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ABSTRACT BOOK



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Enhancing Haematoxylin and Eosin Staining Consistency for Reproducible Histopathology: A Study on pH and Section Thickness

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ABSTRACT

Introduction: Haematoxylin and eosin (H&E) staining remains fundamental to diagnostic histopathology, medical teaching, and tissue-based research. With increasing reliance on digital pathology and AI-assisted interpretation, maintaining consistent, high-quality staining is critical, as automated systems cannot compensate for poor stain variation. Standardised manual protocols are therefore essential across laboratories. This study investigates how haematoxylin pH and tissue section thickness influence staining quality in loose and compact tissues to support more reproducible H&E workflows. **Materials and methods:** Duodenum and heart tissues from male Sprague–Dawley rats were fixed in neutral-buffered formalin, processed into paraffin blocks, and sectioned at 3 μm , 5 μm , and 7 μm . Sections were stained using haematoxylin adjusted to pH 2.3, 2.6, or 2.9. Nuclear staining intensity was quantified using ImageJ. Two-way ANOVA with Tukey's post-hoc test assessed the effects of pH and thickness. **Results:** Both pH and thickness significantly affected nuclear staining intensity in duodenum (pH: $p = 0.008$; thickness: $p < 0.001$) and heart (pH: $p = 0.015$; thickness: $p = 0.001$), with no significant interaction. Haematoxylin at pH 2.3 produced weaker nuclear staining, while pH 2.6 and 2.9 improved colour intensity and definition. Thicker sections (7 μm) consistently showed reduced staining quality. **Conclusion:** Optimal staining was achieved at pH 2.6 for duodenum and pH 2.9 for heart, with 3–5 μm sections recommended for reliable outcomes. These findings highlight that consistent manual staining remains fundamental, reinforcing the importance of robust, standardised H&E workflows for reproducible and clinically meaningful histological evaluation.

Keywords: H&E; pH; reproducible staining; section thickness