BIOLOGICAL STAIN COMMISSION

A Century of Certainty

Biological Stain Commission
University of Rochester Medical Center
Box BSC, 575 Elmwood Avenue
Rochester, NY 14624

Email: chad_fagan@urmc.rochester.edu

2026 NSH San Antonio, TX

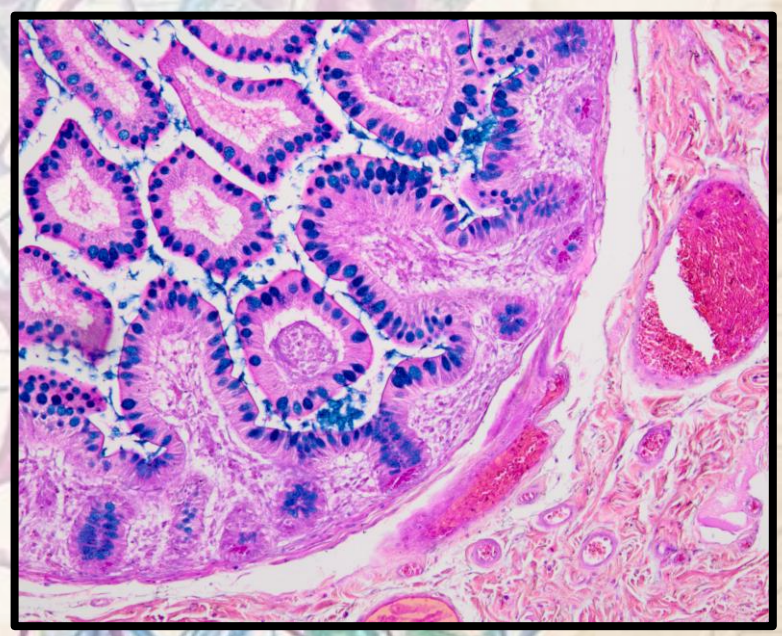
Georg Grübler Dyes

- Development of synthetic dyes: 1856-1883
- Grübler company founded 1880.
- Tested stains for common histological use and bought large batches for sale.

- Circa 1900 –company was sold & split.
- 1914 – WW I
  Both companies went out of business.

Why stain quality matters

- **Variability in dye lots can alter:**
  - **Sharp visual contrast with color intensity and hue.**
  - **Selectivity and specificity of tissue components.**
  - **Diagnostic value.**
- **Consequences of poor-quality stains:**
  - **Misinterpretation**
  - **Loss of reproducibility.**
  - **Wasted time and resources**
  - **Troubleshooting components, reagents, stains and steps.**



Biological Stain Commission

Beginnings: National Research Council (Woodrow Wilson 1918), Chemical Foundation, group of scientists.

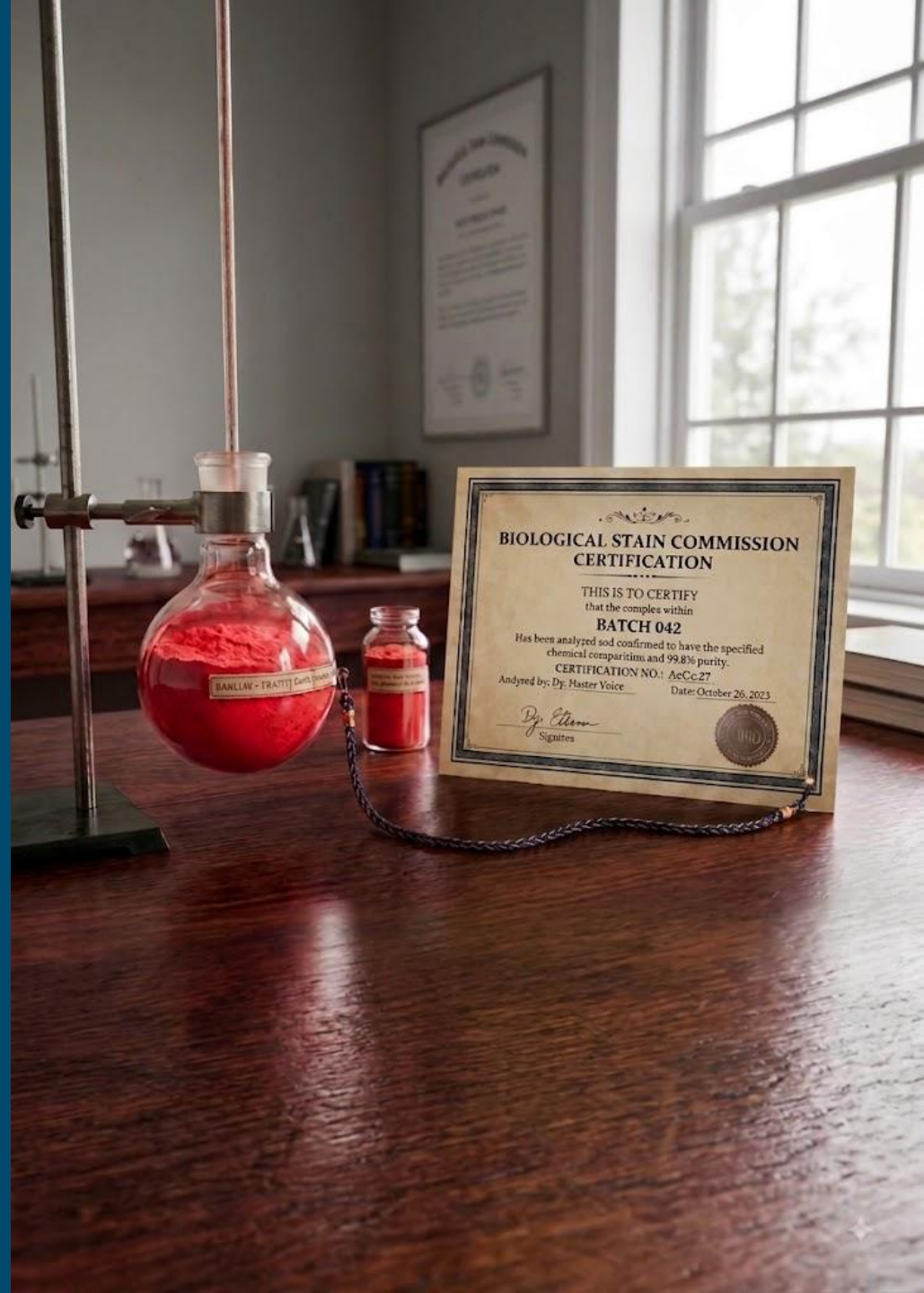
1923 Non-profit organization founded in Geneva, NY

Improve the consistency of biological stains.

Promote & Certify high-quality stains.

What does “Certified” mean?

- Certify
  - A.) To guarantee meeting a standard.
  - B.) To have documentation of certification.
- Traceable
  - A.) To have origins; be traceable.



BSC Assay Laboratory

- BSC is a non-profit non-biased QC Laboratory.

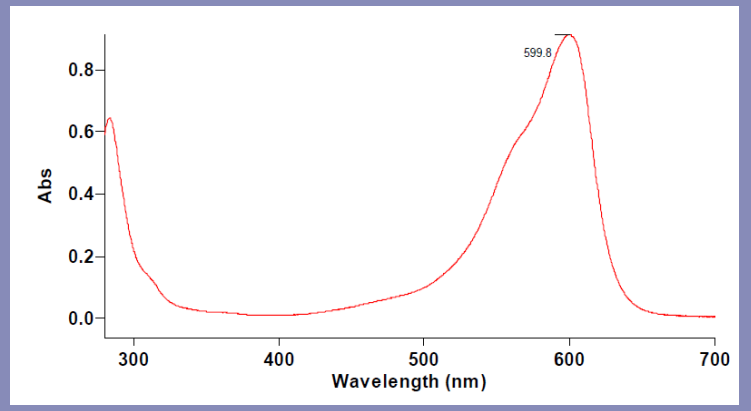
- Certification –
  - comprehensive testing
  - passing criterion
  - Secure documentation.

- Central source of sample archive and records storage.

Analytical testing

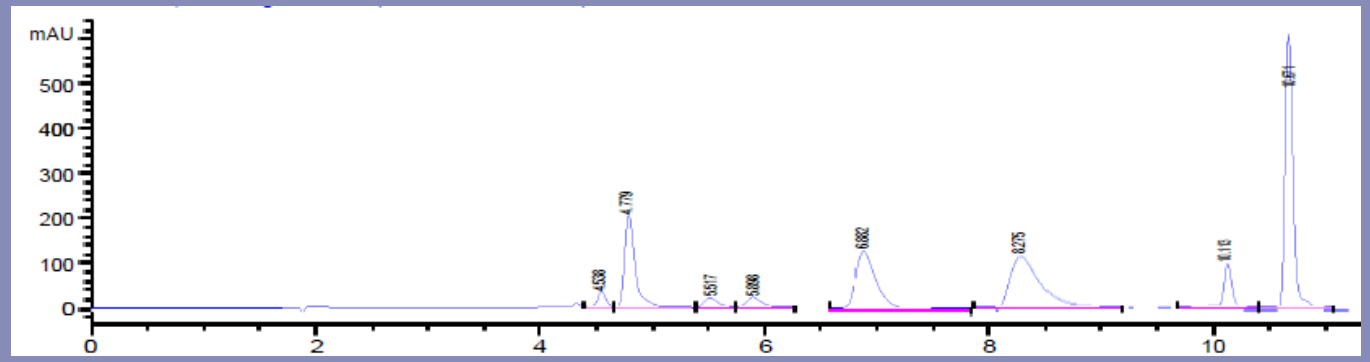
- UV-Vis spectrophotometry
- HPLC analysis
- TLC testing
- Quantitation:
  - Titration
  - Acid precipitation
  - UV-Vis spectroscopy
    - Beer's Law

The Cary 60 UV-Vis Spectrophotometer



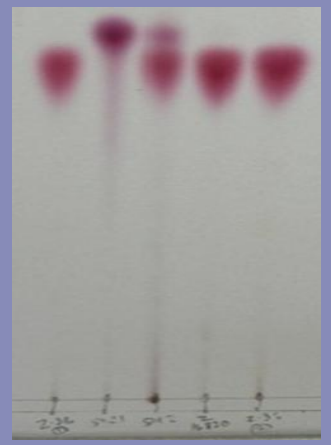
Analytical testing

- UV-Vis spectrophotometry
- HPLC analysis
- TLC testing
- Quantitation:
  - Titration
  - Acid precipitation
  - UV-Vis spectroscopy
    - Beer's Law



HPLC - EMPOWER Arc HPLC

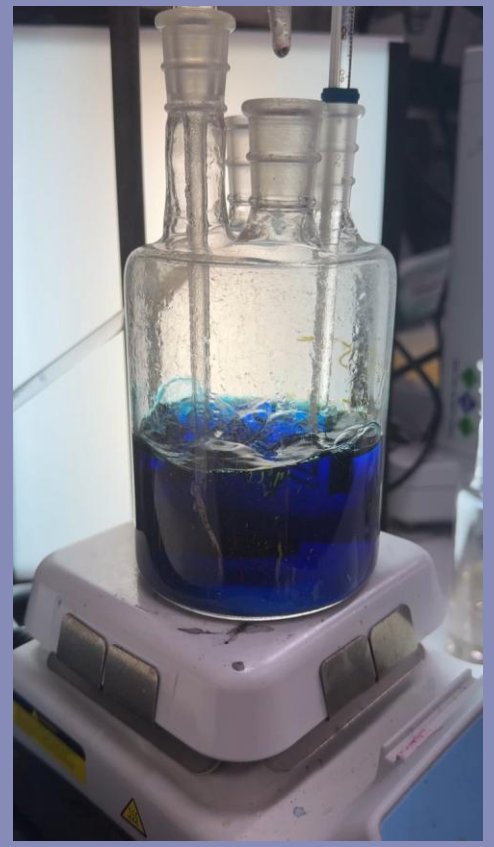
Thin Layer Chromatography



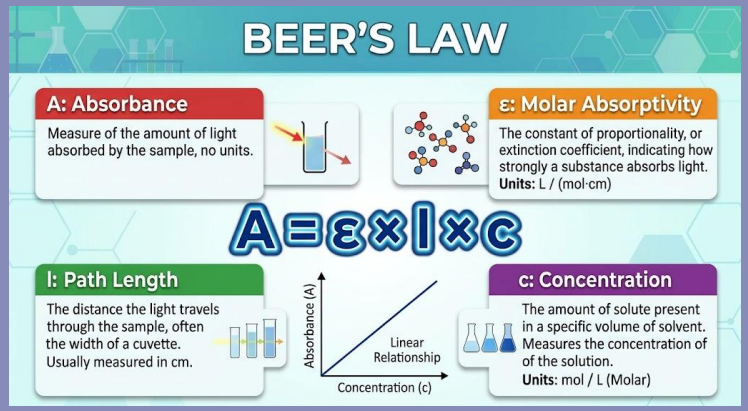
Analytical testing

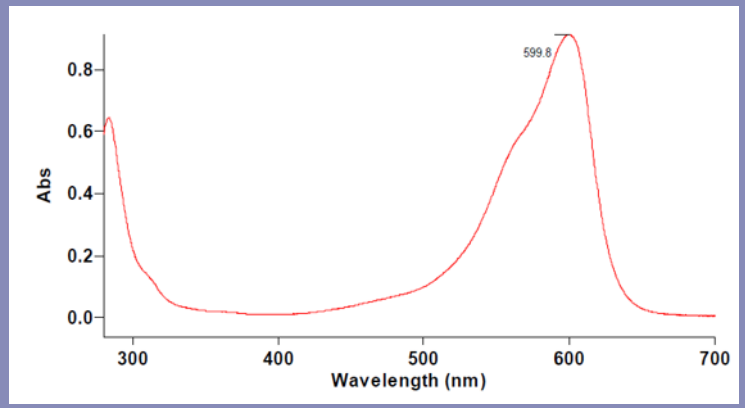
- UV-Vis spectrophotometry
- HPLC analysis
- TLC testing
- Quantitation:
  - Titration
  - Acid precipitation
  - UV-Vis spectroscopy
    - Beer's Law

Titanous Chloride Titration



Beer's Law

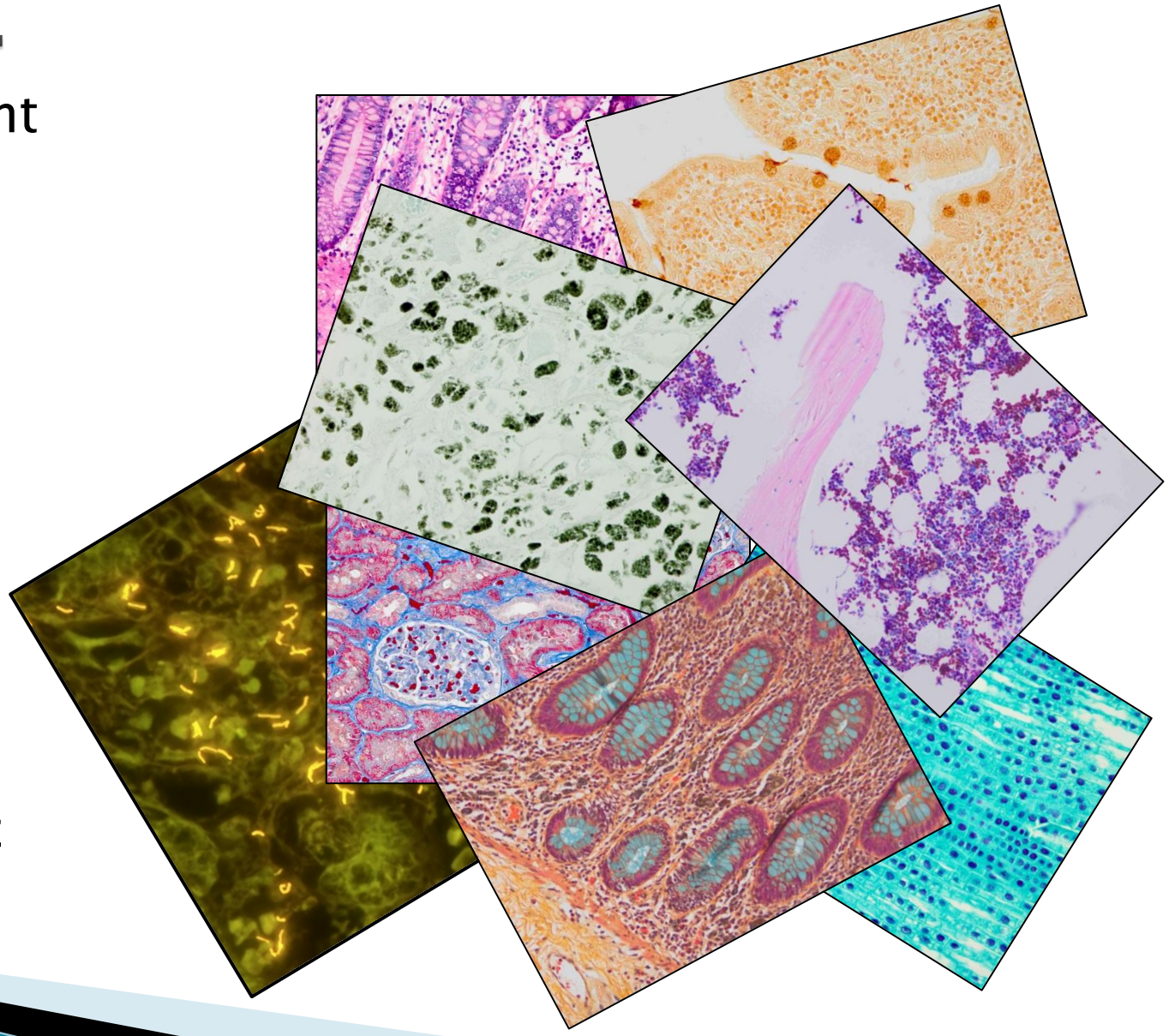




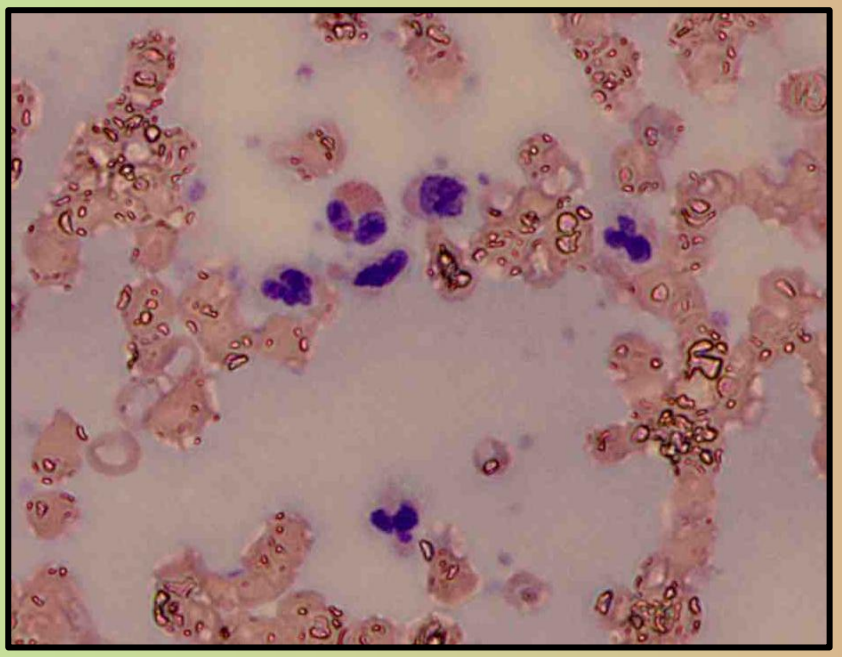
Stain testing batteries specific for common uses of stains.

- Acid Fast/Flourescent
- Bacterial
- Background
- Blood Cell Stain
- Collagen
- Counterstain
- Differential Staining
- Fat
- Metachromatic
- Mucin
- Nucleus

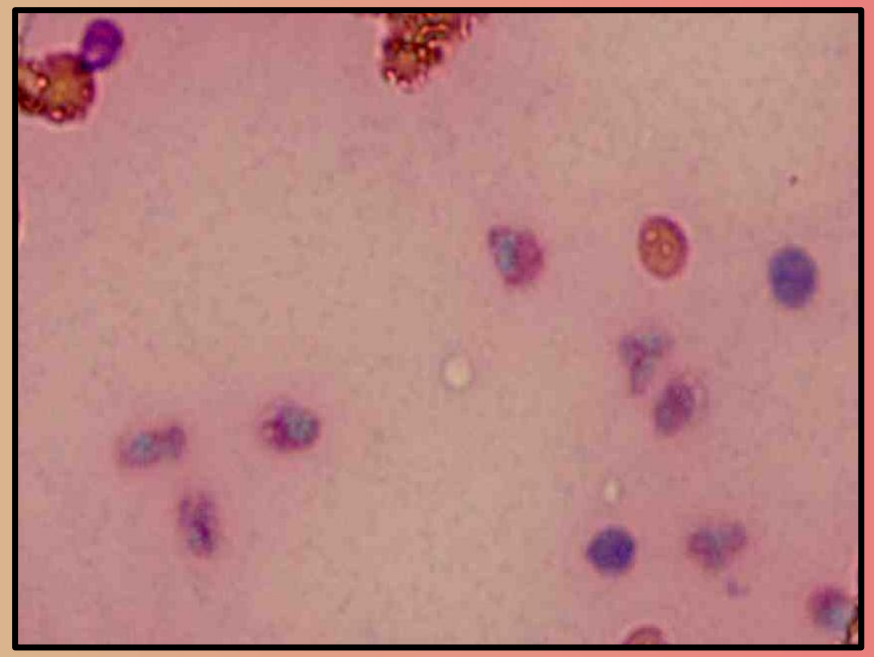
- Other: Bacteriostatic



Satisfactory Staining for Certification



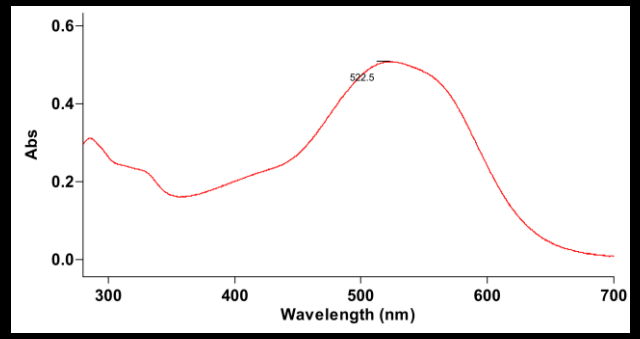
Acceptable



Unsatisfactory

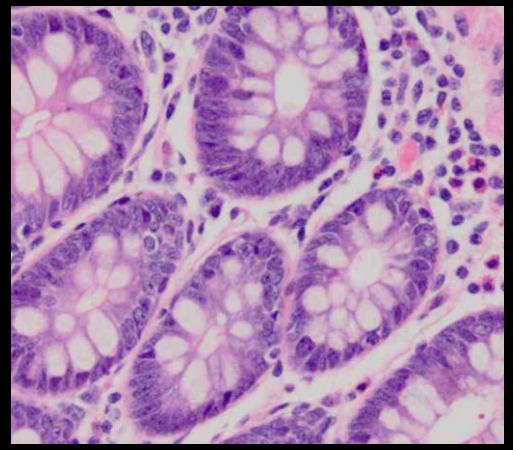
Hematoxylin

- UV-Vis analysis

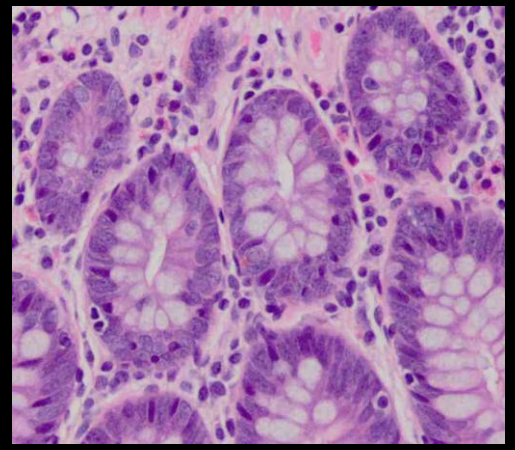


- Staining
  - Harris Alum Hematoxylin
  - Mayer Alum Hematoxylin
  - Heidenhains Regressive
  - Weigert's Iron Hematoxylin

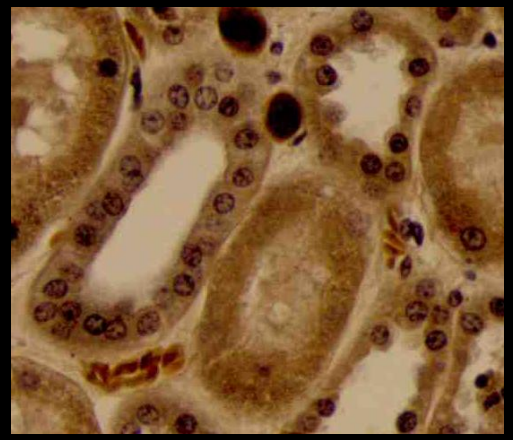
Harris



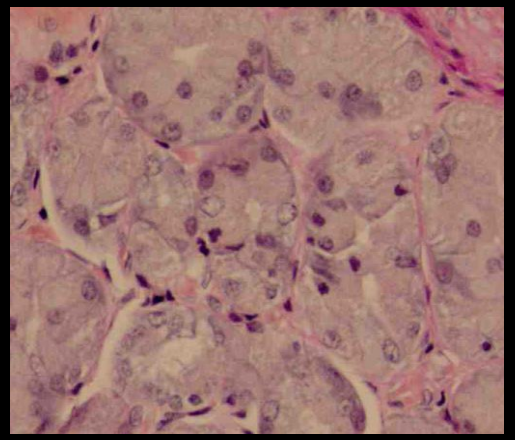
Mayer



Heidenhain



Weigert

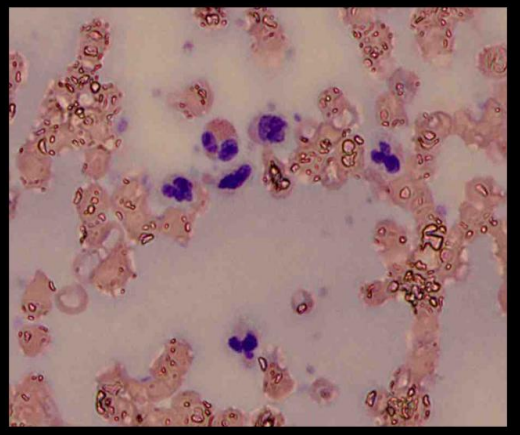


Azure A

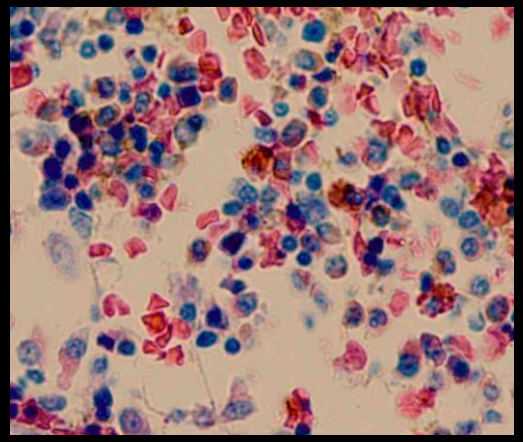
- UV-Vis analysis
  - Peak 628-634 nm
  - Ratio 0.94-1.20

- HPLC – > 33% Azure A
- Titration – 75% Dye Content
- Staining
  - Blood Smear
  - Nocht's on bone tissue

Blood Smear

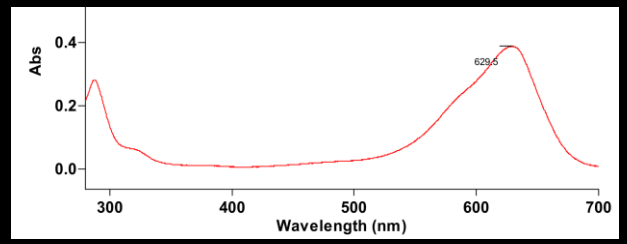


Nocht's - Bone

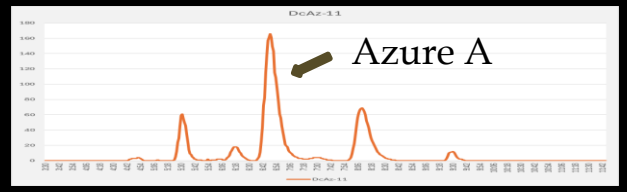


Azure A – CoAz-11

- UV-Vis analysis
  - Peak 630 nm
  - Ratio 0.99



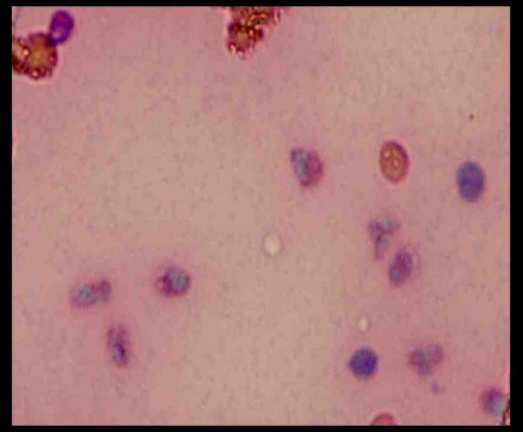
- HPLC
  - 60% Azure A



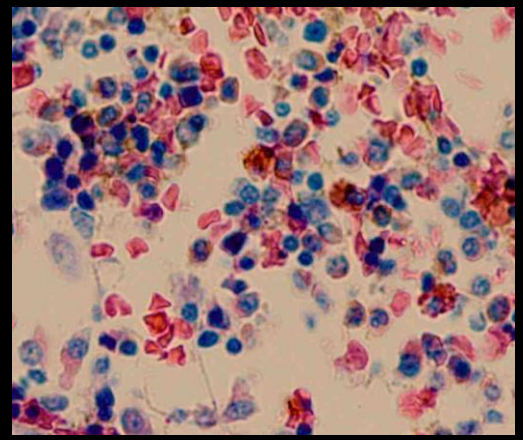
- Titration – 66% (FAIL)

- Staining
  - Blood Smear (FAIL)
  - Nocht's on bone tissue

Blood Smear



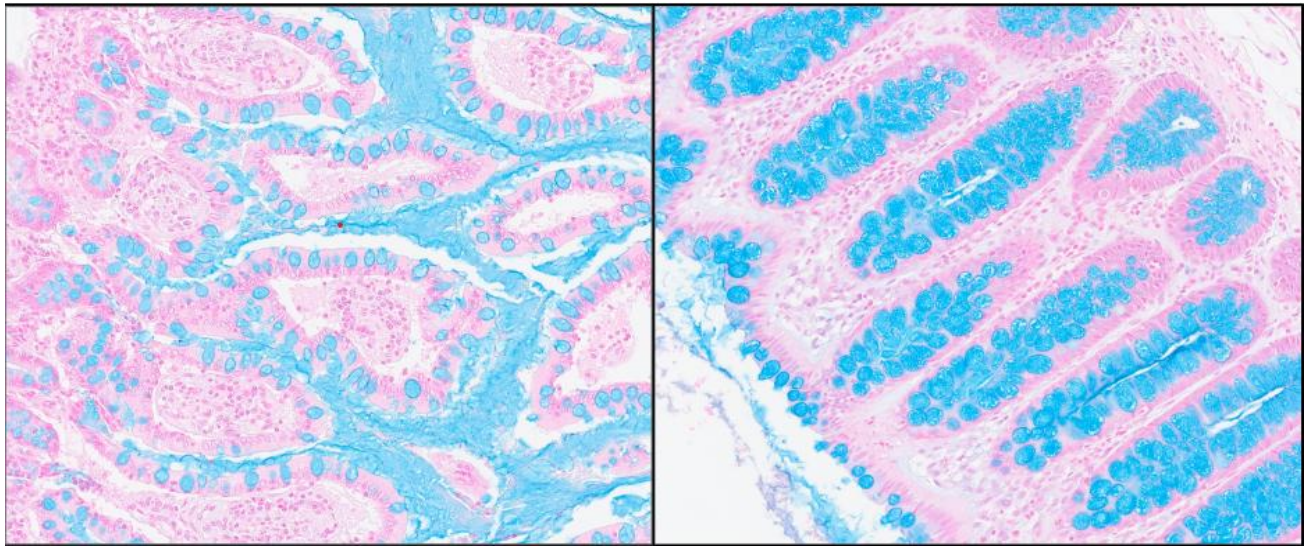
Nocht's - Bone



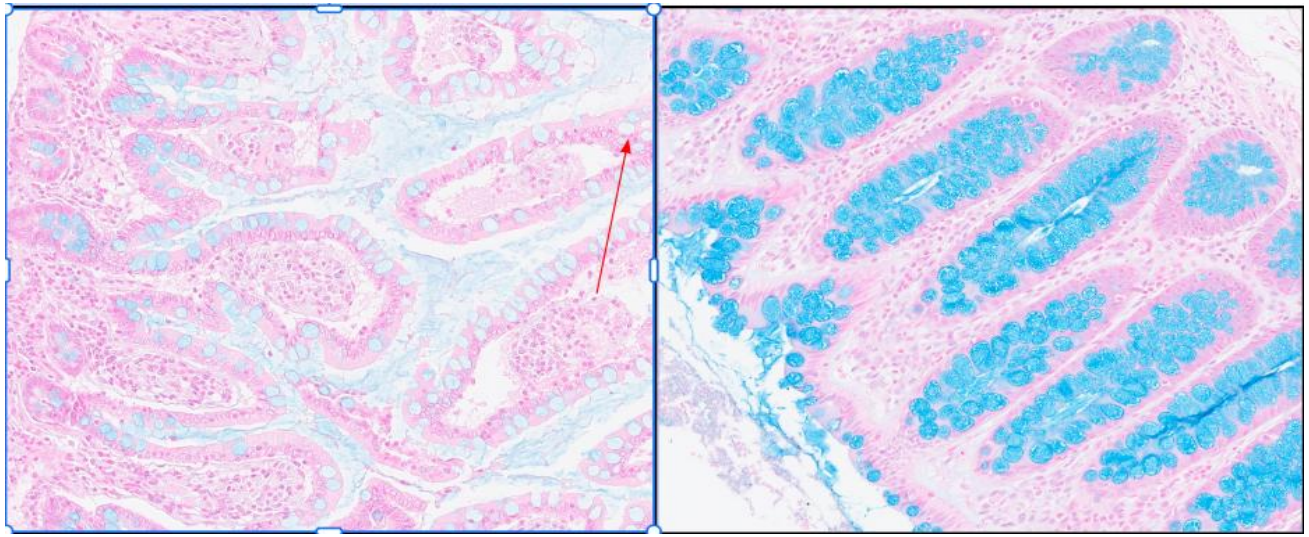
The case of Alcian Blue

- Call from Aug. 2024
- Material lot#18344 purchased in Mar ‘22.
- Material lot#18344 repurchased Apr ‘24

’22 - DcAn-26 Purchased from Vendor



’24 - DcAn-26 Repurchased

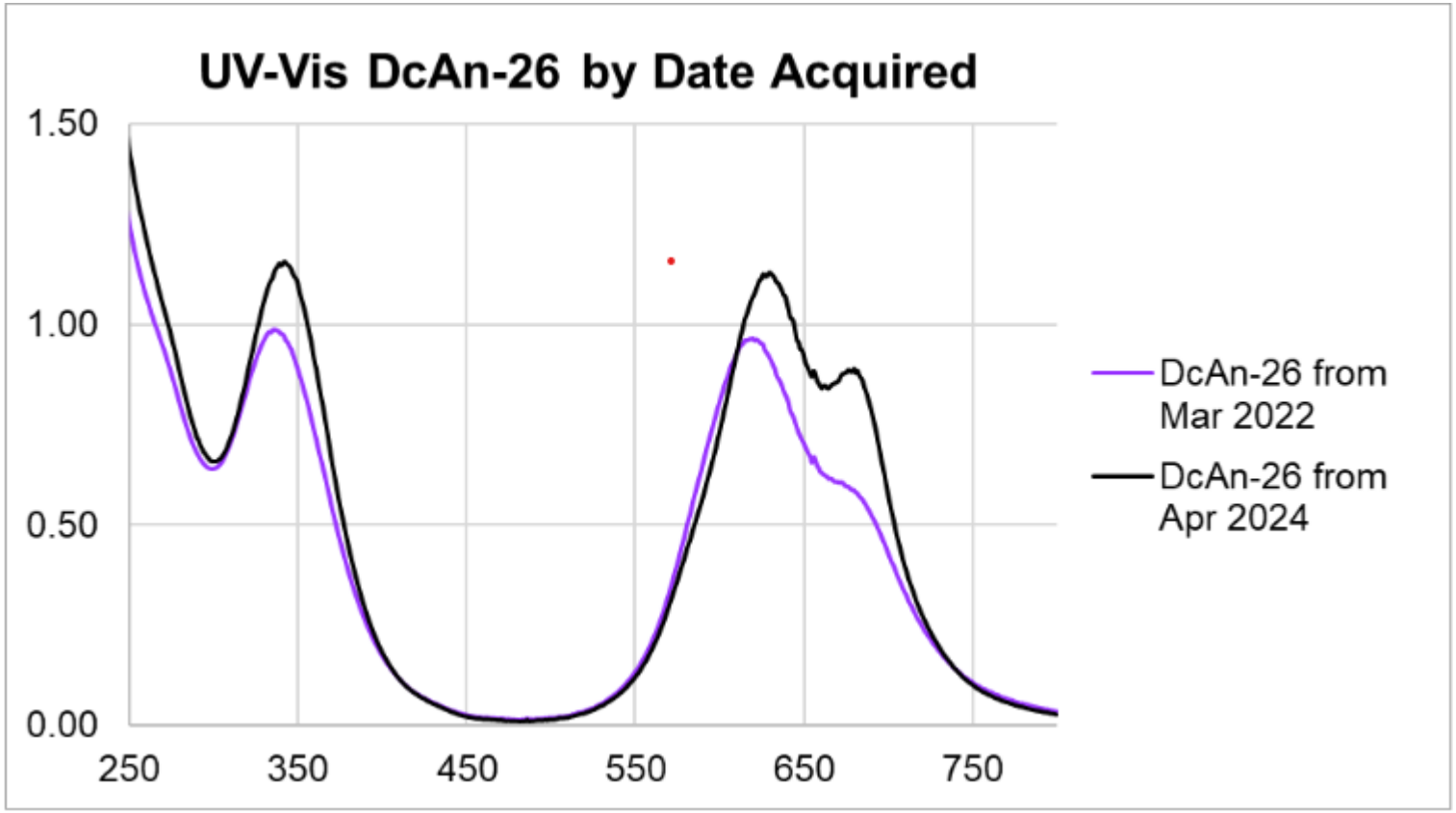


Normal Small Intestine

Normal Colon

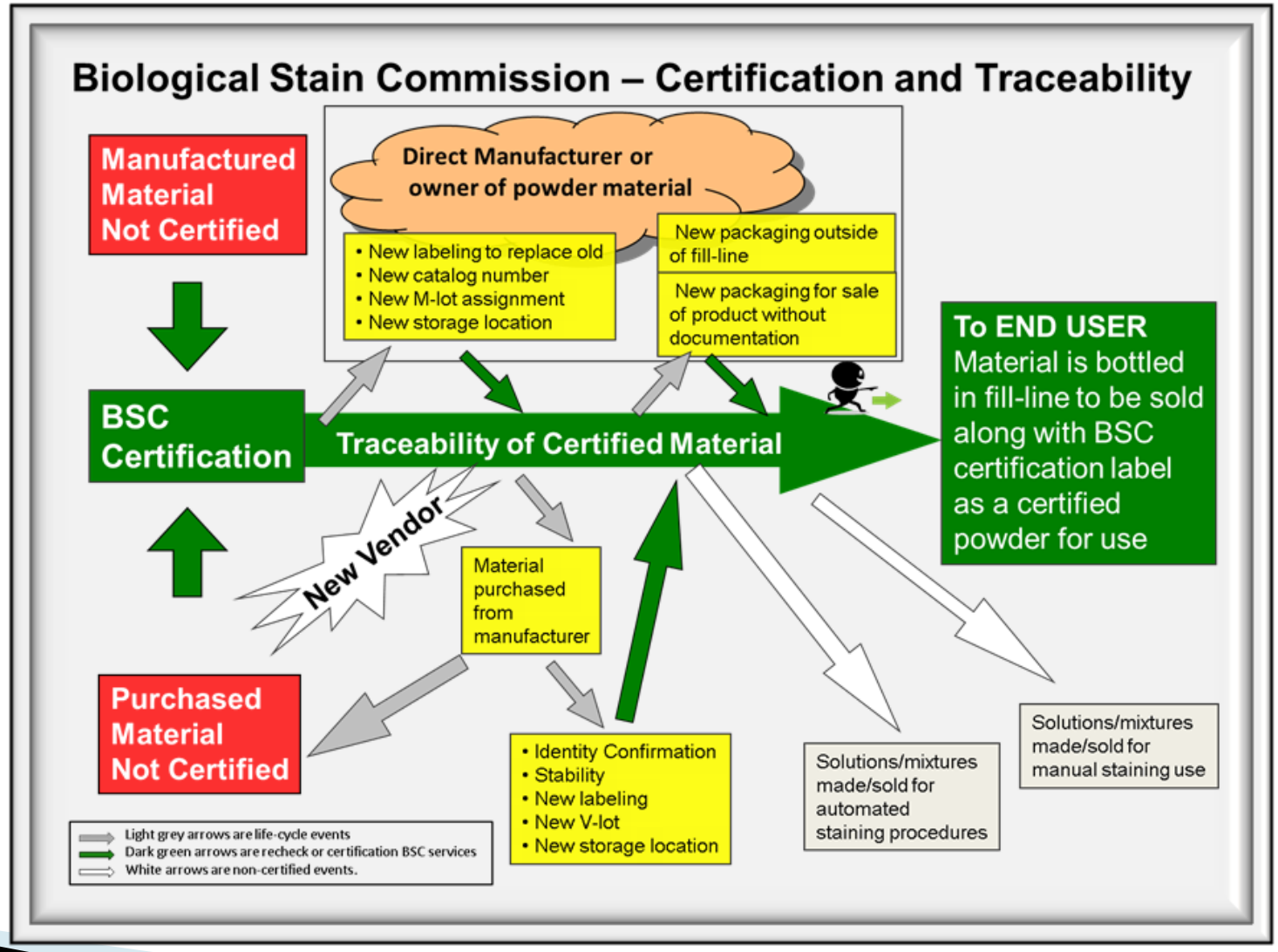
The case of Alcian Blue

- Material identity mismatch.
- Vendor quality deficiency



In house testing

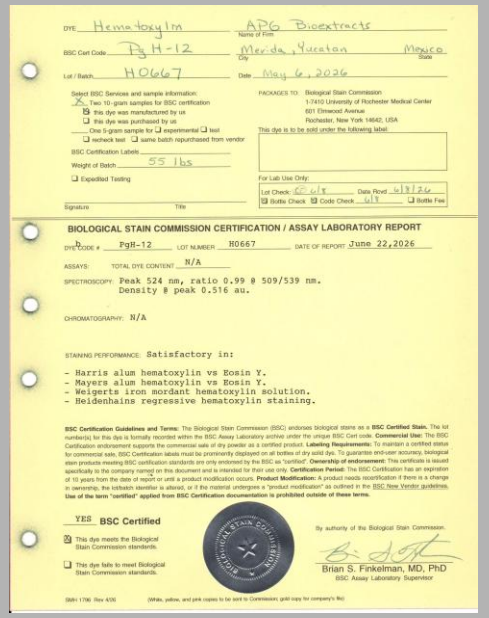
Traceability of a BSC Certified stain



Certification Documentation

- Company specific
  - Lot number
  - BSC Code – CcDd-##
- Lists the lab results.
- Embossed BSC seal with signature.
- Terms & Conditions

Certification



DYE: Hematoxylin — APG Bioextracts
BSC Cert Code: PgH-12 — Merida, Yucatan, Mexico
Lot / Batch: H0667 — Date: May 6, 2026
Weight of Batch: 55 lbs

BIOLOGICAL STAIN COMMISSION CERTIFICATION / ASSAY LABORATORY REPORT

DYE CODE #: PgH-12 LOT NUMBER: H0667 DATE OF REPORT: June 22,2026

ASSAYS: TOTAL DYE CONTENT: N/A

SPECTROSCOPY: Peak 524 nm, ratio 0.99 @ 509/539 nm.
Density @ peak 0.516 au.

CHROMATOGRAPHY: N/A

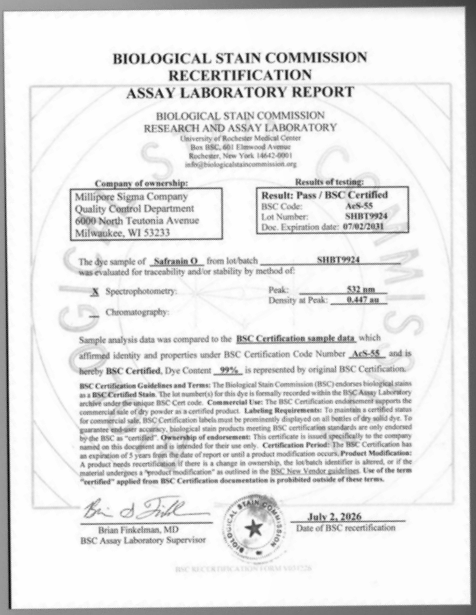
STAINING PERFORMANCE: Satisfactory in:

- Harris alum hematoxylin vs Eosin Y.
- Mayers alum hematoxylin vs Eosin Y.
- Weigerts iron mordant hematoxylin solution.
- Heidenhains regressive hematoxylin staining.

YES BSC Certified

Brian S. Finkelman, MD, PhD
BSC Assay Laboratory Supervisor

Recertification



BIOLOGICAL STAIN COMMISSION
RECERTIFICATION
ASSAY LABORATORY REPORT

BIOLOGICAL STAIN COMMISSION
RESEARCH AND ASSAY LABORATORY
University of Rochester Medical Center
Box BSC, 601 Elmwood Avenue
Rochester, New York 14642-8001
info@biologicalstaincommission.org

Company of ownership:
Millipore Sigma Company
Quality Control Department
6000 North Teutonia Avenue
Milwaukee, WI 53233

Results of testing:
Result: Pass / BSC Certified
BSC Code: AcS-55
Lot Number: SHBT9924
Doc. Expiration date: 07/02/2031

The dye sample of Safranin O from lot/batch SHBT9924 was evaluated for traceability and/or stability by method of:

X Spectrophotometry: Peak: 532 nm, Density at Peak: 0.447 au
— Chromatography:

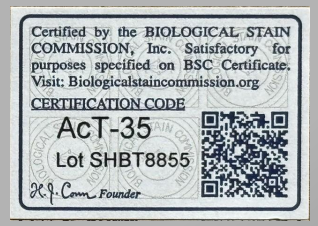
Sample analysis data was compared to the BSC Certification sample data which affirmed identity and properties under BSC Certification Code Number AcS-55 and is hereby BSC Certified, Dye Content 99% is represented by original BSC Certification.

BSC Certification Guidelines and Terms: The Biological Stain Commission (BSC) endorses biological stains as a BSC Certified Stain. The lot number(s) for this dye is formally recorded within the BSC Assay Laboratory archive under the unique BSC Cert code. Commercial Use: The BSC Certification endorsement supports the commercial sale of dry powder as a certified product. Labeling Requirements: To maintain a certified status for commercial sale, BSC Certification labels must be prominently displayed on all bottles of dry solid dye. To guarantee end-user accuracy, biological stain products meeting BSC certification standards are only endorsed by the BSC as "certified". Ownership of endorsement: This certificate is issued specifically to the company named on this document and is intended for their use only. Certification Period: The BSC Certification has an expiration of 5 years from the date of report or until a product modification occurs. Product Modification: A product needs recertification if there is a change in ownership, the lot/batch identifier is altered, or if the material undergoes a "product modification" as outlined in the BSC New Vendor guidelines. Use of the term "certified" applied from BSC Certification documentation is prohibited outside of these terms.

Brian Finkelman, MD
BSC Assay Laboratory Supervisor

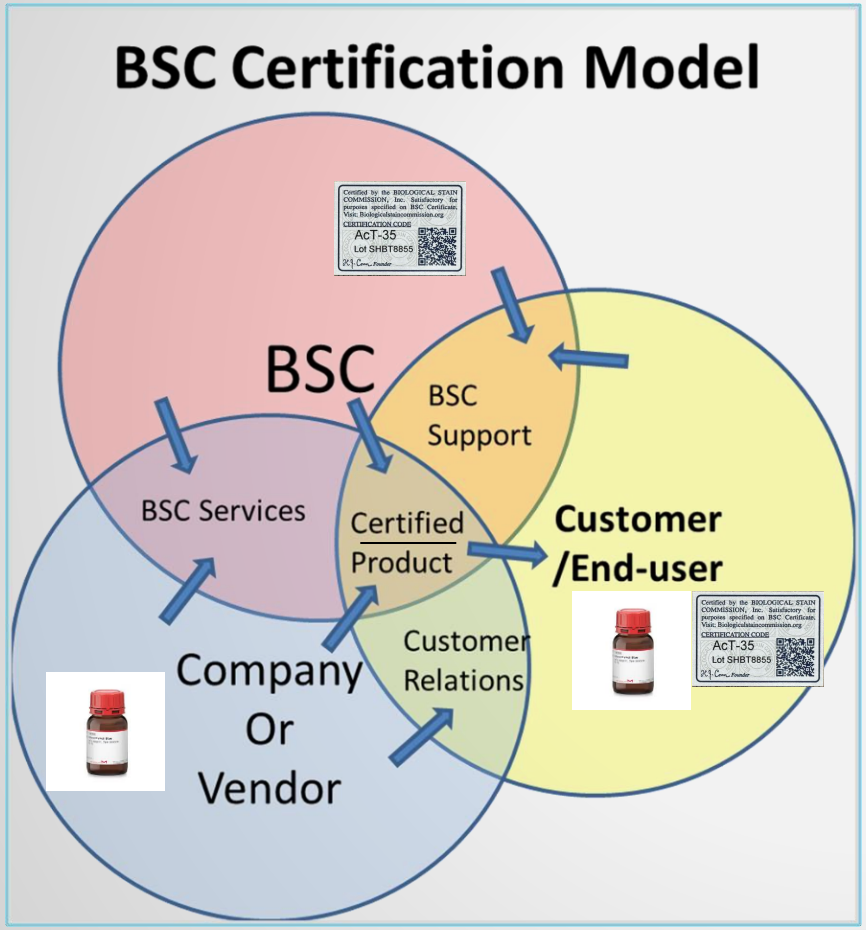
July 2, 2026
Date of BSC recertification

BSC Certification Label



Certified by the BIOLOGICAL STAIN COMMISSION, Inc. Satisfactory for purposes specified on BSC Certificate. Visit; Biologicalstaincommission.org
CERTIFICATION CODE
AcT-35
Lot SHBT8855
H.J. Conn Founder

The BSC, the Vendor and YOU!



- BSC certifies biological stain for commercial sale.
- Vendor sells stain/dye to customer.
- Vendor & BSC help customer.

Understanding quality output

- Certification – between BSC and Vendor
- Recertification – between BSC and Vendor
- BSC Certification labels – BSC, Vendor, and User

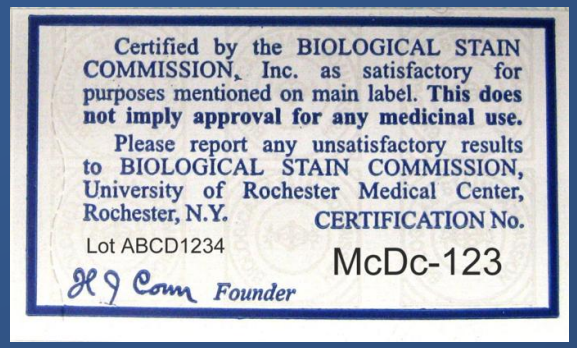
- Unofficial paperwork – Certificate of Analysis
- Claims of “Certified”
  - Website
  - Product label
  - Literature

Look for BSC Certification Label!

- Traceable sticker authenticates "BSC Certified" endorsement.
- Lot number cross checks with lot on company label.
- Security features
  - Cert. Code. – Record of testing results.
  - Lot number: identifies material tested.
- QR code – https://biologicalstaincommission.org/certified-biological-dyesstains/

BSC Certification Label

New 2026 BSC Certification Label



Older 2011– 2026 BSC Certification Label

Benefits of using a Certified stain…

- Increase amount of quality product on the market/better choices.
- BSC helps you to work with vendors – creating more transparency.
- Can use this process to evaluate the quality of vendors.

Biological Stain Commission

Certified Dyes & Stains

Thank you!

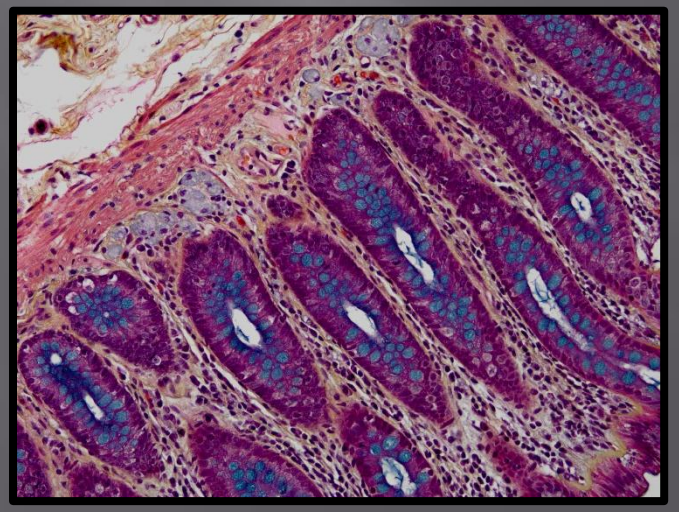
Dyes Certified by the BSC

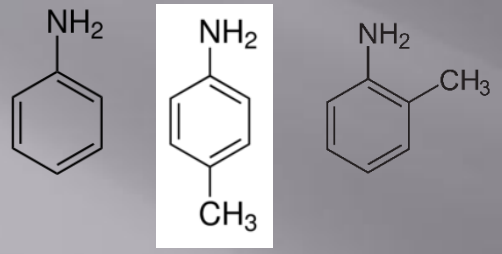
Stain	Stain	Stain
Acid Fuchsin	Eosin B	Nigrosin
Alcian Blue	Eosin Y	Nile Blue A
Alizarin Red S	Erythrosin B	Oil Red O
Aniline Blue,W.S.	Ethyl Eosin	Orange II
Auramine O	Fast Green FCF	Orange G
Azocarmine G	Giemsa Stain	Orcein, Synthetic
Azure A	Hematoxylin	Pararosaniline
Azure B	Indigo Carmine	Phloxine B
Azure C	Janus Green B	Pyronin B
Basic Fuchsin	Jenner Stain	Pyronin Y
Basic Fuchsin, Flagella	Light Green SF Yellowish	Resazurin
Bismarck Brown Y	Malachite Green	Rose Bengal
Brilliant Cresyl Blue	Martius Yellow	Safranin O
Brilliant Green	Methyl Green	Sudan III
Carmine	Methyl Orange	Sudan IV
Chlorazol Black E	Methyl Violet 2B	Sudan Black B
Congo Red	Methylene Blue	Tetrachrome Stain
Cresyl Violet Acetate	Methylene Violet	Thionin
Crystal Violet	Nuclear Fast Red	Toluidine Blue O
Darrow Red	Neutral Red	Wright Stain

Staining and histology

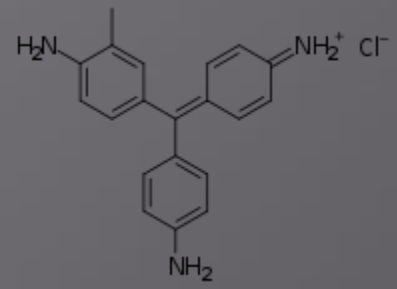
- Histology – study of the microscopic structure of tissues.
- 1.) a branch of anatomy that deals with the minute structure of animal and plant tissues as discernible with the microscope.
- 2.) tissue structure or organization

- Alizarin
- Carmine
- Hematoxylin
- Aniline

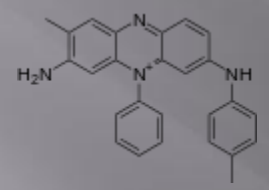




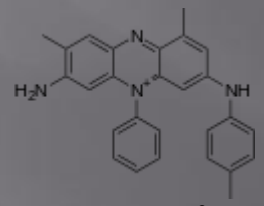
Aniline Dyes



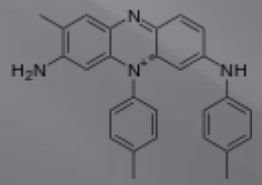
- William Perkin created muavine from coal tar.
- Aniline, p-toluidine, or o-toluidine mixture.
- Excellent silk dye
- Patented process 1856.



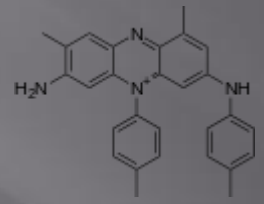
Muavine A



Muavine B



Muavine B2



Muavine C

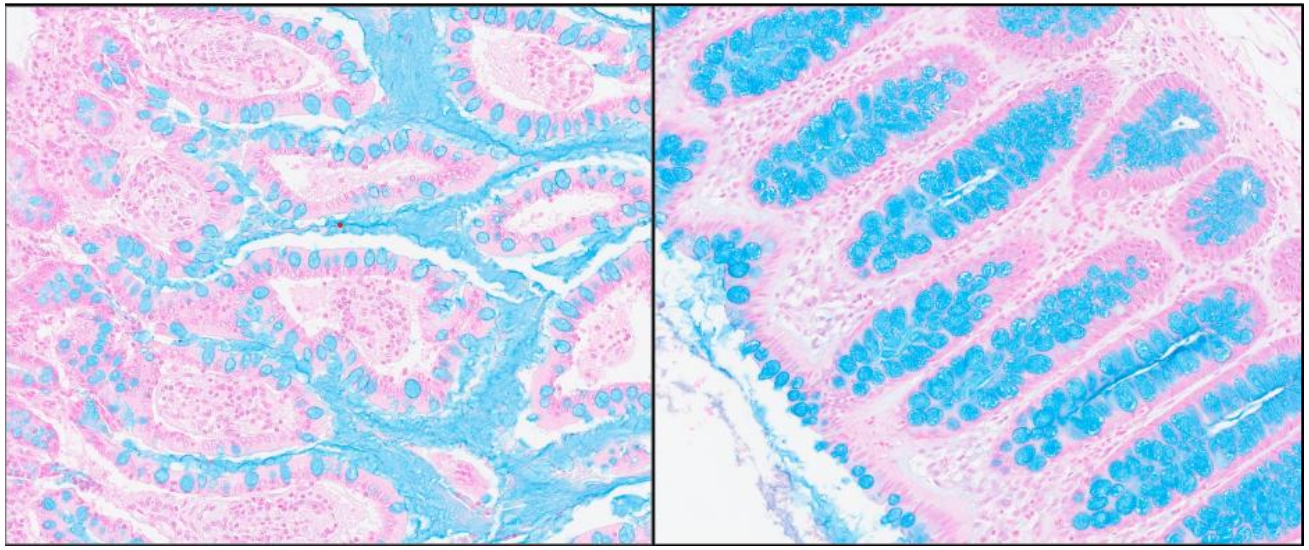
- 1856 Basic Fuchsin
- 1859 Safranine
- 1861 Methyl Violet
- 1862 Aniline Blue
- 1871 Eosin Y
- 1871 Methyl Green
- 1876 Thionin
- 1876 Methylene Blue
- 1877 Acid Fuchsin
- 1878 Orange G
- 1880 Sudan III
- 1883 Crystal Violet

The case of Alcian Blue

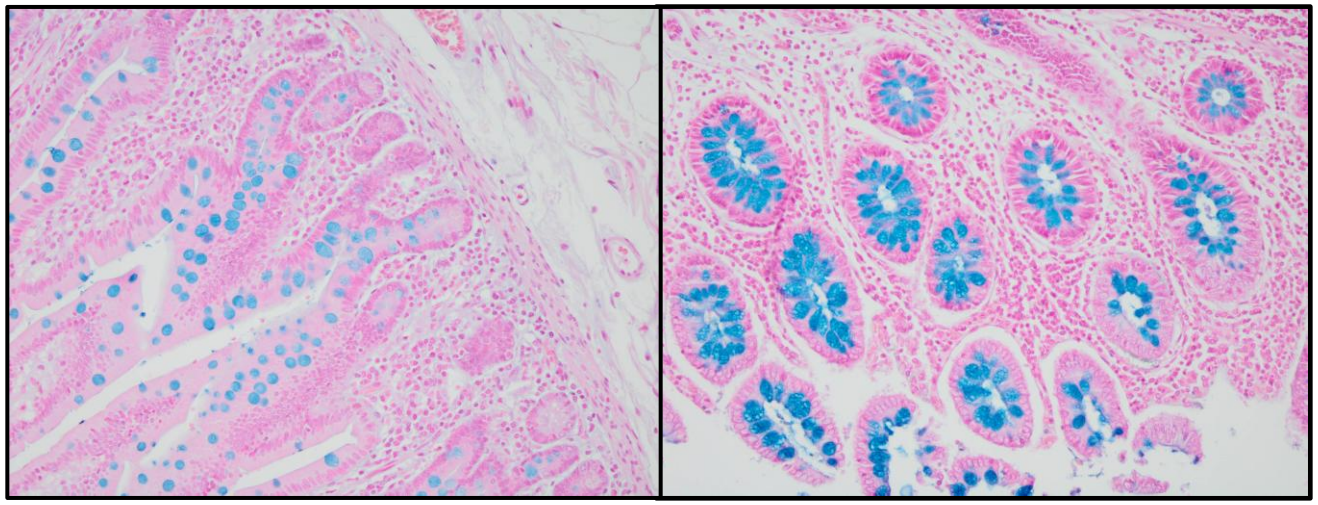
- Certified stain DcAn-26 Call from Aug. 2024
- Material lot#18344 purchased in Mar ‘22.

- BSC Certification staining

DcAn-26 In house staining



Normal Small Intestine Normal Colon



BSC Certification testing

Troubleshooting a biological stain

- Fixation & Tissue processing
  - Autolysis
  - Fix and pH
  - Poor dehydration & clearing
  - Tissue type
- Slide & Tissue
  - adhesion
  - section quality & thickness
  - mounting media & coverslip
- Protocol
  - Improper clearing & hydration
  - Slide drying & rehydration
  - Staining technic
- Reagent & solution quality
  - Dye quality & Certification
  - stability of dye powder
  - solution pH
  - solution integrity
  - contamination

How to get involved

- BSC organization –
  - Archive storage, content creators, documentation, historical preservation, organizational structure, quality, system design, website management.
- Editorial board – professionals, peers, volunteers, students.
- BSC Laboratory – Certification, Technical experience, Testing advancement, Quality, Organization.
- SME's: We'll save you a seat.