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Automated H&E Staining and Coverslipping (Leica)

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1 Works for me dx.doi.org/10.17504/protocols.io.bpyhmpt6

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ABSTRACT

This procedure establishes a consistent process for preparing H&E slides from FFPE tissue samples using the automated H&E Leica Stainer and coverslip instruments. This procedure is performed by trained histology laboratory personnel.

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GUIDELINES

ADDITIONAL STAINING/COVERSLIPPING INFORMATION

1. Changing the Stainer Reagents

a. The regents in the Stainer need to be changed weekly, except for the H and E, which are changed every 2 to 3 weeks. There is a guide on the side of the machine that shows which reagents go in which section of the machine.

Do not fill the wells past the fill line.

b. All of the reagents except for xylene can be disposed per normal protocol. Xylene needs disposed of in the special container that sits underneath the Stainer. When this gets full a chemical waste collection request form will need to be filled out and submitted.

c. Go to http://www.safety.vanderbilt.edu/index.php and find the Chemical Waste Collection Request Form. There is a link for this form on the homepage under the most requested section. Click 'Submit an Online Request for Chemical Waste Collection' and fill out the form that appears.

Figure 10. *Xylene Waste:* Xylene cannot go down the sink. It must go into the special hazardous waste containers and be removed by special services.

2. Changing the Cover-slipper Reagents

a. Fill the mounting media the evening before use if it is running low, make sure it has time to settle so there aren't any air bubbles in it before running the machine. Never fill past the 200 ml mark.

b. When closing the cover-slipper, double check that there is xylene in the small bottle the needle sits in. It can be refilled with the xylene in the drawer below using a dropper.

3. Refilling the Coverslips

a. The black box that the coverslips sit in can be removed from the machine. When new coverslips are placed in the machine, they need to be flush against the bottom of the box, without any sticking out of the pile. Tap the sides of the box to get them to align and use a pair of tweezers to tap down any coverslips that are not in line with the rest of them. The Cover-slipper has trouble grabbing and placing the coverslip if it is not sitting in the box correctly.

4. The Carbon Filter

a. The filter in this machine needs replace every 3 to 6 months. There is a sticker in the machine next to the filter that says the date it was last replaced. Take the filter out by lifting the black cap that holds the filter in place and pulling it out. Place the new filter in the machine and close the cap. There are arrows on the side to guide the user and the black clip will not close properly if the filter is in the machine incorrectly.

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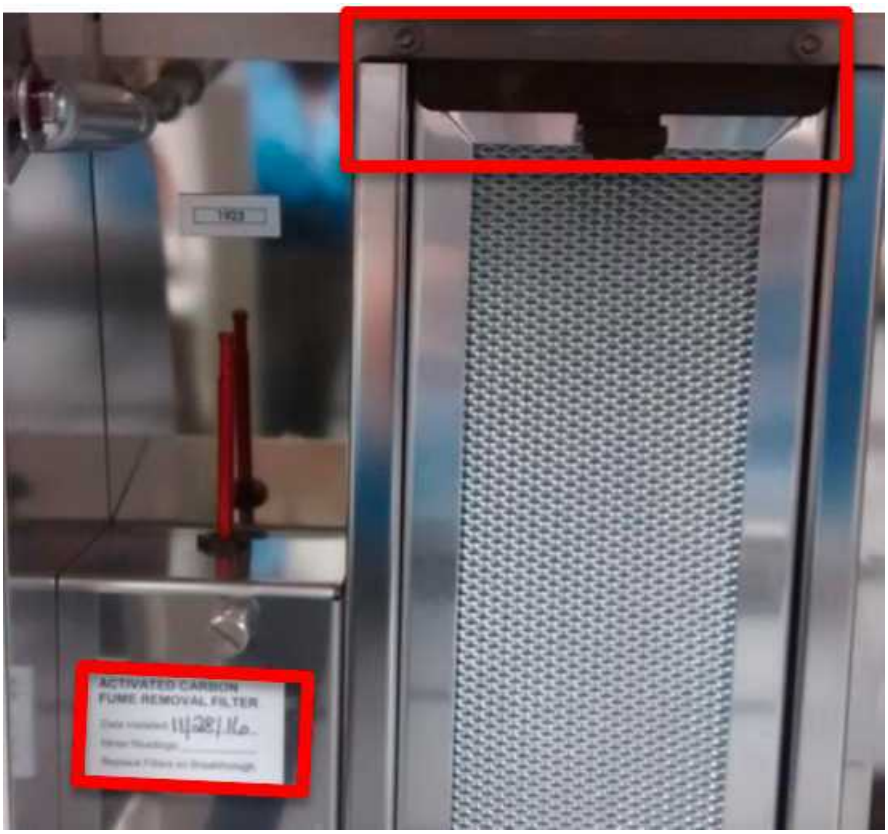

Figure 11. *The Carbon Filter:* The image above shows the black clip that holds the filter in place. It also shows the sticker with the date that the filter was last changed.

MATERIALS TEXT

REQUIRED EQUIPMENT, SUPPLIES AND REAGENTS

1. Equipment

a. Leica ST5020 Multi-Stainer (Serial #0234)
b. Leica CV5030 Robotic Coverslipper (Serial #3761)

2. Supplies

a. Stainer racks and clips
b. Coverslipper racks
c. Coverslips – Leica cat# 3800145ACS

3. Reagents

a. Alcohol – Pharmco cat# 1110002000
b. Xylene – ThermScientific cat# 9990501 (see attachment MSDS)
c. Eosin – ThermoScientific cat# 71204
d. Hematoxylin - ThermoScientific cat# 7211
e. Bluing agent – ThermoScientific cat# 7301
f. Clarifying agent – Richard-Allan Scientific cat# 7401 (see attachment MSDS)
g. Mounting Media – Leica cat# 3801730 (see attachment MSDS)

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

SAFETY

Ethanol is flammable. Store properly in a flammable cabinet.
Wear gloves when using these machines to protect from dyes and reagents.

Automated Staining and Coverslipping

1 Turn on the Automated Robotic Cover slipper and check levels for mounting media and coverslip. After initializing, retrieve the white brush clip from the Xylene well in the drawer on the front and clip it in place.

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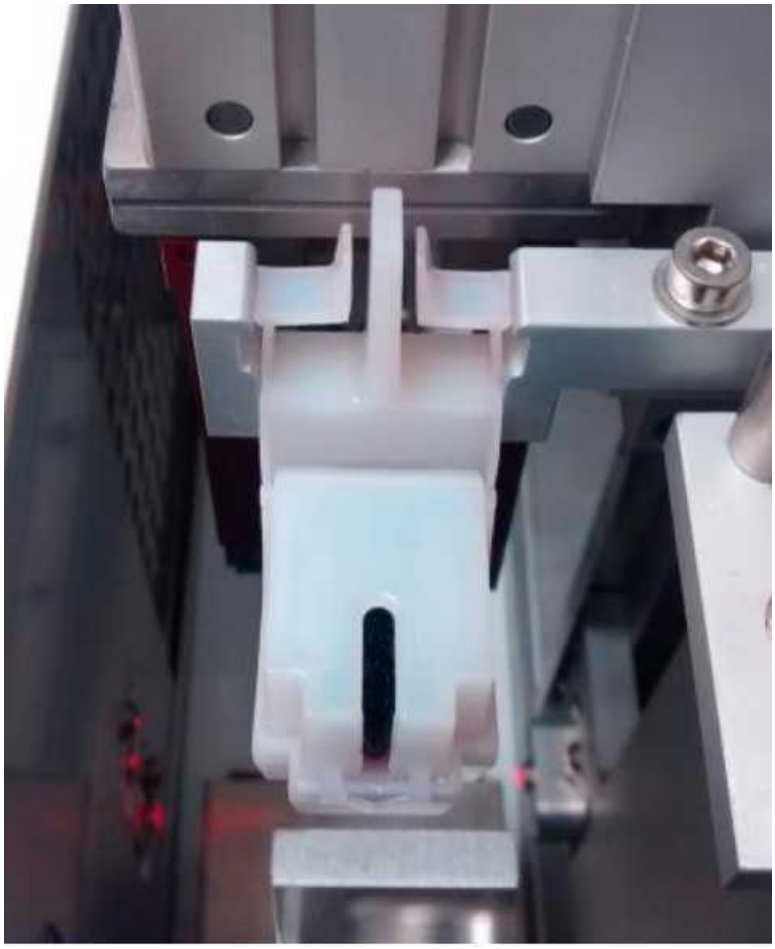

Figure 1. *The Brush Clip:* Pinch together the white pieces at the top of the brush to insert it into the little metal holder. This brush helps keep the needle clean during the cover-slipping process.

2 Take the needle out of the small xylene bottle and press the *'Prime Needle'* button while holding the needle over something to catch the xylene and glue that will come out. An empty coverslip container is kept for this purpose.

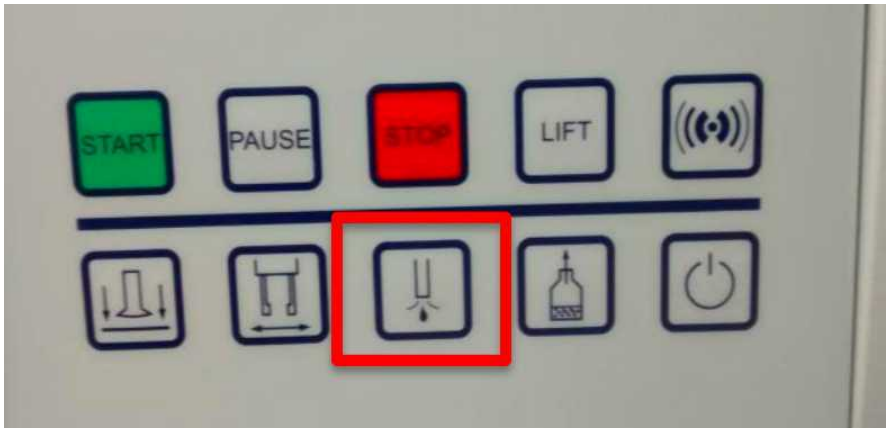

Figure 2. *Prime the Needle:* This button releases the glue and xylene that was in the needle when it was stored so that fresh glue is used with the coverslips.

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3 Move the needle over its holder and push it down into place, aligning the grooves on the side of the needle mechanism with the metal pegs.

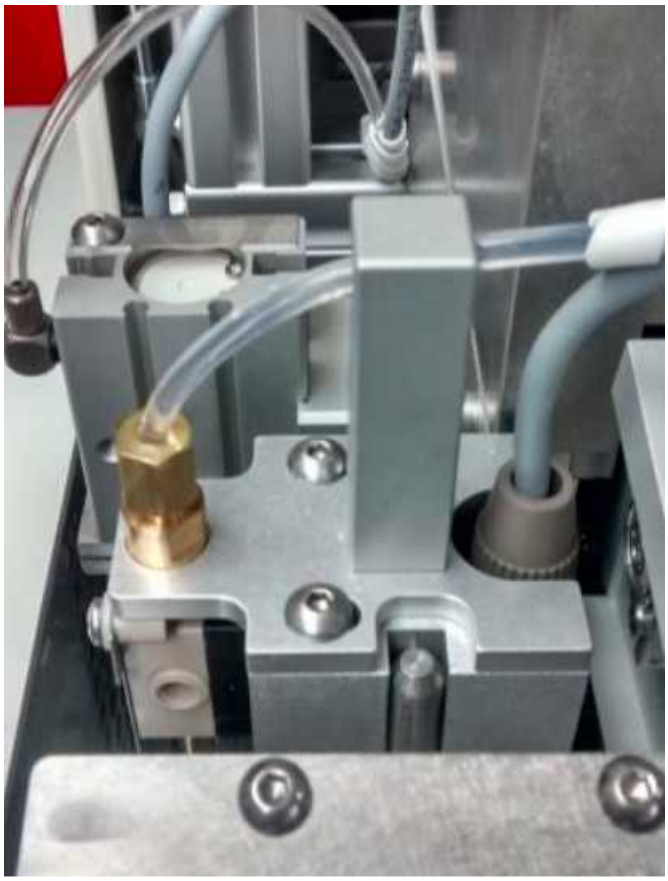

Figure 3. *Needle Placement:* The needle sits between the two metal pegs in front of the brush clip. Push the needle mechanism down with the needle facing the printer.

4 Make sure that the cover-slipper racks are in place to catch the slides.

5 Load 5-6 blank slides in a Stainer rack. All slides should be facing the *'Up'* label. Load rack into the Xylene well in the drawer and press start. Remove rack when coverslipping is complete and discard the blank slides.

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Figure 4. *Slide Racks:* The unstained slides need to be placed with the label area facing this side of the rack. If they are not facing this way, the coverslips will be put on the wrong side of the slide.

6 Open the lid on the Leica Stainer and remove the metal reagent covers. Turn the machine on.

7 The screen should show a representation of the reagent wells. The only program we use to stain is the H&E program, and it should already be set to run. Do not change any of the settings on screen.

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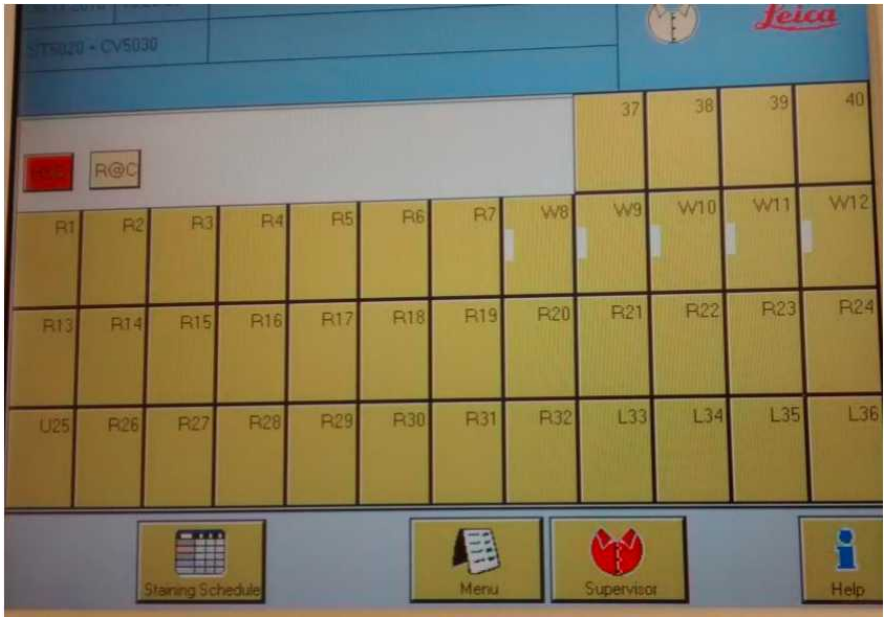

Figure 5. *The Stainer Screen:* The Stainer display will almost always look like the image above. The only time the user will mess with this display is when the Stainer alarm sounds (which happens when the cover-slipper racks are full or when the wrong clip color is used).

8 Place a red clip on the front of the rack (The side labeled *Up*). The red clips must be used with the H&E program. The clip color corresponds to the program being run. The wrong clip color will disable and prevent the Stainer from running the program.

9 Open the drawer on the front of the machine and place the racks inside. The red clips should be facing away from the machine.

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Figure 6. *Putting the Racks in the Stainer:* The racks must be placed with the clips facing out or the machine will not be able to pick the racks up correctly. Up to 4 racks can be placed.

10 The slides will move from the Stainer to the coverslipper automatically. The order the slides are dipped into the reagents and the amount of time they spend in each reagent is set in the program. Once the process has begun the user does not need to do anything to assist the process unless the coverslipper rack fills up, in which case an alarm will sound.

Staining Protocol

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A	B	C
STEP	STATION	TIME
1	1-Xylene	3:00
2	2-Xylene	2:30
3	3-Xylene	1:30
4	4-100% Alcohol	1:00
5	5-100% Alcohol	2:00
6	6-95% Alcohol	1:00
7	7-95% Alcohol	1:00
8	12-Wash	0:30
9	22-Hematoxylin	3:00
10	10-Wash	1:30
11	21-Clarifier	1:00
12	9-Wash	1:00
13	20-Bluing Reagent	1:00
14	8-Wash	1:00
15	19-95% Alcohol	1:00
16	18-Eosin	1:00
17	17-95% Alcohol	1:00
18	16-100% Alcohol	2:00
19	15-100% Alcohol	2:00
20	14-Xylene	2:00
21	13-Xylene	1:00
22	Coverslipper	

11 When the coverslipper sounds an alarm, the Stainer will also sound an alarm to indicate that coverslipper needs attention. Turn off the alarm on the Stainer by hitting the back arrow to get rid of the message that says the coverslipper needs attention.

12 Hit the alarm button on the coverslipper to turn it off.

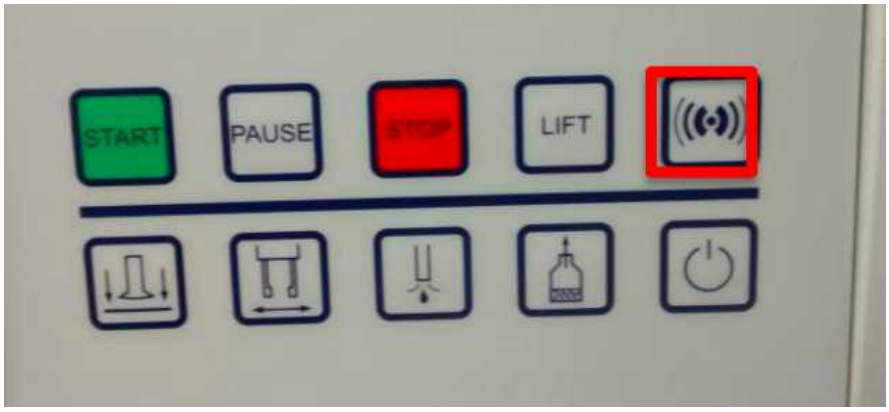

Figure 7. *The Cover-slipper Alarm:* The button that turns off the cover-slipper alarm is outlined in red on the image above.

13 Carefully remove the full racks from the coverslipper and replace them with empty ones.

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14 Once the new racks have been placed, hit *'Start'* to continue the process.

15 When the machines are done, turn off the Stainer and place the metal well covers over the reagents to keep them from evaporating. When the Stainer is turned off, sometimes the cover-slipped alarm will sound. If this happens, turn off the alarm.

Figure 8. *Well Covers:* When the Stainer is not in use, the reagent wells should be covered.

16 Remove the emptied Stainer racks from the machine.

17 Go to the coverslipper, place the needle in the small bottle of xylene kept inside the machine.

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Figure 9. *Turning Off the Cover-slipper:* The glue needle should be placed in Xylene when the machine is not in use. This keeps the glue from drying inside the needle.

18 Take the racks out of the coverslipper and set them aside to the side to dry.

19 Remove the white brush clip and place it in the xylene well. Place the cover over the xylene well before closing the drawer.

20 Turn the coverslipper off.

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