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Comparative Evaluation of Routine Haematoxylin and Eosin Staining with Rapid H&E Technique in Histopathology.

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Abstract

Background: Haematoxylin and Eosin (H&E) staining is the standard histopathological staining method for evaluating tissue morphology. Rapid H&E techniques have been developed to reduce staining time while maintaining acceptable diagnostic quality. ***Objective:*** To compare the staining quality and diagnostic efficacy of Routine H&E staining with the Rapid H&E technique in histopathology. ***Materials and Methods:*** This prospective comparative observational study included 200 histopathological specimens. Two serial sections were prepared from each specimen, with one stained by the conventional Routine H&E method and the other by the Rapid H&E technique. Nuclear staining, cytoplasmic staining, tissue architecture, staining uniformity, background clarity, staining artifacts, overall diagnostic adequacy, and staining time were evaluated by experienced histopathologists. Statistical analysis was performed using SPSS version 25.0, and a p-value <0.05 was considered statistically significant. ***Results:*** Routine H&E showed slightly superior nuclear and cytoplasmic staining quality compared with Rapid H&E. However, the Rapid H&E technique demonstrated excellent tissue preservation and achieved diagnostic adequacy in 97% of cases. Mean overall staining scores were significantly higher with Routine H&E ($p<0.01$), although the differences were small. Rapid H&E significantly reduced staining time (12.7 ± 1.8 minutes) compared with Routine H&E (45.8 ± 3.6 minutes, $p<0.001$), thereby considerably improving laboratory turnaround time. ***Conclusion:*** Rapid H&E staining provides staining quality and diagnostic accuracy comparable to Routine H&E while substantially reducing staining time. Although Routine H&E remains the gold standard, Rapid H&E is an effective, reliable, and practical alternative for routine histopathology, emergency biopsy evaluation, and high-volume diagnostic laboratories.

Keywords: Haematoxylin and eosin, Rapid H&E, routine H&E, histopathology, staining quality, diagnostic accuracy, tissue morphology.

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INTRODUCTION

Histopathological examination is the cornerstone of diagnostic pathology and remains the gold standard for the diagnosis of a wide spectrum of inflammatory, infectious, benign, premalignant, and malignant diseases. Accurate histopathological diagnosis relies on optimal tissue fixation, processing, sectioning, and staining, all of which contribute to the preservation of cellular morphology and tissue architecture. Among the various staining methods available, Haematoxylin and Eosin (H&E) staining is the most universally accepted and routinely employed technique because it provides excellent differentiation between nuclear and cytoplasmic structures, allowing detailed assessment of normal and pathological tissue changes.[1-3]

The conventional H&E staining method has been the standard laboratory procedure for decades due to its reproducibility, reliability, and superior staining quality. Haematoxylin selectively stains cell nuclei a deep blue-purple color by binding to nucleic acids, whereas eosin imparts varying shades of pink to the cytoplasm, connective tissue, and extracellular components. This differential staining enables pathologists to evaluate cellular morphology, tissue organization, inflammatory changes, dysplasia, and neoplastic alterations with high diagnostic confidence. Consequently, H&E staining remains indispensable in routine surgical pathology and forms the basis for most histopathological diagnoses before the application of special stains or immunohistochemistry.[2-5]

Despite its widespread acceptance, the conventional H&E staining protocol is relatively time-consuming, involving multiple sequential steps including deparaffinization, hydration, staining, differentiation, bluing, counterstaining, dehydration, clearing, and mounting. The complete staining process may require 40–60 minutes or longer, depending on laboratory protocols. This prolonged turnaround time can delay reporting, particularly in emergency situations, intraoperative consultations, urgent biopsies, and high-volume pathology laboratories where rapid diagnosis is essential for prompt clinical decision-making and patient management.[4-6]

To overcome these limitations, several modifications of the conventional H&E staining protocol have been developed, among which the Rapid H&E technique has gained considerable attention. By reducing reagent exposure times and optimizing staining steps, Rapid H&E significantly shortens the staining process while aiming to preserve nuclear detail, cytoplasmic contrast, tissue architecture, and overall diagnostic quality. An ideal rapid staining method should not only decrease processing time but also maintain staining consistency and reproducibility comparable to the conventional technique.[6,7]

Previous studies have reported that Rapid H&E staining provides satisfactory morphological preservation and diagnostic accuracy with only minimal compromise in staining intensity. The technique has shown particular usefulness in frozen section interpretation, small biopsy specimens, emergency surgical pathology, cytology cell blocks, and resource-limited settings where rapid reporting is desirable. Nevertheless, concerns remain regarding whether the reduction in staining time may adversely affect nuclear sharpness, cytoplasmic staining, background clarity, or overall diagnostic confidence. Therefore, continued evaluation of Rapid H&E against the conventional method is necessary before its widespread adoption in routine laboratory practice.[7,8]

In view of these considerations, the present study was undertaken to comparatively evaluate Routine Haematoxylin and Eosin staining and the Rapid H&E technique with respect to staining quality, nuclear and cytoplasmic detail, tissue architecture preservation, background staining, diagnostic adequacy, and staining time in routine histopathological specimens. The study aims to determine whether Rapid H&E can serve as a reliable and time-efficient alternative to the conventional H&E staining method in routine diagnostic histopathology.

MATERIALS AND METHODS

Study Design

This was a prospective comparative observational study conducted to compare routine Haematoxylin and Eosin (H&E) staining with the Rapid H&E staining technique in histopathological tissue sections.

Study Setting

The study was carried out in the Department of Pathology of a tertiary care teaching hospital.

Study Duration

The study was conducted over a period of 12 months after obtaining approval from the Institutional Ethics Committee.

Sample Size

A total of 200 histopathological specimens were included in the study.

Study Population

Histopathological tissue specimens received in the pathology laboratory for routine diagnostic evaluation were included.

Inclusion Criteria

- Formalin-fixed, paraffin-embedded tissue specimens.
- Adequately processed tissue blocks with satisfactory morphology.
- Surgical biopsy and excision specimens suitable for routine histopathological examination.
- Tissue sections showing adequate preservation without processing artifacts.

Exclusion Criteria

- Poorly fixed or autolyzed tissue specimens.
- Inadequately processed tissue blocks.
- Tissue sections with excessive folds, tears, or staining artifacts.
- Inadequate biopsy specimens unsuitable for evaluation.

Study Procedure

From each paraffin block, two serial sections of 4–5 μm thickness were prepared using a rotary microtome.

- Section A: Stained using the conventional Routine Haematoxylin and Eosin (H&E) staining protocol.

- Section B: Stained using the Rapid H&E staining protocol.

Both staining procedures were performed under standardized laboratory conditions using freshly prepared reagents and identical tissue processing techniques.

Evaluation of Stained Sections

All stained slides were independently evaluated by two experienced histopathologists who were blinded to the staining technique.

The following parameters were assessed:

- Nuclear staining quality
- Cytoplasmic staining quality
- Nuclear-cytoplasmic contrast
- Staining uniformity
- Tissue architecture preservation
- Background cleanliness
- Presence of staining artifacts
- Overall diagnostic adequacy

Each parameter was graded using a semi-quantitative scoring system:

- 3 = Excellent
- 2 = Good
- 1 = Fair
- 0 = Poor

The total staining score was calculated for each slide.

Outcome Measures

Primary Outcome

- Comparison of overall staining quality between Routine H&E and Rapid H&E staining techniques.

Secondary Outcomes

- Comparison of nuclear detail.
- Comparison of cytoplasmic detail.
- Comparison of staining uniformity.
- Comparison of tissue architecture preservation.
- Comparison of diagnostic adequacy.
- Comparison of staining time required for both techniques.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as frequency and percentage. Comparison between Routine H&E and Rapid H&E staining scores was performed using the paired Student's t-test or Wilcoxon signed-rank test, depending on data distribution. Categorical variables were compared using the Chi-square test. Interobserver agreement between the two histopathologists was assessed using Cohen's kappa coefficient. A p-value <0.05 was considered statistically significant.

RESULTS

A total of **200 histopathological specimens** were included in the study. Each specimen was stained using both the Routine Haematoxylin and Eosin (H&E) method and the Rapid H&E technique. The staining quality, diagnostic adequacy, and staining time were comparatively evaluated.

Table 1. Comparison of Nuclear and Cytoplasmic Staining Quality (n = 200)

Parameter	Routine H&E Excellent n (%)	Rapid H&E Excellent n (%)	p-value
Nuclear staining	190 (95.0)	182 (91.0)	0.118
Cytoplasmic staining	188 (94.0)	179 (89.5)	0.092
Nuclear-cytoplasmic contrast	186 (93.0)	176 (88.0)	0.081
Tissue architecture preservation	194 (97.0)	190 (95.0)	0.344

Routine H&E demonstrated slightly better nuclear and cytoplasmic staining quality than Rapid H&E. However, the differences between the two techniques were **not statistically significant (p>0.05)**, indicating that Rapid H&E produced comparable microscopic details suitable for histopathological interpretation.

Table 2. Comparison of Overall Staining Characteristics

Parameter	Routine H&E n (%)	Rapid H&E n (%)	p-value
Uniform staining	192 (96.0)	184 (92.0)	0.087
Clean background	195 (97.5)	188 (94.0)	0.118
Minimal staining artifacts	193 (96.5)	186 (93.0)	0.146
Overall diagnostic adequacy	198 (99.0)	194 (97.0)	0.248

Routine H&E provided marginally superior staining uniformity and cleaner background. Nevertheless, **97% of Rapid H&E slides were considered diagnostically adequate**, demonstrating that the rapid technique maintains excellent diagnostic utility comparable to the conventional method.

Table 3. Comparison of Mean Staining Scores

Parameter	Routine H&E (Mean ± SD)	Rapid H&E (Mean ± SD)	p-value
Nuclear staining score	2.94 ± 0.19	2.84 ± 0.31	0.003
Cytoplasmic staining score	2.91 ± 0.24	2.80 ± 0.34	0.005
Overall staining score	2.93 ± 0.18	2.82 ± 0.28	0.002

Routine H&E achieved significantly higher mean staining scores than Rapid H&E (**p<0.01**). However, the absolute difference in scores was small, indicating that Rapid H&E still provides excellent staining quality suitable for routine histopathological diagnosis.

Table 4. Comparison of Staining Time

Staining Technique	Mean Time (minutes)	Standard Deviation	p-value
Routine H&E	45.8	3.6	<0.001
Rapid H&E	12.7	1.8	

Rapid H&E significantly reduced the staining time compared with the conventional H&E technique (**12.7 ± 1.8 minutes vs. 45.8 ± 3.6 minutes; p<0.001**). This substantial reduction in processing time highlights the usefulness of Rapid H&E for emergency biopsies, frozen section substitutes, and high-volume histopathology laboratories where rapid reporting is essential.

DISCUSSION

Haematoxylin and Eosin (H&E) staining remains the cornerstone of histopathological diagnosis because of its ability to provide excellent nuclear and cytoplasmic contrast, facilitating accurate assessment of tissue architecture and cellular morphology. However, increasing workload in pathology laboratories has created a need for rapid staining techniques that can reduce turnaround time without compromising diagnostic quality. The present study compared the staining characteristics and diagnostic efficacy of Routine H&E staining with the Rapid H&E technique in 200 histopathological specimens.

The present study demonstrated that Routine H&E produced marginally superior nuclear and cytoplasmic staining quality compared with Rapid H&E. Nevertheless, the Rapid H&E technique achieved comparable staining characteristics with diagnostic adequacy in 97% of cases. These findings indicate that although the conventional technique continues to provide optimal staining quality, the rapid method is sufficiently reliable for routine diagnostic use. Similar findings were reported by Buesa, who emphasized that modifications in staining protocols can significantly reduce processing time while preserving tissue morphology and diagnostic accuracy in routine histopathology laboratories.[9]

The overall staining scores were slightly higher with Routine H&E, whereas Rapid H&E demonstrated excellent preservation of tissue architecture and minimal staining artifacts. These observations are in agreement with the study conducted by Ankle and Joshi, who reported that Rapid H&E staining provides satisfactory nuclear detail, cytoplasmic staining, and overall tissue morphology comparable to conventional H&E, with only minor differences in staining intensity that do not affect histopathological diagnosis.[10]

A major advantage observed in the present study was the marked reduction in staining time. Rapid H&E required approximately 13 minutes compared with nearly 46 minutes for Routine H&E, representing a statistically significant improvement in laboratory turnaround time. Similar observations were reported by Ralpathi et al., who demonstrated that Rapid H&E staining significantly shortens reporting time while maintaining excellent diagnostic concordance with conventional staining.[11]

The diagnostic adequacy of Rapid H&E remained high, with nearly all stained sections being suitable for microscopic interpretation. Patil et al. also concluded that Rapid H&E is a dependable alternative for routine surgical pathology and

emergency biopsy specimens, particularly in laboratories handling a large number of specimens where rapid reporting is essential.[12]

The findings of the present study are further supported by Meshram et al., who reported excellent agreement between Rapid and Conventional H&E staining regarding tissue morphology, nuclear clarity, and diagnostic interpretation in oral biopsy specimens. They suggested that Rapid H&E can effectively supplement conventional staining in routine pathology practice without compromising diagnostic confidence.[13]

The World Health Organization (WHO) continues to emphasize that accurate histopathological diagnosis depends primarily on excellent tissue preservation and adequate staining quality. Regardless of the staining protocol employed, proper tissue processing and adherence to standardized laboratory procedures remain fundamental for achieving reliable diagnostic outcomes.[14]

Overall, the findings of the present study indicate that although Routine H&E remains the benchmark technique, Rapid H&E provides comparable staining quality with a substantial reduction in processing time. Therefore, Rapid H&E represents a practical and efficient alternative, particularly for urgent biopsies, intraoperative consultations, and high-volume diagnostic laboratories.

CONCLUSION

Routine Haematoxylin and Eosin staining continues to provide the highest quality nuclear and cytoplasmic staining and remains the reference standard in diagnostic histopathology. However, the Rapid H&E technique demonstrated comparable staining quality, excellent preservation of tissue morphology, and high diagnostic adequacy with a significantly shorter staining time. The marked reduction in turnaround time makes Rapid H&E a valuable alternative for emergency biopsies and high-volume pathology laboratories. Therefore, Rapid H&E can be safely incorporated into routine histopathological practice as a rapid, reliable, and cost-effective staining technique without significantly compromising diagnostic accuracy.

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