Standard Operating Procedure (SOP) for Hematoxylin and Eosin (H&E) Staining Coverslipping

I. SCOPE AND PURPOSE

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

II. PROCEDURE

A. Safety Procedures

1. Ethanol is flammable. Store properly in flammable cabinet. Use gloves and gown when working will ethanol solutions.
2. Wear gloves and gown when handling unfixed tissue. Read and know all Safety Data Sheets (SDS) for each chemical used. Wear solvent-resistant gloves (e.g., nitrile) and a fluid-impervious laboratory coat at all times when using the stainer. Wear goggles when cleaning and changing solutions on the stainer.
3. All spills are cleaned up according to the Research Institute at Nationwide Children's Hospital policies and procedures.

B. Required Equipment, Supplies And Reagents

1. **Equipment -** Leica Autostainer XL or Multistainer with slide racks
2. **Supplies**
   a. Coverslips - Mercedes Medical cat# CAS942450
   b. Gauze 4x4's – Cardinal Health #KC9134
   c. Control mixed tissue slide (FFPE Mouse or human tissue, deparaffinized, if used for frozen tissue slides), for H&E Control (run daily prior to any samples going through the stainer) - obtained from Morphology Core lab
3. **Reagents**
   a. Alcohol ACS/USP Grade - Fisher cat# 22032600
   b. Xylene - Fisher cat# HC-700 1G
   c. Hematoxylin – Fisher cat# 6765015
   d. Eosin Y- Anatech cat# 832
   e. 10% Neutral buffered Formalin –Fisher cat# SF100-4
   f. Ammonium Hydroxide: Fisher A669-500, diluted in ddH_2O to 1% solution
   g. Mounting medium: Leica cat# 01731

C. Frozen Tissue Slides

1. **Automatic**
   a. The Automated Robotic Coverslipper is turned on and checked for Mounting Media and Coverslip levels. After Coverslipper initializes, press the prime key twice and move the dispenser needle into the coverslipping position.
   b. Turn the stainer on and load the staining containers with the appropriate chemicals (450 mL/ container).
      i. Station 1 - 10% Neutral Buffered Formalin
      ii. Station 2 - water wash
      iii. Station 3 - Hematoxylin

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iv. Station 4 - water wash
v. Station 5 – Bluing (1% Ammonium Hydroxide in ddH_2O)
vi. Station 6 - water wash
vii. Station 7 - 95% Ethanol
viii. Station 8 - Eosin
ix. Station 9 - 95% Ethanol
x. Station 10 - 95% Ethanol
xi. Station 11 - 100% Ethanol
xii. Station 12 - 100% Ethanol
xiii. Station 13 - Xylene
xiv. Station 14 - Xylene
xv. Station 15 - Exit- Xylene

c. Press the "Stain" Button on the LED screen and choose the Stain Program: #1 Frozen Section H&E
d. Prior to running samples, insert a staining basket with 1 H&E Control mixed tissue slide into the Loading Station.
e. Select H&E Frozen program and press the "Load" button. After completion of staining, QC the control slide for color quality. If satisfactory, proceed with staining the freshly cut frozen samples.
f. Insert basket of slides into Loading Station. Select desired program and press the "Load" button. Slides will spend 30 minutes in the oven before proceeding to Station 1.
g. The slides should be picked up after the Exit xylene and moved to the automated coverslipper CV5030 attached to the Leica AutoStainer. After cover-slipping is complete remove output rack and distribute slides.
h. Coverslip with fast-drying MicroMount mounting medium from Surgipath and a glass coverslip using the Leica CV5030 Automated Cover-slipper (or by hand – use 2 drops only of MicroMount.) Do NOT permit the slide to dry out before coverslipping.

2. Hand Staining

a.	10% Formalin	2 min
b.	Running Tap Water	2 min
c.	Hematoxylin	1 min
d.	Running Tap Water	5 min
e.	Bluing	20 sec
f.	Running Tap Water	5 min
g.	Running Tap Water	2 min
h.	95% Ethanol	30 sec
i.	Eosin-Y	1 min
j.	95% Ethanol	30 sec
k.	95% Ethanol	30 sec
l.	100% Ethanol	30 sec

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m. 100% Ethanol 30 sec
n. Xylene 30 sec
o. Xylene 30 sec
p. Xylene 30 sec

D. FFPE Slides

1. Automatic

a. The Automated Robotic Coverslipper is turned on and checked for Mounting Media and Coverslip levels. After Coverslipper initializes, press the prime key twice and move the dispenser needle into the coverslipping position.
b. Turn the stainer on and load the staining containers with the appropriate chemicals (450 mL/ container).
   i. Station 1 – xylene
   ii. Station 2 – xylene
   iii. Station 3 – 100% Ethanol
   iv. Station 4 – 100% Ethanol
   v. Station 5 – 95% Ethanol
   vi. Station 6 - water wash
   vii. Station 7 - Hematoxylin
   viii. Station 8 - water wash
   ix. Station 9- Acid Alcohol
   x. Station 10 – Bluing (1% Ammonium Hydroxide in ddH_2O)
   xi. Station 11 - water wash
   xii. Station 12 - 95% Ethanol
   xiii. Station 13 - Eosin
   xiv. Station 14 - 95% Ethanol
   xv. Station 15 - 95% Ethanol
   xvi. Station 16 - 100% Ethanol
   xvii. Station 17 - 100% Ethanol
   xviii. Station 18 - Xylene
   xix. Station 19 - Xylene
   xx. Station 20 - Exit- Xylene
c. Press the "Stain" Button on the LED screen and choose the Stain Program:#3 FFPE Section H&E. Prior to running samples insert a staining basket with 1 H&E Control mixed tissue slide into the Loading Station.
d. Select H&E FFPE program and press the "Load" button. After completion of staining, QC the control slide for color quality. If satisfactory, proceed with staining.
e. Insert basket of slides into Loading Station. Select desired program and press the "Load" button. Slides will spend 30 minutes in the oven before proceeding to Station 1.

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f. The slides should be picked up after the Exit xylene and moved to the automated coverslipper CV5030 attached to the Leica AutoStainer. After cover-slipping is complete remove output rack and distribute slides.
g. Coverslip with fast-drying MicroMount mounting medium from Surgipath and a glass coverslip using the Leica CV5030 Automated Cover-slipper (or by hand – use 2 drops only of MicroMount.) Do NOT permit the slide to dry out before coverslipping.

2. Hand Staining

a.	Heat fix on Slide warmer @ 60C for 30+ min, then dry in 60C oven for 20-60min	
b.	Xylene or Citrus	3 min
c.	Xylene or Citrus	3 min
d.	100% Ethanol	2 min
e.	100% Ethanol	2 min
f.	95% Ethanol	2 min
g.	Running Tap Water	2 min
h.	Hematoxylin	4 min
i.	Running Tap Water	2 min
j.	Acid Alcohol	10 sec
k.	Running Tap Water	2 min
l.	Bluing	10-30 sec
m.	Running Tap Water	1 min
n.	95% Ethanol	30 sec
o.	Eosin-Y	2 min
p.	95% Ethanol	30 sec
q.	100% Ethanol	1 min
r.	100% Ethanol	1 min
s.	Xylene or Citrus	2 min
t.	Xylene or Citrus	2 min

III. REFERENCES

Carson Frieda L. PhD, HT (ASCP) Histotechnology A Self-Instructional Text 2nd ED, 1996 p. 98 ASCP Press

IV. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 4, Effective Date 8/5/2016
1. Made title not all capitalized
2. Minor language changes

B. Changes made in Version #3, Effective Date 11/26/2014
1. New format used
2. Minor word and grammatical changes made throughout
3. Title updated to include use on FFPE tissues
4. Changed MSDS to SDS to reflect updated terminology

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5. Removed information regarding fresh tissues as it is not applicable in this SOP
6. Added information for FFPE slides
7. Title change

C. Changes made in Version 2 Effective Date 8/22/2012

1. Minor word and formatting changes made throughout
2. Removed references to IGC has it was no longer applicable
3. Added safety precautions and storage requirements when working with frozen tissue
4. Changed all information included in the Quality Control section:
   a. Removed requirement to run a control slide at the beginning of each day
   b. Removed requirements for changing and replacing solutions
   c. Moved the color descriptions for the cell structures to a new section entitled Interpretation/Analysis/ Documentation
   d. Removed requirement for the instrument to be monitored during runs and errors will be immediately corrected or followed-up with a supervisor or service company
   e. Removed requirement for problems to be noted in the log book
   f. Added requirement for tissue storage during use
   g. Added Hematoxylin preparation instructions
   h. Added information regarding Bluing Reagent
5. Added specimen information
6. Updated the required supplies and materials
7. Added requirement to document in Sapphire
8. Clarified parts of the procedure related to loading, including
   a. Changed the Frozen Section program number from #2 to #1
   b. Clarifying the staining basket is inserted prior to running the samples
9. Added running times when running slides by hand
10. Added requirement that the slides should be reviewed for quality

D. Version #1, Effective Date 2/26/2011 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

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