Artifacts in histopathology: An experimental study

OP 26

Introduction

Histopathology is the art and science of tissue diagnosis on constant artifacts that are introduced by standard procedures of fixation, processing and staining, for which we are trained to recognize. It is of prime importance to identify unwanted artifacts and correlate them to the faulty laboratory step and avoid erroneous slide interpretation.

Objective

To correlate artifacts in hematoxylin and eosin stained tissue sections to various errors in fixation, processing and staining.

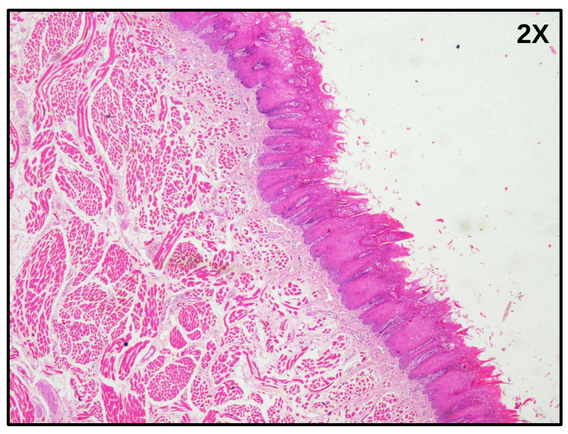
Methodology

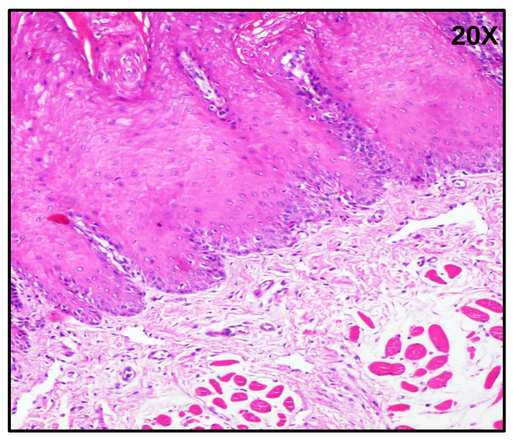
It was a double blind study where, tongue specimens were collected from sacrificed pigs and various fixation, processing and staining errors were performed. The slides were examined by two observers and the findings were correlated to the errors performed in the laboratory.

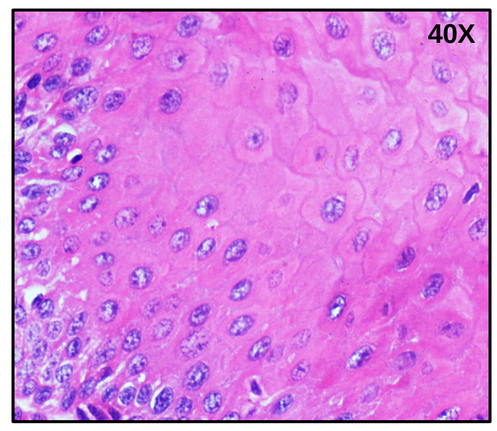
Technical details
Gross Photomicrographs: Canon EOS 550D
Processing : LEICA TP 1020
Photomicrographs : Jenoptik Chip Cool CCD PResC5

Results

Tissue routinely fixed, processed and stained →

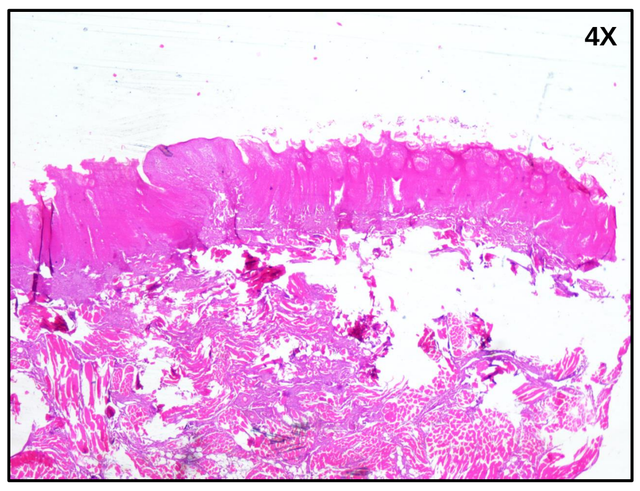




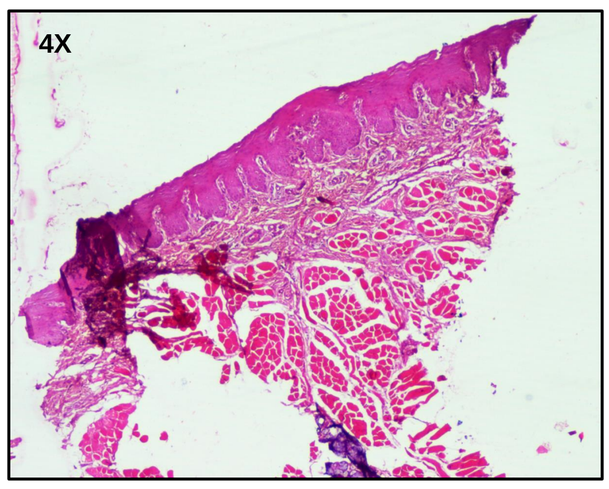


1.The following procedural errors gave rise sectioning artifacts

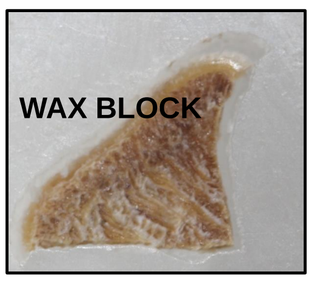
A. When tissue was immersed in spirit more than or equal to15 minutes
B. When tissue was immersed in saline more than 30 minutes
C. When tissues were not dehydrated using graduated concentration of isopropyl alcohol (IPA)
D. When tissues were dehydrated with very low concentration of IPA (25%)
E. When tissue was cleared in xylene for prolonged time (Overnight /8 hours)
F. It was impossible to section the tissues embedded in the blocks when 100% IPA was used as a fixative/ routinely fixed and diluted dehydrant was used and/ when dehydration procedures was skipped.



Tissue routinely fixed and processed but cleared overnight (prolonged) in xylene. (Ref 1E)



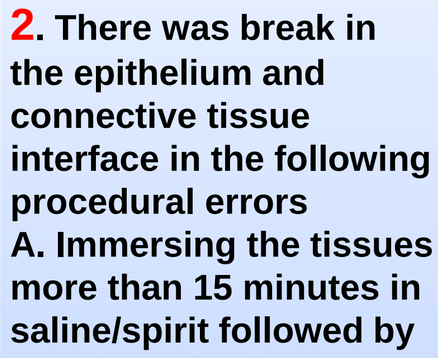
Tissue routinely fixed but processed without graduated IPA (Ref 1E)



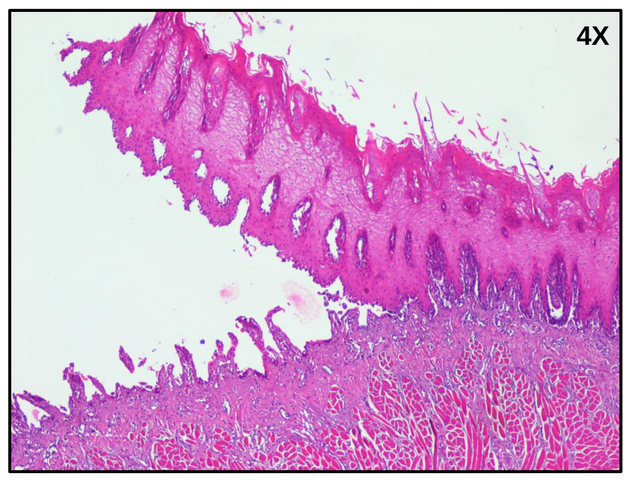
Tissue routinely fixed but was processed with week diluted IPA (50%) Ref 1F

2. There was break in the epithelium and connective tissue interface in the following procedural errors

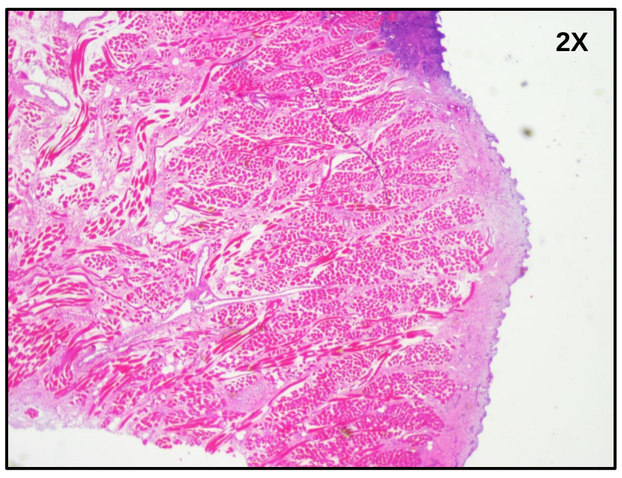
A. Immersing the tissues more than 15 minutes in saline/spirit followed by 10% formalin fixation
B. When 5% or lesser concentration of formalin was used
C. When tissues were immersed in saline, spirit and water without formalin fixation followed by routine processing there was complete loss of epithelium in areas.



Tissue immersed in surgical spirit followed by routine processing. (Ref 2A)



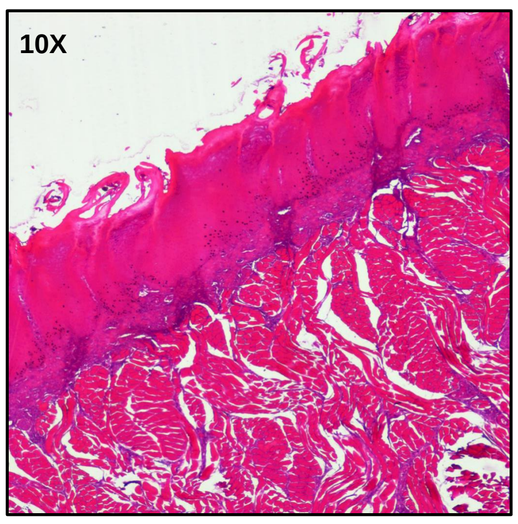
Tissue immersed in Saline for 60 minutes, followed by 10% formalin fixation (Ref 2A)



Tissue immersed in saline followed by routine processing. (Ref 2C)

3. There was loss of connective tissue architectural in the following procedural errors

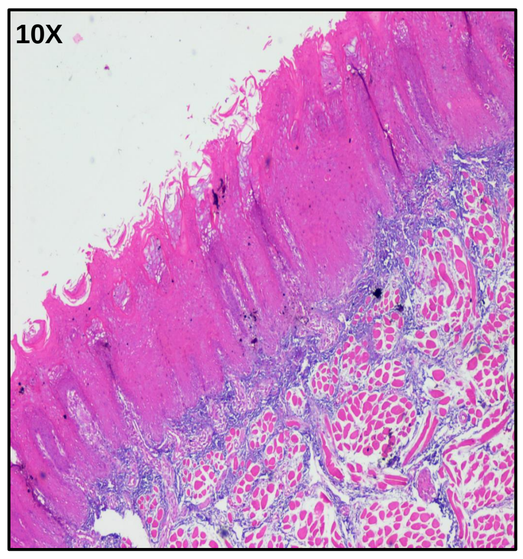
A. When tissue was cleared in xylene for prolonged time (Overnight /8 hours)
B. When clearing and dehydration steps were skipped
C. When tissue was dehydrated using IPA (100%) without graduation.
D. When tissue section was overheated during staining procedure.



Tissue routinely fixed but processed with only 100% IPA without graduation. (Ref 3C)

4. There was increased basophilia in connective tissue in the following procedures

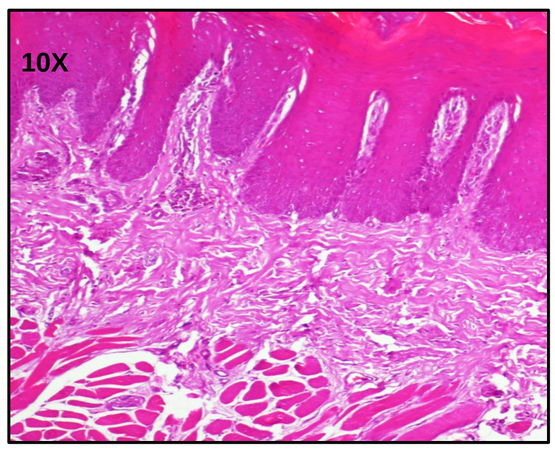
A. When tissue was fixed with basic formalin (10.8pH)
B. When tissue was immersed in saline for more than 45 minutes
C. When tissue was cleared in xylene for prolonged time (Overnight /8 hours)
D. When tissue was fixed in 100% formalin
E. When tissue section was overheated during staining procedure.
F. When bluing step was skipped during staining procedure.



Tissue section overheated during staining procedure. (Ref 4E)

5. Epithelial cell boundaries were poorly distinct in the following procedure

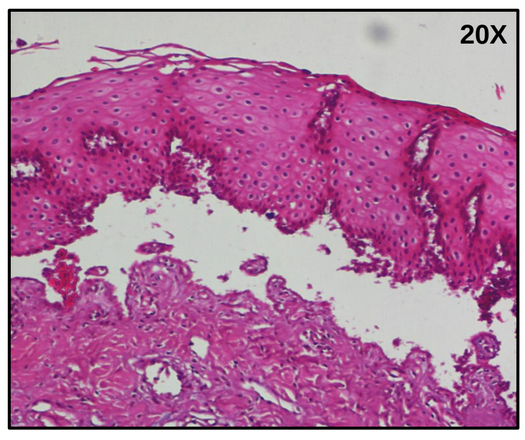
A. When tissue was fixed with higher concentration of formalin (50% & 100%)
B. When tissue was dehydrated using IPA (100%) without graduation.
C. When tissue was processed routinely but clearing step was skipped.



Tissue fixed in 100% formalin Ref 5A

6. Intracellular edema was apparently evident in the following scenarios

A. When tissue was fixed with 5% formalin
B. When tissues were immersed in spirit, saline, water for more than 30 minutes



Tissue immersed in water followed by routine processing Ref 6B

Conclusion

Clinical context is very important as it aids in diagnosis and avoids erroneous interpretation due to such artifacts. Recognition of unwanted artifacts not only aids in proper diagnosis but also acts as an alarm for faulty laboratory procedures .