



Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document

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<i>Number</i>	PHARRISH-EST
<i>Title</i>	Staining of prepared formalin-fixed tissue slide by Harris' Hematoxylin and Eosin (H&E) procedure
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Wisconsin Veterinary Diagnostic Laboratory

Standard Operating Procedure

1 Introduction

The Harris method of the Hematoxylin and Eosin staining method is a regressive method that stains all tissue structures. Hematoxylin, or the more commonly used oxidized form Hematein, are used to stain nuclei. Eosin is used as a counterstain which is selective of bodies rich in ribonucleic acid. A properly stained tissue should present red to reddish-purple nucleoli with blue nuclear chromatin staining.

2 Specimen submission

2.1 Type

Paraffin embedded tissue block

2.2 Special requirements for collection - NA

2.3 Handling conditions - NA

2.4 Criteria for rejection of sample -- NA

3 Materials

3.1 Equipment & Instrumentation

1. Automatic Stainer
2. Incubator 50 to 70°C
3. Slide racks and slide rack holders
4. Plastic carboys for holding reagents
5. Fume hood
6. Large glass funnel
7. Funnel holder and stand

3.2 Reagents & Media

1. Eosin, 1% Alcoholic
2. Ethanol 70%, 95% and 100%
3. Glacial Acetic Acid
4. Hydrochloric acid 35% to 38% (12M)
5. Hematoxylin, Harris Modified
6. Lithium carbonate
7. RO/DI H₂O
8. Xylene

3.3 Supplies

1. All-purpose wipes
2. Disposable weigh boats
3. 27.0 cm to 32.0 cm #1 Filter paper

4 Safety Management

4.1 Required Safety Training:

- Chemical fume hood operation -Mandatory use when working with Xylene
- Hazardous chemical use and disposal training

4.2 Required personal protective equipment (PPE):

- Minimum: Lab coat, Safety glasses and closed toe shoes– upon entry of lab
- Gloves: Nitrile
 Chemical resistant – when immersing fingers in Xylene

4.3 Hazard Communication

A. Chemical: See MSDS

Ethanol (MSDS-260 and 261)

- Flammable liquid and vapor

Glacial Acetic Acid (MSDS-31)

- Flammable liquid and vapor.
- Causes severe burns by all exposure routes.

Hydrochloric acid (MSDS-826)

- Causes burns by all exposure routes. May be harmful if inhaled.

Xylene (MSDS-1952)

Emergency Overview

DANGER! Harmful or fatal if swallowed. Vapor harmful. Affects central nervous system. Causes severe eye irritation. Causes irritation to skin and respiratory tract. May be harmful if absorbed through the skin. Chronic exposure can cause adverse liver, kidney and blood effects. Flammable liquid and vapour.

B. Biological: Biosafety Level 2

C. Physical: N/A

D. Electrical: N/A

E. Sharps: N/A

F. Ergonomics: N/A

G. Other: N/A

4.4 Waste Disposal

- Xylene is discarded in a designated waste xylene carboy and recycled using the in-house xylene recycler.
- Ethanol, Ethanol contaminated with xylene, histological stains, heavy metal stains and acids must be dumped into a designated waste carboy. Document the type and amount of each solution on the Chemical Inventory Analysis For Waste Solvents In Carboys. Once the carboy is full, transport the carboy with the completed documentation to room 1101 for safe storage until it can be picked up by UW-Safety.
- All broken glassware and/or slides are disposed in the broken glass box located in Room 1124.

5 Preparation for procedure

5.1 Equipment and instrumentation preparation

- Fill containers with appropriate solutions as labeled. The containers shall be filled to the top line with solution.
- Turn automatic stainer on.

5.2 Reagents and media preparation

- The following solutions in the stainer shall be exchanged every two weeks:
 - Xylene
 - 100% Ethanol
 - 95% Ethanol
 - 70% Ethanol
 - RO/DI H₂O
 - Eosin
 - 1% Acid Alcohol
 - Lithium Carbonate
- The solutions in the stainer can be re-filled to the top line as necessary. Generally it is necessary to re-fill the solutions at the beginning of the off weeks of use.
- The Hematoxylin must be changed every 6 weeks and filtered daily prior to use.

Eosin Working Solution

95% Ethanol.....	450 mL
Eosin Stock Solution, 1% Alcoholic.....	150 mL
Glacial Acetic Acid.....	3.0 mL
Stability: 6 months at room temperature	

Saturated Lithium Carbonate

RO/DI H ₂ O water.....	500 mL
Lithium carbonate.....	7.0 gm or until saturated (lithium carbonate falls out of solution/ accumulates on bottom of bottle)
Stability: 6 months at room temperature	

1% Acid Alcohol

70% Ethanol.....	3960 ml
Hydrochloric Acid (12M).....	40 ml
Stability: 6 months at room temperature in a closed container	

5.3 Standards/controls preparation

Prior to daily staining of diagnostic slides, stain a validated H&E control slide cut at 5 μ m (PSECTIONING). Enter the control ID and the number of slides that were stained into the Histology Database under Histology Production tab.

5.4 Specimen preparation

- The slides shall be placed in a brown Sakura slide rack after sectioning.
- The unstained tissue slides shall be heated for 10-20 minutes at 50 to 70°C in an incubator before being placed in the automatic stainer.
- Attach a slide rack holder to the handle of the slide rack prior to placing the rack in the stainer.

6 Performance of procedure

The following procedure is applied to the daily H&E control slide and all diagnostic slides:

1. Open the stainer cover and door and place the racked slides into the start position of the DRS automatic stainer. (The stainer door can be opened by pushing on the top left portion of the door where there is a label "PUSH". The door will open down. The cover can be opened by lifting the cover up and out. The cover will slide back on top of the unit.)
2. Close the cover and door.
3. Press "Start" on main menu screen.
4. Press "Method".
5. Select desired method
 - a. "Cu dehydrate" – Counterstain and dehydration for (PRHODANINEST)
 - b. "H&E" - standard stain intensity
 - c. "H&E Heat" – standard stain intensity with pre-heat
 - d. "H&E H2O" – used for Melanin bleach technique
 - e. "H2O" – Deparaffinize and hydrate for special stains
 - f. "H2O w/o Heat" – H2O cycle with pre-heating done outside stainer
 - g. "NWHC H&E" – Deeper stain intensity
 - h. "PAS Dehydrate" – End of PAS stain with dehydration
 - i. "Specials kwik" - Dehydration for special stains
6. Press "Start". The staining method will begin.
7. When the alarm signifies completion, press "Remove".
8. Open the lower door of the stainer.
9. Press "Confirm".
10. Wait for the metal bar to quit moving and re-center in the stainer and then remove the slide rack.
11. Place a paper towel or all-purpose wipe underneath slide rack/s after removing from the stainer to prevent spilling xylene on self or floor.

12. Close the stainer upper cover and/or lower door.
13. Coverslip the slides (PGLASCOVERSLIP).

7 Interpretation of results

1. Slides can be checked by the unaided eye for appropriate staining. If the staining is either too dark, too light, or predominantly one color (blue or pink), the slides/tissue shall be checked using the microscope.
2. When stained properly, the tissue shall appear as follows:

Nuclei.....	Blue
Erythrocytes and eosinophilic granules.....	Bright pink to red
Cytoplasm and most other tissue structures.....	Varying shades of pink

8 Report of results

1. Record the completion and validity of the daily H&E control slide in the Histology Database ([\\wvdlfs\wvdl-m\Path\Shortcut](#) to Histology.accde)
2. The accession number for each case along with the pathologist's initials, species, and block/tissue count will be recorded in the Histology database on the day that the H&E slides are stained.
3. The slides shall then be checked to be sure that the number of slides produced matches the number of blocks recorded on the paperwork.
4. WVDL Pathologist slides, see AHISTOSPECMNGT.
5. National Wildlife Health Center (NWHC)
 - a. Place the slides in slide boxes.
 - b. Organize the blocks numerically in a block organizer drawer.
 - c. Send NWHC an e-mail with the paperwork attached.
 - d. The NWHC slides and blocks will be picked up by their employees.
6. Other Submitters
 - a. Place the slides in slide boxes.
 - b. Organize the blocks and place in an appropriate container.
 - c. Notify the submitter of the completed case.

9 Procedure notes

9.1 Details and helpful hints

1. The "H&E" method will take approximately 25 minutes.
2. The "H&E heat" method does not require the slides to be pre-heated. This staining option takes about 45 minutes to complete.
3. Cover the solutions in the stainer at the end of the day to minimize evaporation and to prevent the hygroscopic solutions from up taking moisture from the atmosphere.

4. In the event of a stainer malfunction, the procedure can be done manually. The steps for performing the stain manually are described in the Quick Procedure Reference below.
5. If problems with the stain arise, use the troubleshooting section (REF 10.1).
6. The stations of the automatic stainer can be used even if the stainer is not functioning properly.

9.2 Limitations of procedure - NA

10 References

1. Carson, Freida L. Histotechnology: A Self-Instructional Text. Second edition. ASCP Press: Chicago 1997; 91-102.
2. Laboratory Methods in Histotechnology: 1992, American Registry of Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306-600. Page: 56-57.
3. Sakura Tissue-Tek DRS 2000 Automatic Slide Stainer Operating Manual.
4. Histology Autostainer Programs (RES-P-36).

11 Summary of Current Revisions

1. Section 8: Removed WVDL slide flattening procedure, as noted in AHISTOSPECMNGT.
2. Section 5.5.2: Changed volume of glacial acetic acid to 3.0 mL for eosin production, per supplier instructions.
3. Sections 5.5.3, 6.1, and 8: Added mention of using daily H&E control slide prior to staining diagnostic slides.

12 Supplemental Information

12.1 Quick Procedure Reference

Harris Hematoxylin and Eosin Staining Method (manual)

1. Place slides in the slide dryer for 10 – 20 minutes.

In each of the following steps, dip the slide rack 5 times in each station and then allow the slides to sit in each station for the designated time period unless otherwise specified. Proceed directly from one step to the next.

2. 1st **xylene** (station 1) – 2 minutes.
3. 2nd **xylene** (station 2) – 2 minutes.
4. 3rd **xylene** (station 3) – 2 minutes.
5. 1st **100% ethyl alcohol** (station 4) – 1 minute.
6. 2nd **100% ethyl alcohol** (station 5) – 1 minute.
7. 1st **95% ethyl alcohol** (station 6) – 1 minute.
8. 2nd **95% ethyl alcohol** (station 7) – 1 minute.
9. Running tap water (station 21) – 1 minute.
10. **Harris hematoxylin** (station 24) – 2 minutes.
11. Running tap water station 22) – 1.5 minutes.

12. **1% Acid alcohol** (station 20) – 5 quick dips only!
13. Running tap water (station 21) – 1 minute.
14. **Saturated lithium carbonate** (station 8) – 10 seconds.
15. Running tap water (station 22) – 1 minute.
16. **70% ethyl alcohol** (station 9) – 45 seconds.
17. **Eosin** (station 10) – 1 minute.
18. **95% ethyl alcohol** (station 11) – 30 seconds.
19. **95% ethyl alcohol** (station 12) – 30 seconds.
20. **100% ethyl alcohol** (station 13) – 1 minute.
21. **100% ethyl alcohol** (station 19) – 1 minute.
22. **100% ethyl alcohol** (station 18) – 1 minute.
23. **xylene** (station 17) – 1 minute.
24. **xylene** (station 16) – 1 minute.
25. **xylene** (station 15) – 1 minute.
26. **xylene** (station 14) – 1 minute.
27. Coverslip (PGLASCOVERLIP)

Interpretation of results

1. Use the microscope to check the control slide.
2. When stained properly, the slide will look as follows:

Nuclei.....	Blue
Erythrocytes and eosinophilic granules.....	Bright pink to red
Cytoplasm and most other tissue structures.....	Varying shades of pink

12.2 Flow Diagram

12.3 Manufacturer's Information

Newcomer Supply
2217B Parview Rd
Middleton, WI 53562
Phone 608-831-7888

- Catalog# 1201 - Hematoxylin, Harris Modified
- Catalog# 1070 - Eosin, 1% Alcoholic