Automated Robust Image Analysis Qualifying H&E Staining Adequacy with Varying Hematoxylin and Eosin Incubation Times

Satabhisa Mukhopadhyay, PhD[1], Tathagata Dasgupta, PhD[1], Christopher Higgins[2], Alec DeGrand, MBA BS[2], Lisa M Williams, PhD[1], Nicolas Orsi, MBChB PhD MRCPath[1], Judy Lapetino[3], Erico von Bueren, MD PhD MOR[3]

[1]4D Path, [2]Evident Scientific; [3]Sakura Finetek USA

Disclosures: all authors are employed with their respective companies.
Disclaimer: this study does not promote any in-vitro diagnostic use.

Background

Inconsistencies in H&E staining protocols are a well recognized feature across different histopathology laboratories. The aim of the study was to validate an automated image analysis system (IAS) to qualify H&E staining adequacy with varying incubation times (INC) for hematoxylin (HEM) and eosin (EOS).

Material and methods

30 serial sections from a designed control block (DxOptim, LLC) containing 7 human tissues (liver, oncocytoma, spleen, stomach fundus, colon, colorectal carcinoma and skin) were cut at 5 µm, uncharged slides were labeled, and the tissue sections were mounted. 30 protocols were programmed on the Tissue-Tek Prisma® Plus Automated Stainer [PP] (Sakura Finetek USA, Torrance, California [SFA]), each being a unique combination of incubation times [INC] of hematoxylin Gills' II [HEM] and eosin Y [EOS]. HEM-INC were: 30, 60, 120, 180, 240 and 300 seconds. EOS-INC were: 10, 20, 30, 45 and 60 seconds.

Slides were placed into 30 baskets, sequentially loaded into the PP with their unique stain protocol. The Tissue-Tek Prisma® Stain Kit #2 was used to stain the slides, using the factory pre-loaded configuration, and a slide drying step of 15 min was included. Slides were coverslipped using the linked Tissue-Tek Film® Automated Coverslipper (SFA) using the Tissue-Tek® Coverslipping Film (SFA).

The dried coverslipped slides were scanned using the Slideview VS200 scanning system (Evident Scientific, Waltham, Massachusetts) with a UPLXAPO 20x objective and converted to DICOM (WG26 Supp 145) format (Fig 1 and 2).

Image analysis was performed on the 30 DICOM-converted images using the 4D Q-Plasia OncoReader™ [QPOR] (4D Path Inc., Newton, Massachusetts) workflow sequentially providing three routine image quality control parameters for all tissues at the whole slide image (WSI) and individual tissue level: (1) adequacy of tissue content (Tissue Adequacy, TA), (2) analyzable tissues selected by QPOR (Analyzability Adequacy, AA), and (3) QPOR-identified tissue regions of interest (ROI Adequacy, ROIA). Heat maps were used for visualization.

Results

- All seven tissues demonstrated comparable good performance in AA across all 30 protocols.
- Five out of seven tissues (spleen, oncocytoma, stomach fundus (Fig 3), benign/malignant colon (Fig 4)) achieved good performance in ROIA for more than 70% of the staining protocols (21/30). In general, more than 90% (9/10) of protocols having HEM-INC between 120 and 180 sec demonstrated good performance in ROIA for these tissues.
- The protocol for (HEM-INC 120, EOS-INC 20 or 30) and the protocol for (HEM-INC 30, EOS-INC 30 sec) demonstrated the best and the worst performance in ROIA respectively, across the five tissues.

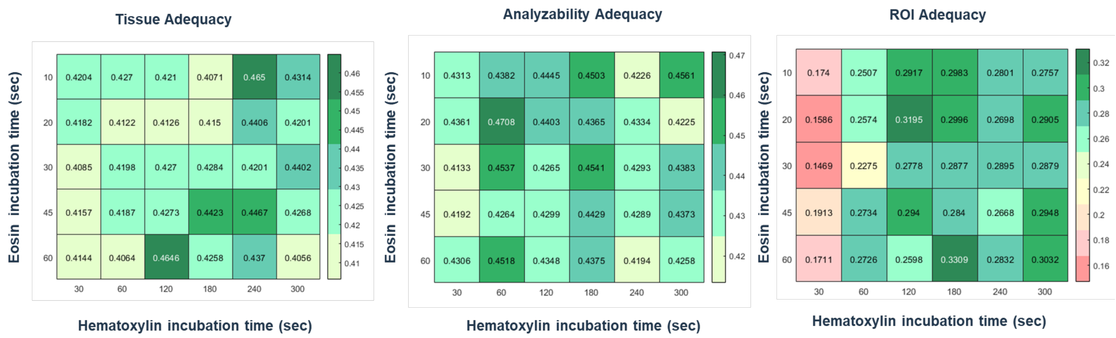

Fig 1: Sequential validation steps of 4D Q-Plasia OncoReader™ for stain adequacy analysis.

Fig 2: (columns 1) AA performance analysis for colorectal carcinoma and oncocytoma.
(column 2, 3) ROIA performance analysis for colorectal carcinoma, oncocytoma, stomach fundus, spleen.

Conclusions

- 4D Q-Plasia OncoReader™ identified 2 preferred H&E protocols with HEM-INC 120 | EOS-INC 20 or 30 sec.
- All 7 tissues were adequate for analysis by 4D Q-Plasia OncoReader™ across all the protocols.
- 5 out of 7 tissues could be used to assess stain adequacy levels of 30 H&E stain protocols.
- Analysis of more tissues with different disease states is suggested to design an H&E control block for this stain evaluation system.

Contact information

1 4D Path, Newton, MA, U.S.A. | Satabhisa Mukhopadhyay, PhD | E: smukhopadhyay@4dpath.com
2 Evident Scientific, Waltham, MA , U.S.A. | Janeen Manning | E: janeen.manning@evidentscientific.com
3 Sakura Finetek USA, Torrance, CA, U.S.A. | Erico von Bueren | E: evonbueren@sakuraus.com

Acknowledgements: We would like to thank Dr. Julia Dahl at DxOptim, LLC for designing the multi-tissue blocks and creating 30 unstained slides.

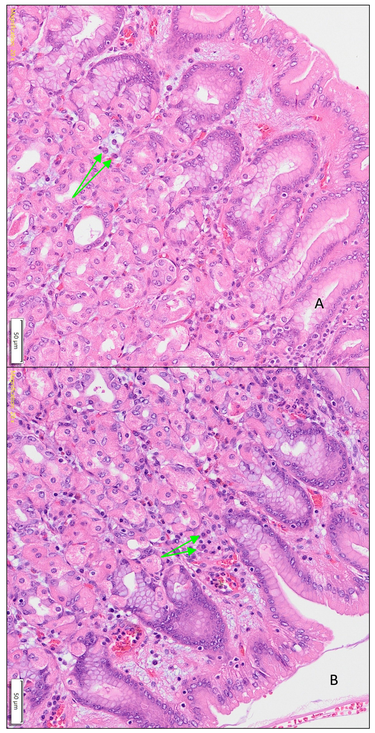

Fig 3: Stomach fundus. The desired H&E staining protocol depicts a greater contrast in parietal cells with peripheral nuclei and abundant eosinophilic cytoplasm, plasma cells with abundant amphophilic cytoplasm and eccentric nuclei with clumped chromatin and eosinophils within its tissue microenvironment (B) compared to the imbalanced protocol (A).

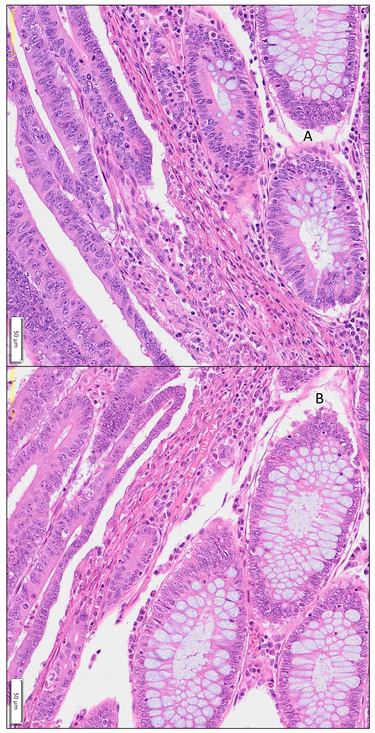

Fig 4: Colorectal carcinoma. The desired H&E staining protocol depicts a greater contrast in invasive colorectal carcinoma with tumors cells exhibiting nuclei with increased nuclear: cytoplasmic ratio, hyperchromasia, nuclear pleomorphism and prominent nucleoli (A) compared to the imbalanced protocol (B)