



DEPARTMENT OF PATHOLOGY Digital Pathology

Coverslipping of Frozen Section Slides by Ultraviolet (UV) Crosslinking

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Purpose

To assure that frozen section slides inserted into whole slide imaging and telepathology instruments (e.g. Leica LV1) are 1) free of excess mounting medium, and 2) their coverslips are firmly affixed to the slide and will not shift during routine handling by the instrument.

Materials

- Microprocessor-controlled UV crosslinker. The Spectroline model XLE-1000A has been validated for this procedure. (Spectronics Corp., Westbury, NY)
 - *Note: the critical feature of this instrument is that it emits UV radiation at 365 nm, which is the appropriate wavelength for the medium below.*
- “EUKITT UV-R” mounting medium Mfr: O. Kindler, Div. of ORSA-tec GmbH (Germany). Distributed in the US through EMS, Hatfield, PA. Cat #15320-35 R.
 - *Please note: the “-R” suffix in the product description is extremely important. ORSA-tec GmbH manufactures a similar medium simply called “EUKITT UV.” However, this product is much more viscous and inappropriate for this procedure.*
 - *The product can be ordered either through Fisher Scientific (preferred vendor) or directly from EMS (above).*
- Platform to elevate the slides to within approximately 3 cm of the UV light source within the UV crosslinker instrument. Custom-made wooden “tables” were manufactured for this purpose.
- Extra coplin jar of xylene

Procedure

Part A. UV crosslinking

1. Cut and stain frozen sections per usual procedure.
2. Remove slide from last xylene wash and place 2-3 drops of EUKITT UV-R mounting medium on the slide.

3. Coverslip as usual, pressing out excess mounting medium.
4. A platform needs to be positioned inside the crosslinker to elevate the slides to within 3 cm of the bulbs.
5. Place up to four slides at a time on top of the platform.
 - a. **Important note!** The platform must be positioned all the way to the front of the crosslinker to maintain exposure times <1 min. A small sensor on the rear floor of the instrument is liable to be partially blocked if the platform is allowed to shift. This will result in a near-doubling of exposure times. In addition, any slide trays inserted into the crosslinker must not extend beyond the Masonite backing of the platform.
6. Turn on Spectrolinker XLE-1000A via rocker switch at front.
7. Press button "ENERGY." Left-most of three red LED lamps should light.
8. Enter **250** on keypad.
9. Press START.
10. UV lamps will flicker on and the display will count down to zero. At zero, the machine will beep. At this point the machine can be safely opened and the slides retrieved.
 - a. **NOTE:** any excess mounting medium will NOT be cured and will smear if touched. Coverslips themselves will be securely mounted to the slide and will not come off with routine handling/moderate finger pressure.
11. If for any reason the coverslips are NOT securely affixed to the slide (i.e. crosslinked), replace slides in crosslinker and go back to step 9.
 - a. If crosslinking with coverslip securely affixed to the slide does not occur with UV light exposure, treat the slide as a typical non-UV crosslinked (Permount coverslipped) slide. Care should be taken: 1) to ensure the coverslip is positioned appropriately on the slide and 2) to insert the slide within any whole slide imaging or telepathology instrument without disruption of the coverslip.

- b. **Notify Peter Ouillette** (764-4003; pouillet@med.umich.edu) if this happens.
12. Dip the crosslinked slides into an extra coplin jar of xylene and wipe them with a paper towel to remove excess mounting medium. Removal of excess mounting medium is necessary to eliminate the chance of smearing or its unwanted introduction into an instrument.
 - a. **NOTE:** Excess mounting media can severely damage a telepathology instrument and will cause unnecessary downtimes and workflow disruptions.
 - b. Wiping these slides without first dipping in xylene will cause smearing.
13. Repeat step 12 once or twice if necessary to remove all excess mounting medium.

Part B. Operation and maintenance of the Spectroline XLE-1000A UV crosslinker

I. Normal use

- a. Crosslinking of UV-curable mounting medium (see protocol above for details)

II. Safety

- a. The crosslinker door has a built-in safety interlock which automatically shuts off the UV tubes when the door is not securely closed.

III. Display

- a. When using the ENERGY mode, the X100 μ J/cm² LED lamp will be lit.
- b. Energy per unit area (μ J/cm²) equals Intensity (μ W/cm² x Time (sec))
- c. A built-in UV integrator computes the energy dosage delivered, thereby *automatically* compensating for the decline in UV intensity output of the tubes as they age.
 - i. If the time to crosslink slides goes above 60 sec when following the above protocol, please contact Peter Ouillette (764-4003; pouillet@med.umich.edu) to troubleshoot the device.

IV. Photo sensor

- a. There is a small sensor on the floor of the chamber at the rear. This sensor determines the amount of UV energy present within the chamber. Do not block

this sensor when irradiating slides. The platform must remain in physical contact with the front frame of the instrument. In addition, any tray used to carry slides must not be inserted farther than the Masonite backing of the platform.

V. Bulbs: type, catalog #, replacing

- a. The XLE-1000A holds five 8-watt bulbs.
- b. Catalog #: BLE-8T365.
- c. Description: TUBE, replacement, 8 watt, 365 nm

VI. Quality Control

a. Timing check

- i. Must be performed weekly and documented
- ii. Start a timer at the same time you press START, with ENERGY setting at 250 (i.e., routine use)
 1. Ensure the wooden platform is present within the device before performing the timing check. Test slides do not need to be present for the timing check.
- iii. Normal range: 20-35 sec with ENERGY setting at 250
- iv. Out of range: > 60 sec
- v. Record on written spreadsheet. Save these.
- vi. If out of range:
 1. Check that the wooden platform is all the way to the front of the instrument and secured in place by Velcro strips.
 2. Check that nothing is blocking the UV sensor at the rear floor of the instrument.
 3. Perform intensity check (see b. below). If "BULB" appears, replace UV bulbs (see below in part VII: Troubleshooting; replace all 5 bulbs at one time).

b. Intensity check

- i. Only perform an intensity check if the timing check is out of range
- ii. Remove wooden platform (will have to detach Velcro first)
- iii. Press INTENSITY, then START
- iv. The value displayed is the irradiance level at the center of the chamber. The unit (bulbs) remains on until "RESET" is pressed.

- v. Press reset after taking power reading on display (wait for a number to appear on the display first).
- vi. Reading on the display should be above 1500 ($\mu\text{W}/\text{cm}^2$). Record this number within the written spreadsheet (separate tab).
- vii. If “BULB” appears (intensity < 1500 $\mu\text{W}/\text{cm}^2$), please contact Peter Ouillette (764-4003; pouillette@med.umich.edu).

VII. Troubleshooting

- a. If the countdown is slower than normal:
 - i. Be sure that the UV sensor is not blocked during operation.
 - ii. Look through the window in the chamber door during operation to see if all the tubes are lit.
 - iii. In the INTENSITY mode, make sure the irradiance is greater than 1500 $\mu\text{W}/\text{cm}^2$. If the chamber output falls below 1500 $\mu\text{W}/\text{cm}^2$ with all tubes lit, it is recommended that you replace the *complete* set of tubes.
 - iv. Should “BULB” be displayed, the UV tubes need to be replaced.
 - 1. UV Tube (bulb) replacement:
 - a. Unplug the unit.
 - b. Grasp the tube by the metal bases located at each end. Applying even pressure, gently rotate the tube a quarter turn until it loosens. The tube may now be easily removed from its sockets.
 - c. Install new tube (bulb) by reversing the above procedure.
 - v. Note: over time, the output of the tubes will degrade and the countdown will become slower.
 - vi. If, after changing bulbs, the unit countdown continues to be slower than normal or “BULB” is displayed, then Spectrolinker should be sent back to Spectronics Corp for recalibration, or a new unit should be purchased.
- b. Vendor contact information: 1-800-274-8888.

Flowchart: Not applicable.

References: Not applicable.

Signature Manifest

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Collaboration

Name/Signature	Title	Date	Meaning/Reason
Peter Ouillette (POUILLET)		15 Feb 2019, 11:20:38 AM	Complete
Brian Smola (BSMOLA)	Lead Cytotechnologist	15 Feb 2019, 04:23:47 PM	Complete

Pathology Informatics Digital Pathology Director A

Name/Signature	Title	Date	Meaning/Reason
David McClintock (DSMCCLIN)		21 Feb 2019, 10:37:45 AM	Approved

CLIA Director Final Approval

Name/Signature	Title	Date	Meaning/Reason
Riccardo Valdez (RCDOVLDZ)		04 Mar 2019, 05:34:29 PM	Approved

PI-DIGPATH-BCSC only

Originator

Name/Signature	Title	Date	Meaning/Reason
Peter Ouillette (POUILLET)		05 Mar 2019, 03:02:59 PM	Approved

Brighton Center for Specialty Care CLIA Director

Name/Signature	Title	Date	Meaning/Reason
Lee Schroeder (LEESCHRO)		21 Mar 2019, 04:33:35 AM	Approved