

EFFECT OF pH ON H&E STAINING IN SMALL AND LARGE INTESTINES OF RATS

Hazulin Mohd Radzuan^{1*}, Wan Fatein Nabeila Wan Omar^{1*}, Mohd Dhiyaulhaq Halim¹

¹Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University, Malaysia.

*Corresponding email: fateinwanomar@iium.edu.my

Background: An optimized hematoxylin and eosin (H&E) histotechnique ensures reproducible and good staining quality. The common practice in determining when to discard the solutions depends largely on frequency and duration of usage. This practice suits labs with many active users, but not practical in less active labs. During this pandemic time, there is a substantial decrease in lab-based activities and replenishment of research materials. Hence, knowing the effect of pH of these solutions on staining quality could assist in deciding if they need to be replaced.

Purpose: To investigate the effect of hematoxylin pH on H&E staining outcomes.

Method: This is an experimental study performed on formalin-fixed paraffin embedded intestines of three healthy female Sprague-Dawley rats. The standard H&E protocol was applied on 3 µm tissue sections using hematoxylin of four serial pH (2.5, 2.75, 3.0 and 3.5). Eosin of pH 5.0 was used as the counterstain.

Results: At pH 2.5, the slides appeared acidic, and the epithelial lining was not distinctive. At pH 2.75, the hematoxylin dye was most prominent with good balance of staining, and 'crisp' epithelial lining was observed. At pH 3.0 or higher, although the lining was 'crisp', bluestaining mucin were observed.

Conclusion: The pH 2.75 appears to be the most optimal to achieve balance of coloration and definitive epithelial lining in H&E staining and with increasing pH overstaining occurs.

Keywords: pH, H&E Staining, Small Intestine, Large Intestine, Rats