



# Comparison of Hematoxylin and Eosin Stains on Automated Stainers

## Instruction Manual

## Purpose

This study was conducted to test the validity of automated H & E staining, on routinely formalin fixed and paraffin embedded prostate chips, colon and kidney tissue.

## Principle

Differentiation is crucial for desired nuclear stain in regressive methods.

In progressive staining, immersion times are important to achieve good staining intensity.

## Equipment

1. Sakura Autostainer - Prisma 81D
2. Sakura Autostainer Coverslipper
3. Leica Autostainer XL
4. Fisher Histomatic Autostainer

## Technique

1. Paraffin sections were cut at 3 microns for slides with prostate chips, colon, and kidney samples
2. Slides with sections of colon, tonsil and placenta samples were cut at 4 microns
3. Skin samples were cut at 7 microns for frozen section
4. Slides were baked for 30 minutes in a 58°C oven

## Reagents

- Xylene, Reagent Grade
- Alcohol, 95%, 100%
- 1N HCl to make 0.5% acid water
- Polysciences, Inc. Gill's #2 hematoxylin (cat. #24243)
- Polysciences, Inc. Gill's #3 hematoxylin (cat. #24244)
- Polysciences, Inc. Harris hematoxylin (cat. #24245)
- Polysciences, Inc. Eosin Y, 0.5% (cat. #09859)
- Polysciences, Inc. Eosin Y, 1.0% (cat. #17269)
- Polysciences, Inc. Ammonium Blue (cat. #24819)
- Polysciences, Inc. Lithium Blue (cat. #24820)
- Polysciences, Inc. Scott's Bluing Reagent (cat. #24605)
- Polysciences, Inc. Poly-Mount (cat. #08381)
- 0.045% Acid Alcohol (2600 ml of 70% alcohol and 1.2 ml of concentrated HCl)
- Ethyl alcohol
- 0.3% in-house ammonia water

## Procedure for Automated Staining

1. Deparaffinize slides to water
2. Stain sections on automatic stainer
3. Times for Polysciences' Harris and Gill's hematoxylin were both the same for regressive stains
4. Coverslip slides

## Procedure for Manual Staining

1. Fix frozen sections in 95% alcohol for 1 minute, then wash and air dry
2. Deparaffinize sections to water and air dry
3. Dip sections in water and stain in hematoxylin for 1 minute
4. Wash slides in water for about 20 dips
5. 10 dips in bluing reagent
6. Wash in water for 20 dips
7. Put slides in 95% alcohol for 10 dips
8. Treat slides with Eosin Y for 10 seconds
9. Dip 5-7 times in 95% alcohol
10. Two changes of absolute alcohol, 10 dips each
11. Two changes of xylene substitute, 10 dips each
12. Coverslip slides

## Results

Stained nuclei appeared blue and cytoplasm was pink. Polysciences' Harris and Gill's hematoxylin needed 7-8 minutes and yielded good results. All Polysciences, Inc. bluing reagents demonstrated desirable results. Sections cut at 3 microns were stained lighter than those at 4-5 microns, which is ideal.

## Table 1 Times for Sakura Autostainer - Prisma 81D

All buckets for the Sakura Autostainer - Prisma 81D hold 250 ml of reagent.

Step	Reagent	Polysciences' Harris/Gill's Regressive (time)	Polysciences' Gill's Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	1:00	1:00
4	0.5% Acid Alcohol	0:01	—
5	Wash	1:45	—
6	Bluing	0:30	0:30
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	1:00	1:30
10	95% OH	0:10	0:10
11	100% OH	0:10	0:10
12	100% OH	0:30	0:30
13	100% OH	1:30	1:30
14	Xylene	1:00	1:00
15	Xylene	1:00	1:00
16	Exit Xylene	1:00 - 3:00	1:00 - 3:00

## Table 2 Times for Leica Autostainer XL

All buckets for the Leica Autostainer XL hold 450 ml of reagent.

Step	Reagent	Polysciences' Harris Regressive (time)	Polysciences' Gill's #2, #3 Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	1:00	1:00
4	0.045% Acid Alcohol	0:01	—
5	Wash	1:10	—
6	Bluing	0:30	0:30
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	2:30	2:30
10	95% OH	0:07	0:10
11	95% OH	0:10	0:10
12	100% OH	0:30	0:30
13	100% OH	1:00	1:00
14	100% OH	—	—
15	Xylene	1:00	1:00
16	Xylene	0:30	0:30
17	Exit Xylene	1:00 - 3:00	1:00 - 3:00

**Table 3 Times for Fisher Histomatic Stainer**

All buckets for Fisher Histomatic Autostainer hold 600 ml of reagent.

Step	Reagent	Polysciences' Harris Regressive (time)	Polysciences' Gill's #2, #3 Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	0:50	0:50
4	0.045% Acid Alcohol	0:01	—
5	Wash	2:00	—
6	Bluing	0:20	0:20
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	2:00	3:00
10	95% OH	0:10	0:10
11	100% OH	0:30	0:30
12	100% OH	1:00	1:00
13	Xylene	1:00	1:00
14	Xylene	1:00	1:00

**Table 4****Nuclear and Cytoplasmic Staining Intensity on the Sakura Autostainer - Prisma 81D**

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin Y	Good
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, provided good contrast
Polysciences' Gill's #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Average
Polysciences' Gill's #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good, no bluish tinge
Polysciences' Gill's #3	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Good, no bluish tinge
Polysciences' Gill's #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Good

**Table 5**  
**Nuclear and Cytoplasmic Staining Intensity on Leica Autostainer XL**

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin	Good combination with fresh acid alcohol
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Gill's	—	Polysciences' Ammonium Blue	Polysciences' Eosin	Good
Polysciences' Gill's	—	Polysciences' Lithium Blue	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Gill's	—	Polysciences' Scott's Bluing	Polysciences' Eosin	Good, crisp stain

**Table 6**  
**Nuclear and Cytoplasmic Staining Intensity on Fisher Histomatic Autostainer**

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Average
Polysciences' Gill's #2	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good
Polysciences' Gill's #2	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, crisp stain

**Table 7**  
**Nuclear and Cytoplasmic Staining Intensity with Manual**  
**Regressive / Progressive Stain**

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin	Excellent
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent

This study was conducted by Sakina Sadiq, B.S., HT (ASCP), HTL (ASCP) for Polysciences, Inc.