COMMON ERRORS IN HISTOPATHOLOGY

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Introduction

- The primary role of the histology laboratory is the provision of a diagnostic service through the preparation, analysis and interpretation of tissue samples. Given that the procedures involved are so complex, it is inevitable that errors can transpire throughout stages of the entire process.
- Identifying those errors and their root causes in the clinical setting can be perplexing. Often partitioned into clerical, processing, technical and documentation categories, errors in cellular pathology are more traditionally subdivided into pre-analytical, analytical and post-analytical phases.

ERRORS CAN OCCUR AT PRE-ANALYTICAL, ANALYTICAL AND POST-ANALYTICAL PHASES, WHICH CAN BE CLASSIFIED AS THE FOLLOWING

- SAMPLE RECEIVING (REGISTRATION) (PRE-ANALYTICAL)
- FIXATION
- PROCESSING
- EMBEDDING
- SECTIONING (ANALYTICAL)
- STAINING
- MOUNTING
- REPORTING AND DISPATCHING (POST-ANALYTICAL)

PRE-ANALYTICAL
SAMPLE RECEIVING (REGISTRATION)

Preanalytical phase comprises of **test selection, patient identification, collection of the sample, handling of the sample, sorting out.** Negligence in any of these steps can lead to erroneous results attributed to preanalytical phase.

For example :

- mislabeling a patient's specimen with another patient's identification details.
- Differences in site of a sample taken. (e.g., right or left)
- Proper fixative (e.g., 10% buffered formalin) or normal saline for immunofluorescence test

ANALYTICAL FIXATION

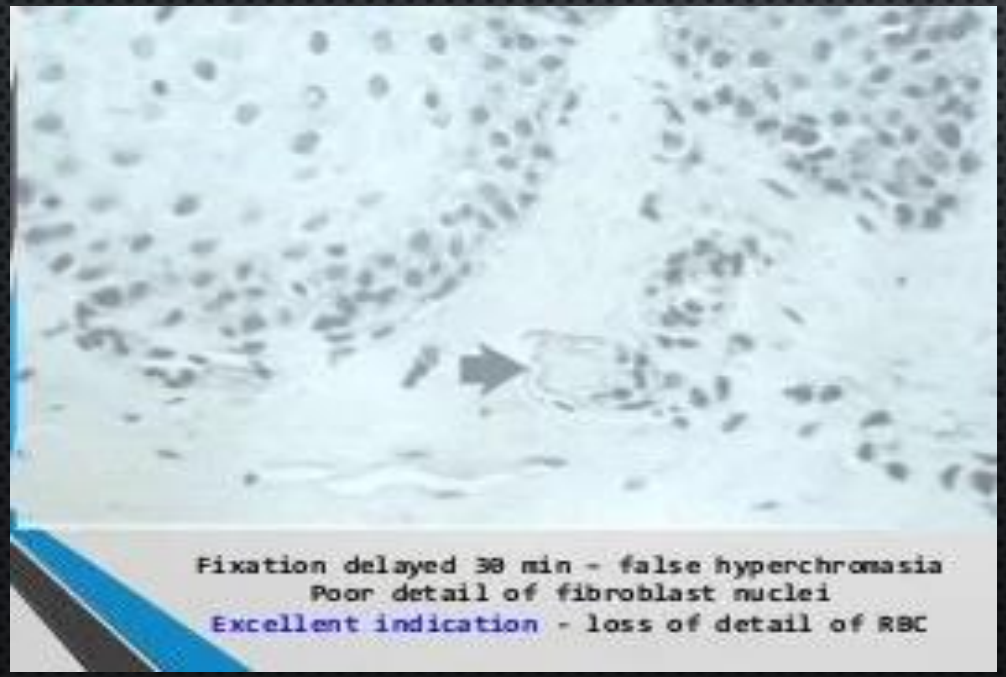
BASIC DEFINITION OF FIXATION.

- Fixation is a process which is necessary to prevent tissue from diffusion of soluble component there by prevent autolysis and putrefaction.
- Depends on the nature and quality of the fixative agent artifact can occurs. The volume of the fixative is 20 times more than that of the specimen (less than 6mm). normally 10% formalin is used as fixative

ERRORS IN FIXATION

DELAY IN FIXATION & INADEQUATE FIXATION TIME PRODUCES SIMILAR CHANGES

- THE SPECIMEN WILL LACK THE DETAIL AND LOSS OF CELLULARITY DUE TO AUTOLYSIS.
- THE STAINING QUALITY OF THE CELLS IS ALTERED, AND THE CELL CYTOPLASM AND NUCLEAR STRUCTURE APPEARS COMPLETELY INDISTINCT.
- INSUFFICIENT TIME FOR FIXATION, TOO LARGE A SPECIMEN AND REAGENT WITH POOR PENETRATION RATE,



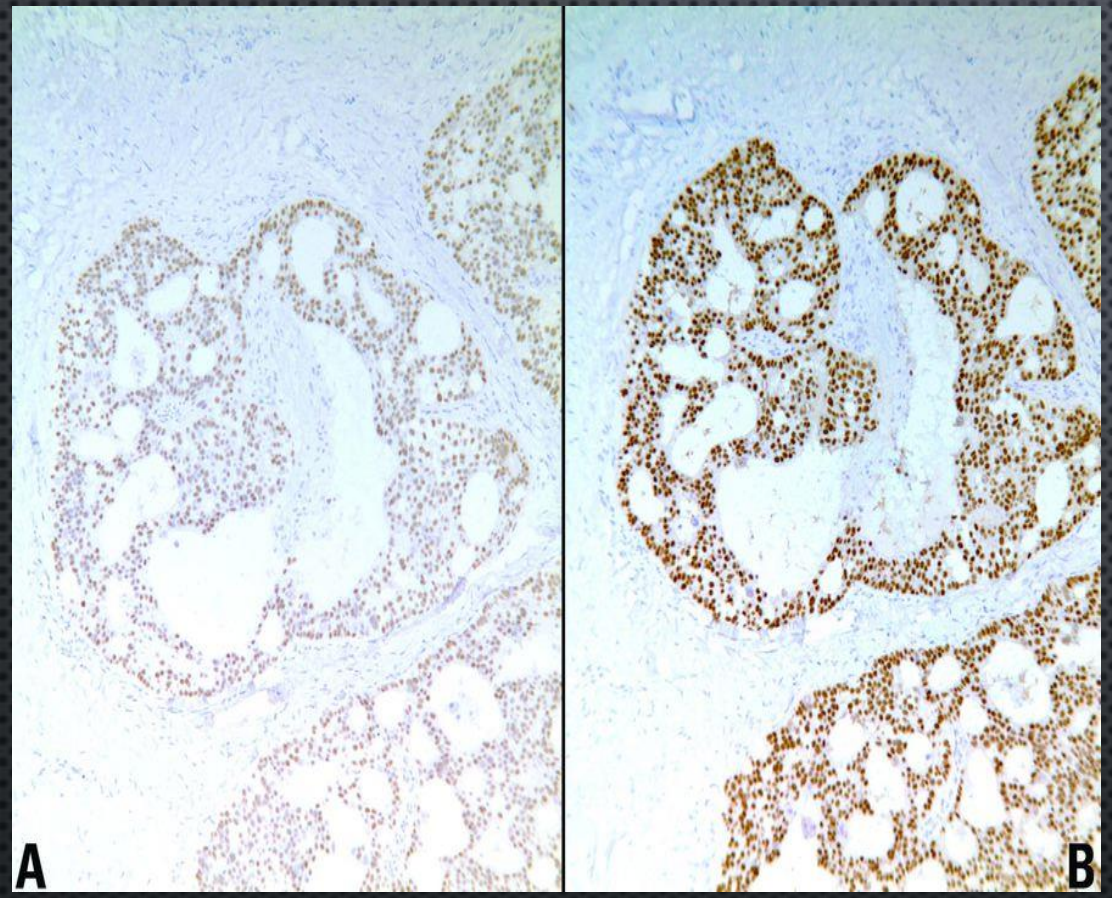


Figure 1. Estrogen receptor (ER) staining of breast carcinoma.

A. Tissue block fixed for 3 hours in 10% formal saline showing weak demonstration of ER positive cells.
B. Tissue block fixed 8 hours in 10% formal saline showing strong demonstration of ER positive cells

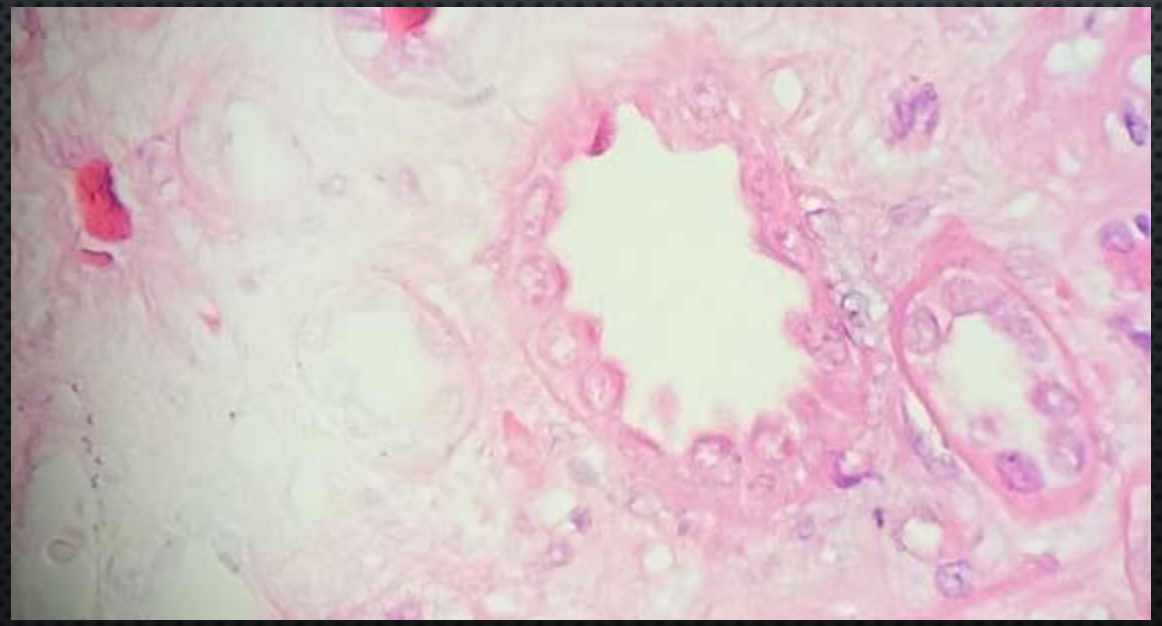
PROCESSING

a) IMPROPER DEHYDRATION

- Too long treatment in higher concentration of alcohol
- results in high degree of shrinkage of the tissue.
- Too long treatment in lower dilution of alcohol macerates the tissue seen as vacuolization. These two procedures will also make the tissue brittle and interfere with staining properties
- When tissue is not completely dehydrated, the paraffin will not infiltrate properly, and the block is difficult to cut resulting in tearing and holes.
- When this occurs, rehydrate the tissue section and repeat the processing. Dehydration should be always gradual starting from 50%.

B) Improper Clearing

- Prolonged treatment in xylene will make the tissue brittle, leading to crumbling and crystallization during cutting.
- Conversely, if specimen is not cleared properly in xylene, the paraffin will not impregnate properly and will lead to distortion of tissue during sectioning



The uneven staining in this section is a direct result of not enough time in in xylene to remove all the paraffin from the tissue. This errors is also seen when the support media for frozen sections has not been adequately remove.

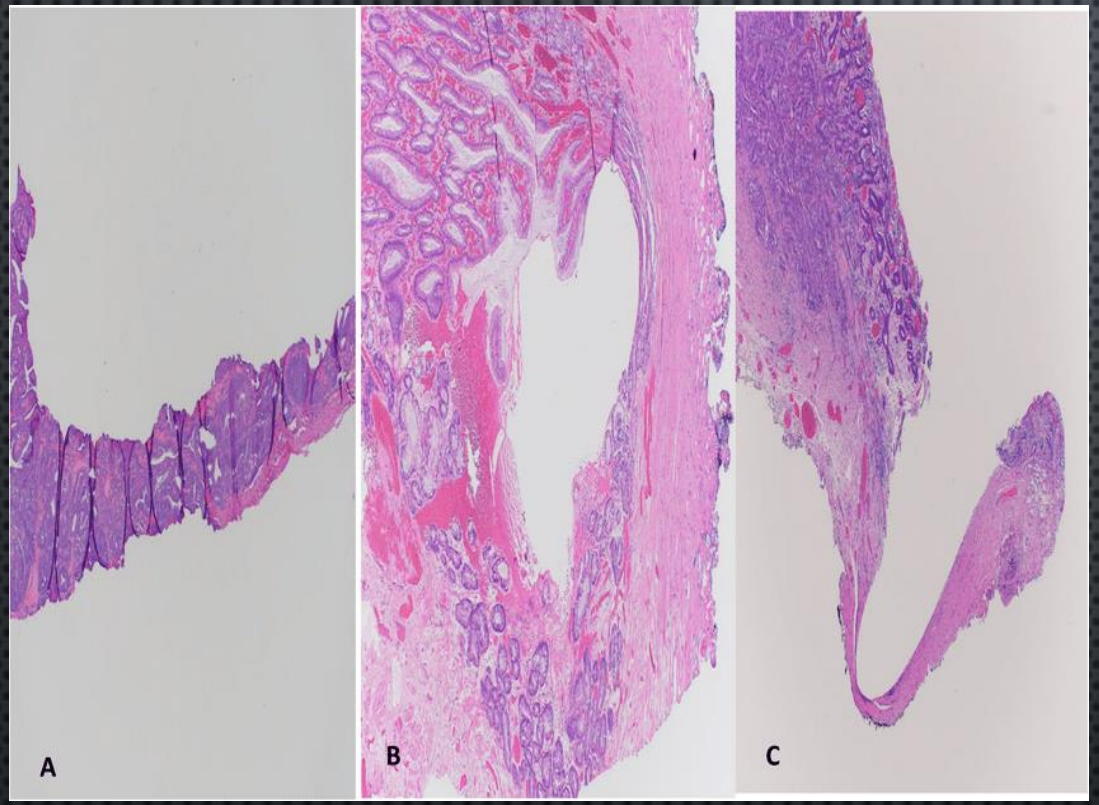
EMBEDDING

A) IMPROPER EMBEDDING

Exposing the specimen too long during embedding procedure causes excessive hardening so that it becomes friable, and sectioning will give rise to cracks

B) IMPROPER ORIENTATION.

- Improper orientation will lead to disorderly arranged histological features in slide. For orientation of skin, it must be positioned so that the epithelial edges, the subcutaneous tissue and deeper layers all flat to the bottom so that all strata will be seen in the finished slide.
- When embedding more than one specimen, all the pieces of tissue should be embedded firmly to the bottom of the container so that the cut section will present a valid presentation of the tissue submitted



A. TISSUE FOLDING DUE TO IMPROPER PROCESSING AND EMBEDDING. HAEMATOXYLIN AND EOSIN STAIN × 20.

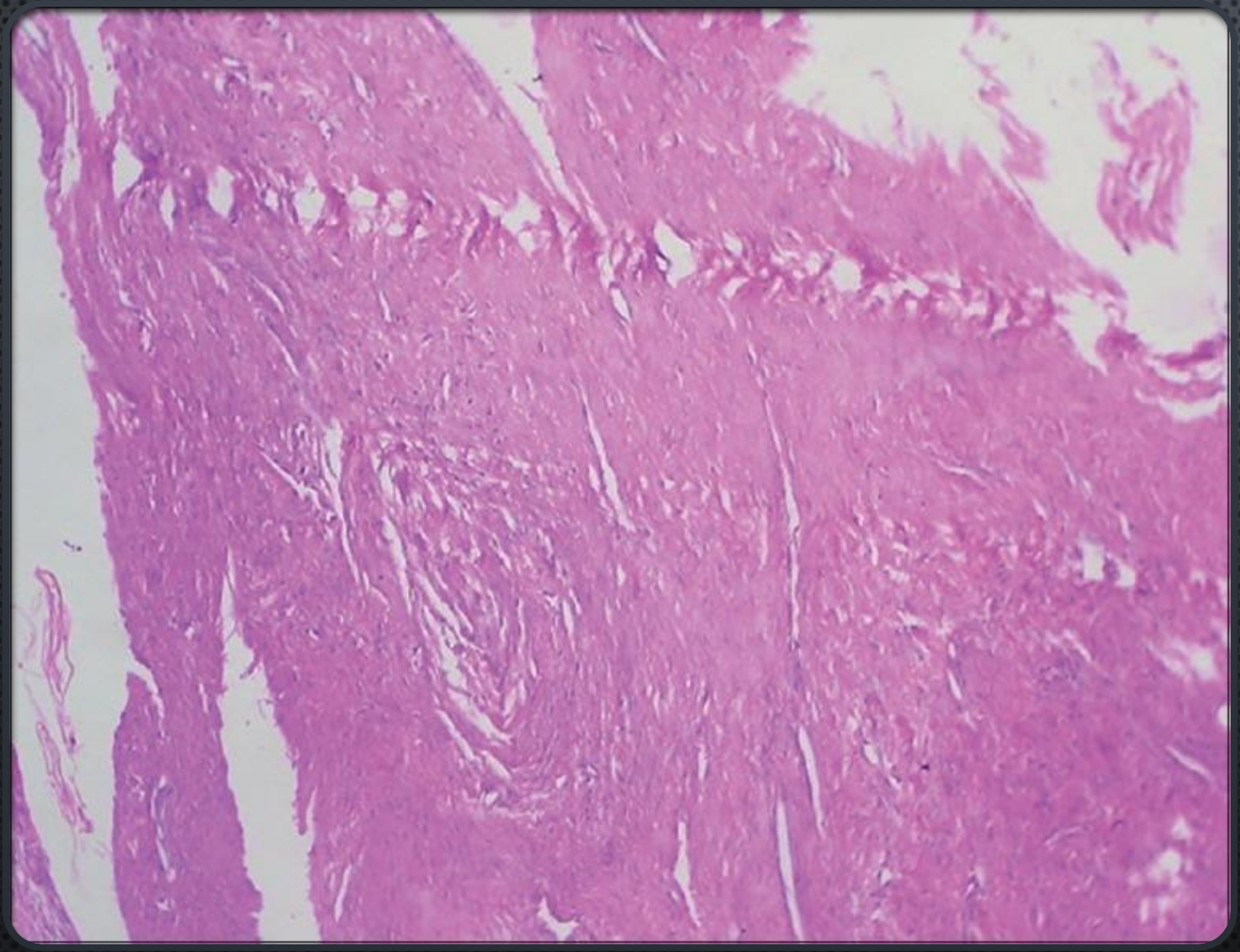
B. PINHOLE CAUSING CURLING OF TISSUE AT EDGES LEADING TO DIFFICULTY IN PERIPHERAL MARGIN INTERPRETATION. HAEMATOXYLIN AND EOSIN STAIN. × 40.

C. LARGE PINHOLE A CAUSING DISRUPTION OF TISSUE AT THE EDGE. × 20

SECTIONING.

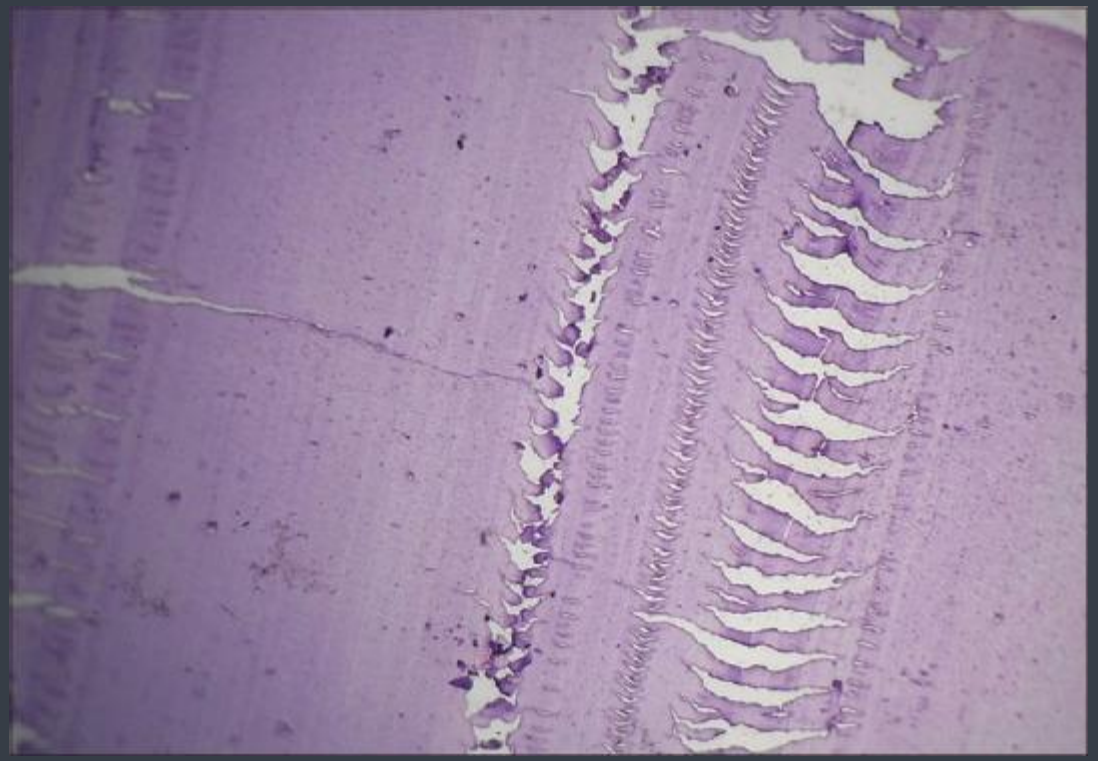
a) Scores and tearing in sections

- These are caused either by a nick or blemish in the knife edge and when sectioning hard particles such as foci of calcification, debris within the block. In the former instance, the tear usually extends across the whole section.
- It is avoided using different part of knife or changing with a new blade . In the latter, it may start from that point.
- It is avoided by removing the hard particle with fine sharp-pointed scalpel or by re-embedding in fresh filtered wax or by surface decalcification before cutting.



HISTOPATHOLOGICAL IMAGE SHOWS SCORING AND TEARING OF SECTION DUE TO NICK IN KNIFE EDGE (H&E, ×10)

HISTOPATHOLOGICAL IMAGE SHOWS VENETIAN BLIND DUE TO VIBRATION OF KNIFE EDGE (H&E, ×10)



CONTINUE…..

These are caused by:

- **Tiny vibrations in the knife edge:**

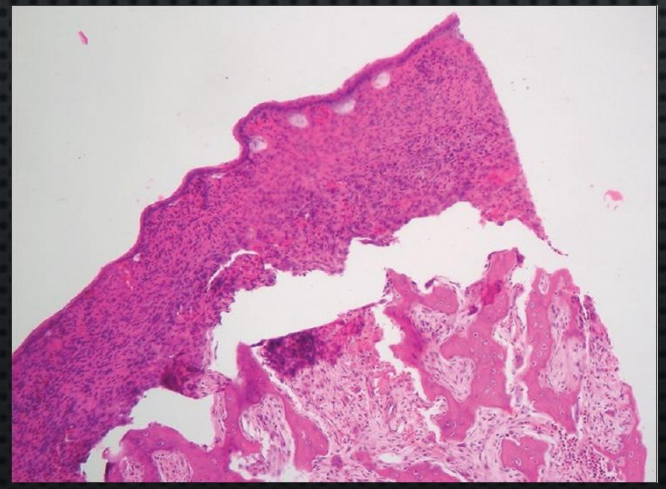
Ensure that the knife is securely clamped into its holder and the holder to the microtome

- **Excessive hardness and brittleness of the block:**

Cutting thinner sections or softening the blocks (softening fluid or surface decalcification), using sharp heavy-duty knife or heavy-duty microtome

B) COMPRESSION ARTIFACT

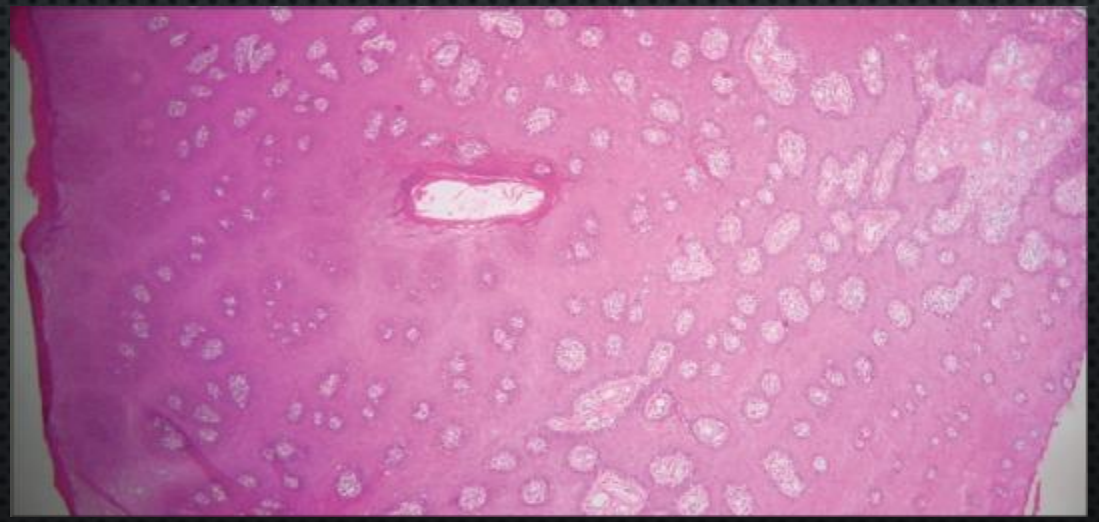
- **BLUNT KNIFE**: DISPLACEMENT OF TISSUE COMPONENTS, ESPECIALLY BONE IS A COMMON FINDING .
- **BEVEL OF THE KNIFE TOO WIDE**: RESHARPEN TO PRODUCE SECONDARY BEVELS OR HAVE REGROUND
- WAX TOO SOFT FOR TISSUE OR SECTIONING CONDITION: COOL BLOCK WITH ICE OR USE HIGHER MELTING POINT WAX.



Histopathological image shows displacement of bone during microtomy in association with the use of dull knife (H&E, ×10)

C) MOUTH-EATEN EFFECT (HOLES FROM ROUGHING)

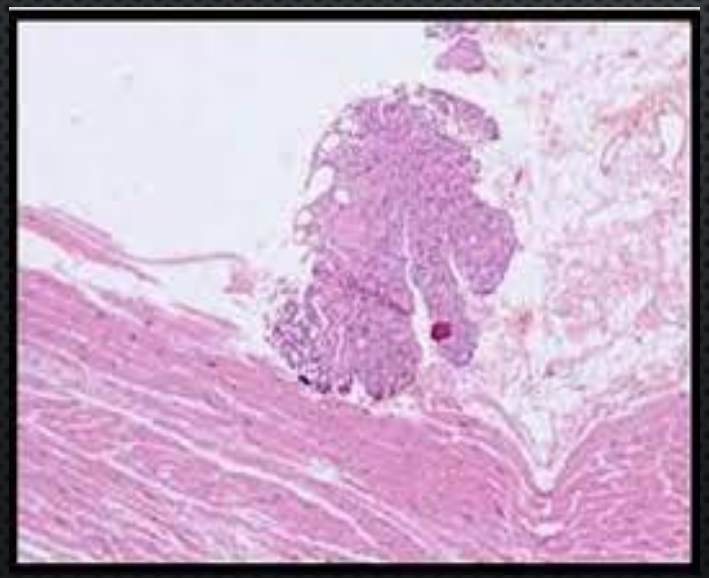
- It occurs due to excessively rough trimming of the paraffin blocks with greater thickness. This pulls out the tissue fragments from the block face and these appear as void spaces or holes in subsequent thin sections with their long axis parallel to the knife edge. To avoid these errors, the knife should be changed regularly, and the rough cutting carried out at a lower thickness setting. If the tissue is cut tangentially, the connective tissue cores may become entrapped within the epithelium, giving a false impression of invasive squamous cell carcinoma



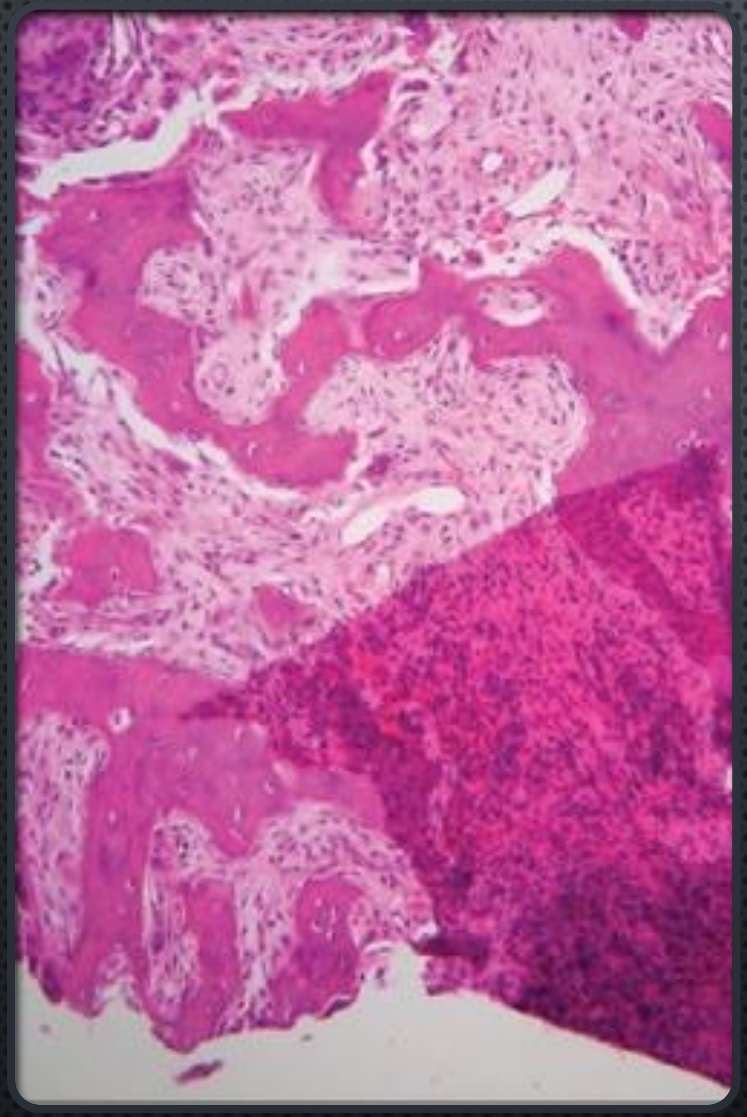
Histopathological image shows tangential cut artifact (H&E, ×10)

D) FLOATER

- Floaters are pieces of tissue that appear on slides that do not belong there. They may have floaters during processing and may result from sloppy procedures on cutting bench using dirty towel, knife, gloves and improper cleaning of water bath. These can have tissues that are carried over to the next case.



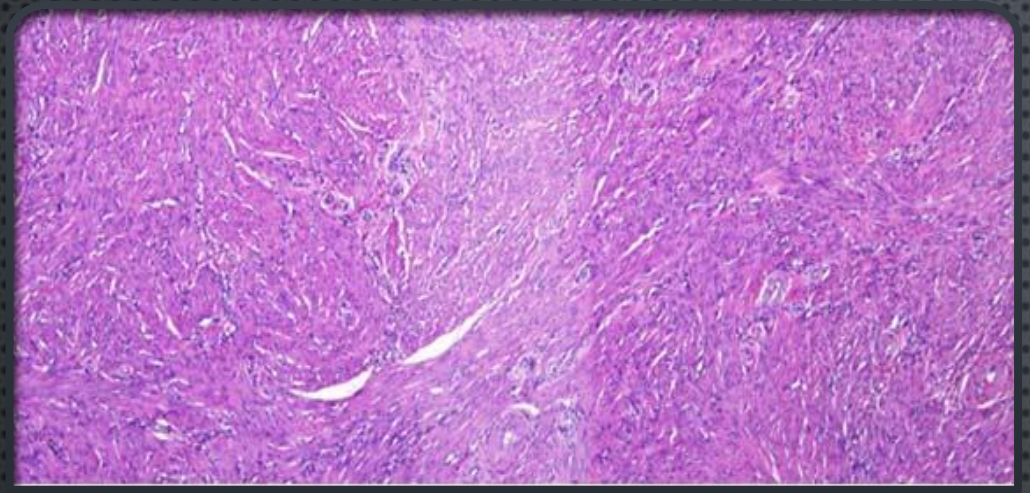
Specimen-specimen contamination: A section of cardiac muscle with a piece of extraneous thyroid tissue present against one surface; H&E.

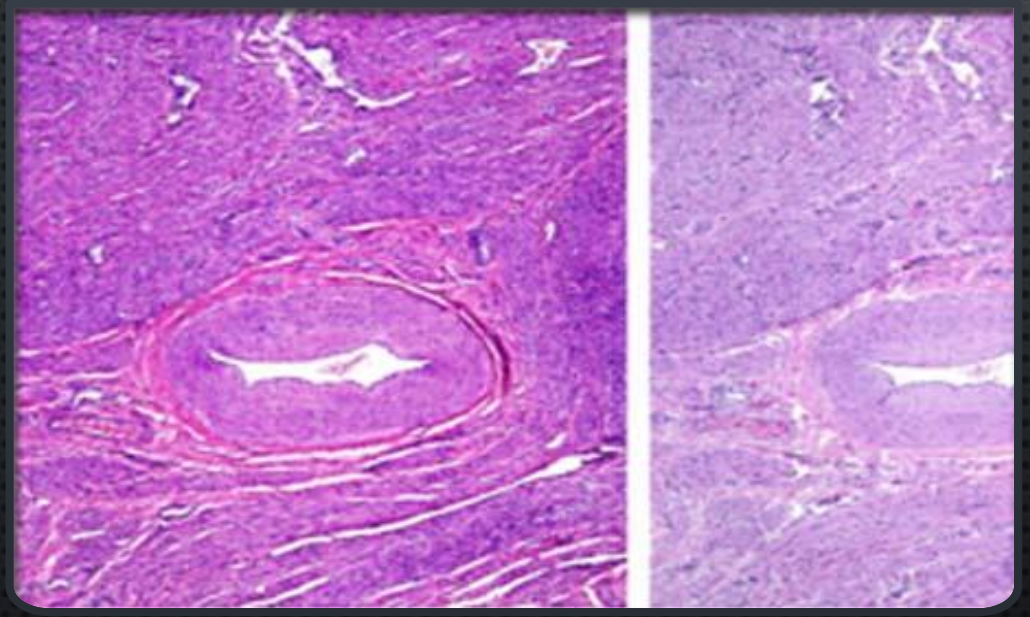


E) ALTERNATE THICK AND THIN SECTIONS

ALTERNATE THICK AND THIN SECTIONS ARE PRODUCED WHEN THE WAX IS TOO SOFT FOR TISSUE, BLOCK OR BLADE IS LOOSE, CLEARANCE ANGLE IS INSUFFICIENT, OR MECHANISM OF MICROTOME IS FAULTY. REMEDY IS TO COOL THE BLOCK WITH ICE, TIGHTEN THE BLADE AND INCREASE THE CLEARANCE ANGLE. ALSO, WRINKLING AND CURLING IN TISSUE CAN OCCUR AT THIS STAGE

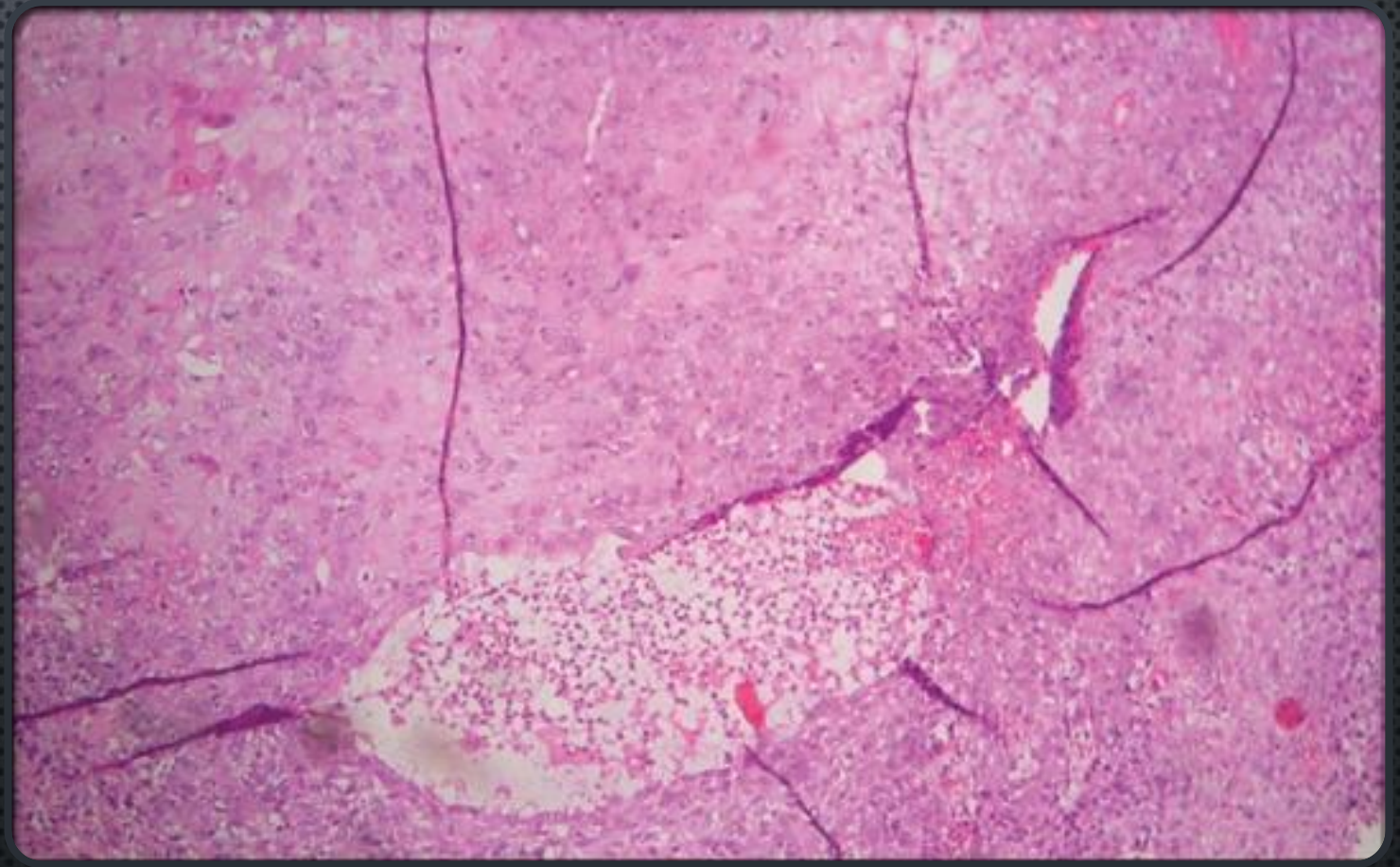
Histopathological image shows curling due to folding of tissue due to blunt microtome knife (H&E, ×10)





Note difference in coloration between these identical sections (Figure 1). Thick and thin sections can also be seen within the same tissue section (Figure 2).

HISTOPATHOLOGICAL IMAGE SHOWS WRINKLES AND FOLDS DUE TO UNEVEN STRETCHING OF TISSUE SECTIONS (H&E, ×10)



STAINING ARTIFACT

A) DUE TO RESIDUAL WAX

- RESIDUAL WAX IN A SECTION WILL PREVENT PENETRATION OF BOTH AQUEOUS AND ALCOHOLIC DYE SOLUTIONS LEAVING AREA TOTALLY DEVOID OF STAIN, TRACES OF RESIDUAL WAX HAVE A SUBTLE EFFECT ON NUCLEAR STAINING PRODUCING SMALL PATCHES IN SECTIONS WHERE NUCLEI APPEAR MUDDY AND LACK DETAIL PROLONGED XYLENE TREATMENT AND RE-STAINING WILL OVERCOME THIS PROBLEM.

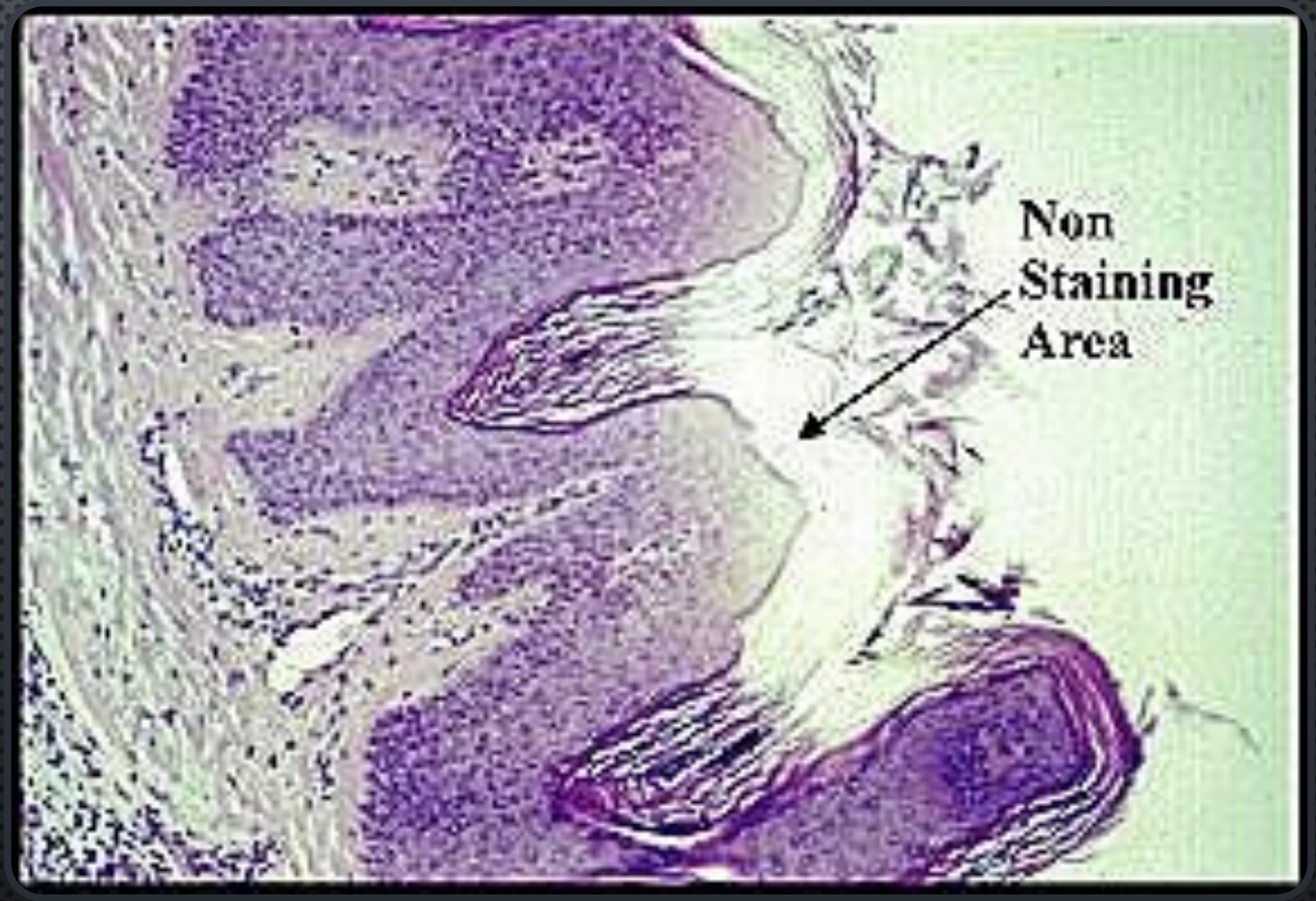
B) DUE TO STAIN DEPOSITS

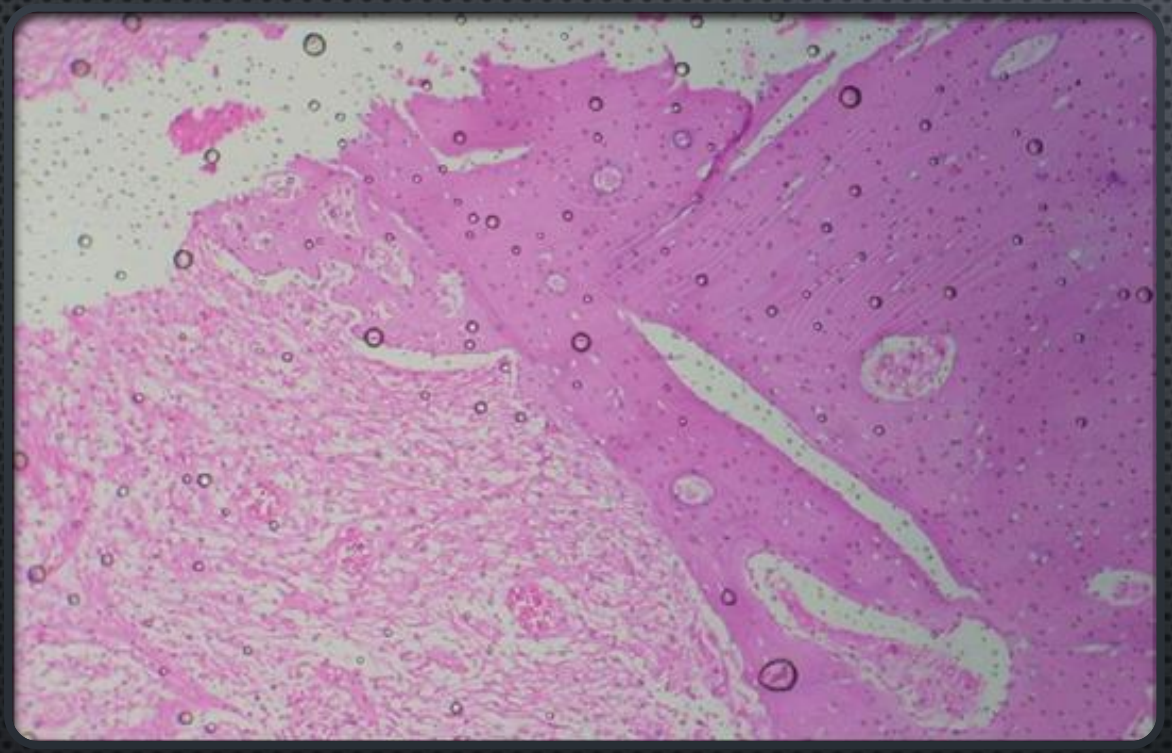
- UNDISSOLVED AND PRECIPITATED STAIN WILL LEAD TO DEPOSITION ON THE SECTIONS.

C) DUE TO INCOMPLETE OR UNSTAINED AREAS

- INADEQUATELY FILLED STAINING DISH OR ACCUMULATION OF STAINING SOLUTION AT THE TOP OF SLIDE WILL CAUSE SUCH ARTIFACTS [

- This micrograph shows a section of skin where the superficial epidermis and keratin have failed to stain with both hematoxylin and eosin because of residual wax. The final clearing of the section prior to cover slipping has removed all traces of the wax leaving no evidence of the cause of the patchy staining. Correct answer failure to stain because of residual wax on the section.





MOUNTING

Residual water and air bubbles

- Air bubbles are formed under the cover-slip when the mounting medium is too thin and as it dries, more air gets sucked under the edges. This can be prevented by using mounting medium of adequate thickness and removal of air bubbles from under the slide.

A) Dry mounting

- The slides should not be allowed to dry during the application of coverslip. An ERROR with the following three distinct features can be produced:
- Section may exhibit brown stippling which resembles pigment
- Highly refractile lines outlining cells and tissue
- Trapped air may be seen in the nuclei leading to dark nuclei lacking details (corn-flake artifact).

This error can be corrected by removing coverslip in xylene and placing in xylene for few minute and then remounting before drying the slide

B) related to excessive use of mounting media

- Excessive use of mounting media or the mounting media will result in foggy appearance. This error can be prevented by using adequate number of mounting media with proper consistency

POST ANALYTICAL

Internal reports (Ramsay Sime Darby medical center)

- All the internal reports need to be validated directly in the system (HMIS)within the timeframe and send it out to the respective physician.

External reports (besides Ramsay Sime Darby medical center)

- all the external reports need to be sort out accordingly and precisely based on the patient's details by our reference business center (RBC) staffs, also fax to the requesting hospital or physician.

CONCLUSION

Hence, proper handling of tissue pre-analytical, analytical and post-analytical phases will reduce the errors in histopathology.

THANK YOU