 6/30/17 |
|  |  | | | | | | | | **Issue date for training** |
| **Annual Review** | **Designee** | | | **Signature** | | | | **Date** |
| Technical Specialist | | | **Signature** | | | |  |
|  | | | Dave Slinger | | | | 6/22/09 |
|  | | | Melissa Turner | | | | 5/4/10 |
|  | | | Melissa Turner | | | | 7/15/10 |
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|  | APS | | | Dave Slinger | | | | 8/5/12 |
|  | APS: Revised | | | Dave Slinger | | | | 4/2/13 |
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| **Revised** | Histology Lead | | | Angela Dubbelde | | | | 5/14/2019 |