Name:	Leica Autostainer XL Procedure	**Created:**	September 11, 2025
Number:	Histo-5	**Revised:**	
Category:	Instrument Operation	**Author(s):**	Katie Tooley

1.0 Purpose

The purpose of this SOP is to describe the proper procedure of use of the Leica Autostainer XL, located in the histology core, Bertelsmeyer 220.

2.0 Policy

2.1 The use of all histology equipment in the histology core lab at Missouri S&T is currently managed by the Center for Biomedical Research (CBR) staff.

2.2 All personnel working in the histology core are required to complete general laboratory safety and BSL-2 training through EHS.

- 2.2.1 More training may be required in the future. Please check with CBR staff before beginning work to ensure all required training is complete.

2.3 Eating or drinking is not permitted in the lab.

2.4 PPE is required for all users. This includes, at minimum, gloves and a lab coat. A mask and/or goggles should also be worn if working with noxious chemicals (i.e. xylene) or chemicals with the potential to splash.

2.5 Bertelsmeyer 220 is a shared lab space, therefore all users must be familiar with the supplies and equipment available to them before keycard access to the lab will be granted.

2.6 All samples should be labeled with your name, date, and sample identification. **Any samples not labeled will be thrown out.**

2.7 Each user is required to sign in and out of each equipment logbook while working in the histology core.

- 2.7.1 Please also schedule all equipment use through the online Outlook calendar for the Histology Users – MST Outlook group.
  - 2.7.1.1 Please view the Outlook calendar instructions pdf sent with the Outlook group invite for further information.

2.8 Each person working in the lab is responsible for cleaning work surfaces, such as benches, and any used equipment before leaving.

- 2.8.1 Cleaning tasks must be documented daily on the provided checklist.

2.9 Each person leaving the lab, including temporary visitors, is required to wash their hands before leaving.

2.10 No user fee is currently being charged, however, a list of supplies to be provided by users is outlined in the Supplies section below.

2.11 The operator is responsible for carefully following all steps outlined in this SOP, performing the required cleaning after each use, and immediately reporting any equipment malfunctions, damage, or safety concerns to the lab supervisor.

2.12 Please contact Anna Chernatynskaya or Katie Tooley if you have any

questions.

3.0 Autostainer Procedure

3.1 Before operating the stainer, remember to bake all tissue samples onto the slides for about 30 minutes at 60C on the slide warmer.
- 3.1.1 This step does not apply to frozen sections, only paraffin processed sections.

3.2 Sign in to the embedder/stainer logbook located in the drawer underneath the embedder.

3.3 Raise the sash on the fume hood as far as needed to open the stainer lid and silence the alarm.

3.4 Turn on the stainer using the switch located on the right side of the instrument.

3.5 Remove all lids of the reagent containers and stack them on the left side of the fume hood.

3.6 Place all slides in the metal slide rack.
- 3.6.1 Make sure the slides are placed with the frosted end facing up.
- 3.6.2 The slide rack can hold up to 30 slides. If you have more slides, you will have to run more than one cycle.

3.7 Place the slide rack in the loading station.
- 3.7.1 **IMPORTANT!** Make sure the handles on the stainer head are lined up with the handles on the rack before moving forward. The stainer head will **not** automatically adjust to the right position. If you do not do this, the stainer head will not pick up the rack correctly, and your slides **will** break.

3.8 On the main menu in the control panel on the righthand side of the stainer, select "Stain".

3.9 Select the program you wish to use using the up and down arrows.
- 3.9.1 Paraffin processed tissues should use Program 2. Frozen sections from the cryostat should use Program 5.
- 3.9.2 Do NOT edit any of the programs yourself. Contact Anna Chernatynskaya or Katie Tooley if you need adjustments to a program.

3.10 The machine will initialize.

3.11 Once initialization is done, press the "Load" button located underneath the loading station.

3.12 The process will run for about 45 minutes.

3.13 Once the program is finished, the rack should be located at the exit station. Remove the rack and press the "Exit" button located underneath the station.

3.14 Place the rack on top of a paper towel in the fume hood and allow the slides to dry for a few minutes.

3.15 After the slides are no longer dripping, they can be moved to the slide drying rack located next to the slide warmer.

3.16 Replace all reagent container lids and lower the fume hood sash.

3.17 Sign out of the logbook.

3.18 Allow the slides to fully dry overnight.

4.0 Coverslipping Procedure

4.1 After allowing the slides to dry overnight, you may begin to coverslip them.

4.1.1 You **MUST** coverslip slides if you want to analyze them under the microscope.

4.2 Retrieve a box of coverslips from the drawer underneath the microtome.

4.2.1 Two different sizes of coverslips are available. Choose the best size for the number of samples on your slide. You may need to use the larger coverslips if you have multiple samples on one slide.

4.3 Set out a slide and coverslip on a kimwipe or clean surface in the fume hood.

4.3.1 Coverslipping should be performed in the fume hood. Toluene-based media produces noxious vapors.

4.4 You can coverslip slides in one of two ways depending on preference.

4.5 **First option:**

4.5.1 Using the glass stirrer next to the media container, allow a small amount of media to drip directly onto the slide.

4.5.1.1 Do **NOT** touch the glass stirrer directly on the slide.

4.5.1.2 Set the glass stirrer on a paper towel. Do **NOT** let it touch the benchtop with mounting media on it.

4.5.2 Lay the coverslip slowly onto the slide at an angle, allowing any air bubbles to be released.

4.6 **Second option:**

4.6.1 Using the glass stirrer next to the media container, dip out a small amount of media directly onto the coverslip.

4.6.1.1 Set the glass stirrer on a paper towel. Do **NOT** let it touch the benchtop with mounting media on it.

4.6.2 Flip the slide upside down and slowly place it onto the coverslip, allowing the weight of the slide to push out any trapped air bubbles.

NOTE: It may take some practice to learn how much mounting media you need for coverslipping. We encourage you to practice your technique on practice slides before beginning work on experimental slides.

4.7 After mounting the coverslip, place the slide back on the slide drying rack next to the slide warmer.

4.8 Repeat the above steps on the remaining slides.

4.9 Once finished coverslipping all slides, clean up any used kimwipes and paper towels.

4.10 Pour out some xylene onto a paper towel and use it to wipe off all remaining media on the glass stirrer and any drops or smears of media in the fume hood.

4.11 Make sure to recap the media and place the glass stirrer next to the media bottle for the next person to use.

4.11.1 Do **NOT** use this glass stirrer for anything else.

4.12 Allow the slides to dry for a few days before storing or analyzing under the microscope.

5.0 Supplies provided by users:

5.1 **Blades for microtome and cryostat**: Epredia HP35 Ultra microtome blades (please stick with this exact product, our microtome is set up for easy change-outs of these specific blades) - $289.09 for a pack of 50, Catalog #31-537-35, https://www.fishersci.com/shop/products/thermo-scientific-ultra-disposable-microtome-blades-

2/3153735?searchHijack=true&searchTerm=thermo-scientific-ultra-disposable-microtome-blades-2&searchType=Rapid&matchedCatNo=3153735

5.2 **Camel hair brushes, small** (links include what we use, but feel free to shop around, must be camel hair) - $70.75 for a pack of 12, Catalog #1910, https://www.fishersci.com/shop/products/cryotome-cryostat-accessories-camel-hair-brush/1910#?keyword=1910%20brush

5.3 **Camel hair brushes, large** - $26.28 each, Catalog #03-661, https://www.fishersci.com/shop/products/fisherbrand-long-handled-camel-s-hair-brush/03661#?keyword=03661

5.4 **1 gallon of Fisher histological grade ethanol** (only needed if you'll be processing more than 50 samples) - $113.10, Catalog #A405F-1GAL, https://www.fishersci.com/shop/products/ethanol-anhydrous-histological-fisher-chemical-3/A405F1GAL?searchHijack=true&searchTerm=ethanol-anhydrous-histological-fisher-chemical-3&searchType=Rapid&matchedCatNo=A405F1GAL

5.5 **Glass microscope slides** (charged slides are best for tissue retention during staining) – Fisherbrand Superfrost Plus Microscope Slides, $47.66 for pack of 144 slides, Catalog # 22-034979, https://www.fishersci.com/shop/products/fisherbrand-superfrost-plus-stain-slides/22034979?searchHijack=true&searchTerm=fisherbrand-superfrost-plus-stain-slides&searchType=Rapid&matchedCatNo=22034979

5.6 **Glass cover slips** (personal preference, but this is what we use) - Epredia Signature Series Cover Glass, $83.55 for a pack of 10 boxes, Catalog #22-050-232, https://www.fishersci.com/shop/products/signature-series-cover-glass-24-x-50mm/22050232#?keyword=22050232

5.7 **Microscope slide box** (feel free to shop around, item linked is an example) – Fisherbrand Microscope Slide Box, 100 slots, $9.58, Catalog #03-446, https://www.fishersci.com/shop/products/fisherbrand-microscope-slide-boxes-numbered-slots-3/03446#?keyword=03-446

6.0 References

6.1 Rankin Biomedical Leica Autostainer XL Manual https://rankinbiomed.com/wp-content/uploads/Leica-ST5010-Operator-Manual.pdf

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
1	9/11/25	Created
2		