2nd Practical 422

✓Collection of specimen from the oral bucal cavity

✓Demonstration of Staining methods

Prepared By

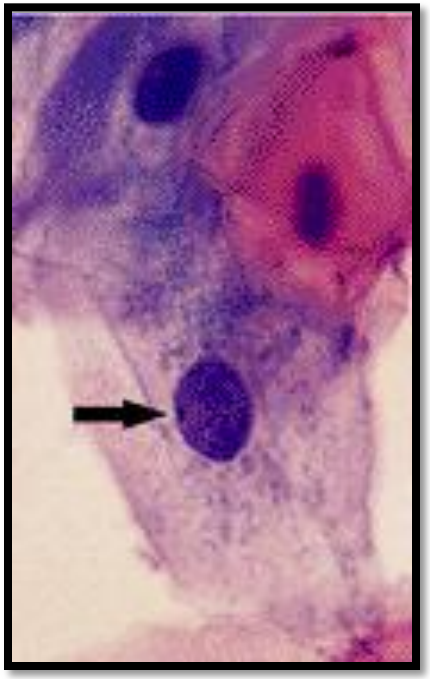
Jocelyn Palao and Layla Faqih

Supervised by

Dr. Sabah Ansar

ORAL CAVITY CYTOLOGY

*Purpose of the smear taken from the buccal cavity:

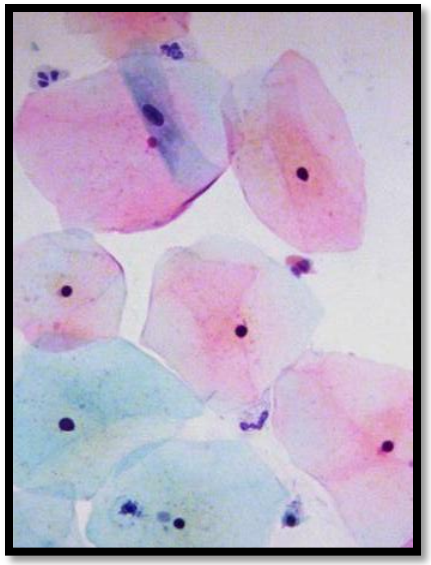
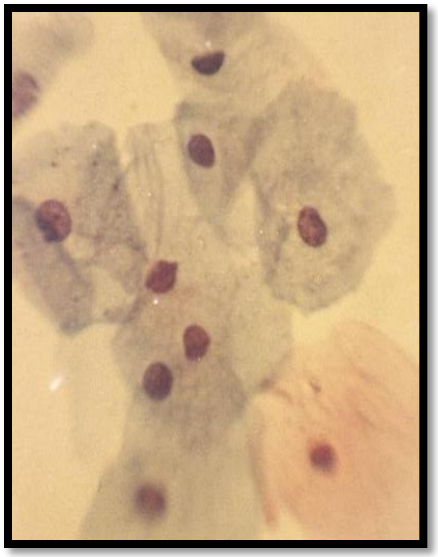
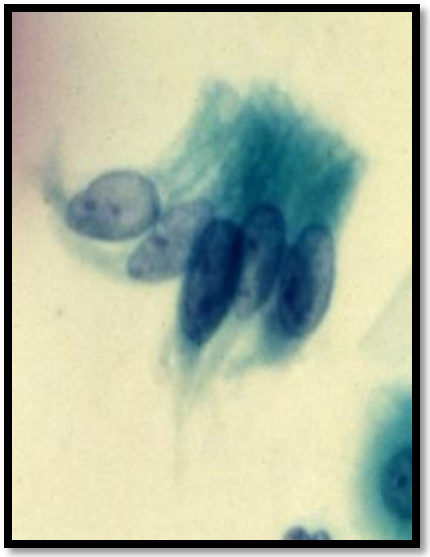


1. Buccal smear is useful for sex chromatin studies.
   - "Barrbodies" normally can be seen in female.
   - Abnormal in male sex chromosomal abnormality.
   - Barr bodies: oval in shape lying against nuclear membrane, usually affect intermediate squamous cells.
2. Early detection of precancerous lesions of oral cavity.
3. Diagnosis of inflammatory lesions of oral cavity.
4. Detection of the benign and malignant lesion of oral cavity.

*Sample collection technique:

- Scraping of oral (buccal) mucosa by using spatula.
- Smear fixed in 95% alcohol.
- Stain using the Papanicoloau staining method.

Normal cytology of the Oral Cavity:

1- Superficial Squamous epithelium	2-Intermediate Squamous epithelium	3-Annucleated squamous epithelium	4-Columnar cells from: A-Saliva secretion B-Salivary gland ducts
			

Progressive pap stain

Use:

1- For staining exfoliated cells in cytological specimens

2- Demonstrate the nuclear content.

***Summary and Explanation:**

The Papanicolaou Staining procedure is used for examining exfoliated cells in secretions, exudates, transudates or scrapings of various internal organs and tissue. Cells are fixed to a slide and stained first with Hematoxylin, which stains the nuclei followed by OG-6 and EA-50 or EA-65 as a counterstain.

*Reagents:

- **EA-50 Multiple Polychrome Stain, Filter before use.**
- **EA-65 Multiple Polychrome Stain, Filter before use.**
- **OG-6 Orange G Stain, Filter before use.**
- **Harris Hematoxylin, Filter before use.**

✓ **EA-50 and OG-6 were developed as general stains for vaginal smears. EA-65 is a modification of EA-50.**

Storage and Stability:

- **Store at 15°-30°C. Reagents are stable until expiration dates shown on the label.**

Warnings and Precautions:

- **Staining reagents, xylene and alcohol are flammable, skin irritants and toxic. Keep away from sources of ignition. In case of contact with eyes, rinse immediately with water and seek medical advice. May be fatal or cause blindness if swallowed. Dispose of waste in accordance with applicable laws.**

***Procedure:** **Filter the Harris Hematoxylin immediately before use**. (regressive stain)

1. Leave slide to fix with **5% alcohol**.
2. Dip slide(s) gently **5-10 times in** **95% ethanol.**
3. Dip slide(s) gently **5-10 times in** **70% ethanol.**
4. Dip slide(s) gently **5-10 times in** **distilled water**.
5. Stain **5 minutes in** **Harris Hematoxylin**.
6. Place smears **in distilled water**.
7. Rinse in successive changes of distilled water until the water remains colorless.
8. Dip slide(s) gently **5-10 times in** **70% ethanol.**
9. Dip slide(s) in a **1% solution of HCl in** **70% ethanol** until the smear shows a salmon colour.
10. Rinse slide(s) well in **2 changes of** **70% ethanol.**
11. Dip slide(s) gently in a 3% solution of ammonium hydroxide in **70% ethanol** until the smear takes on a blue colour.
12. Rinse the slide(s) in two changes of **70% ethanol**.
13. Dip slide(s) →→→**5-10 times in** **95% ethanol**.
14. Stain slide(s) in **OG-6 for** **2 minutes.**
15. Rinse slide(s) in two changes of **95% ethanol.**
16. Stain slide(s) in **EA-50 or EA-65 for** **3-6 minutes**.
17. Rinse slide(s) well in two changes of **100% methanol**.
18. Rinse slide(s) in one **part absolute methanol** one part **xylene.**
19. Clean **smear in xylene.**

Results:*

Nuclei are stained blue while cytoplasm displays varying shades of pink, orange, green and blue.

NOTE: Careful in dipping the slide in order not to dislodge the cells/ specimen.

Limitations:

Proper specimen collection and fixation of cells is essential for interpretation.

*Please Note:

1- The pap stain is useful polychromatic stain because it provides good differentiation of the various cellular components important for diagnosing of malignancy and for cell type classification.

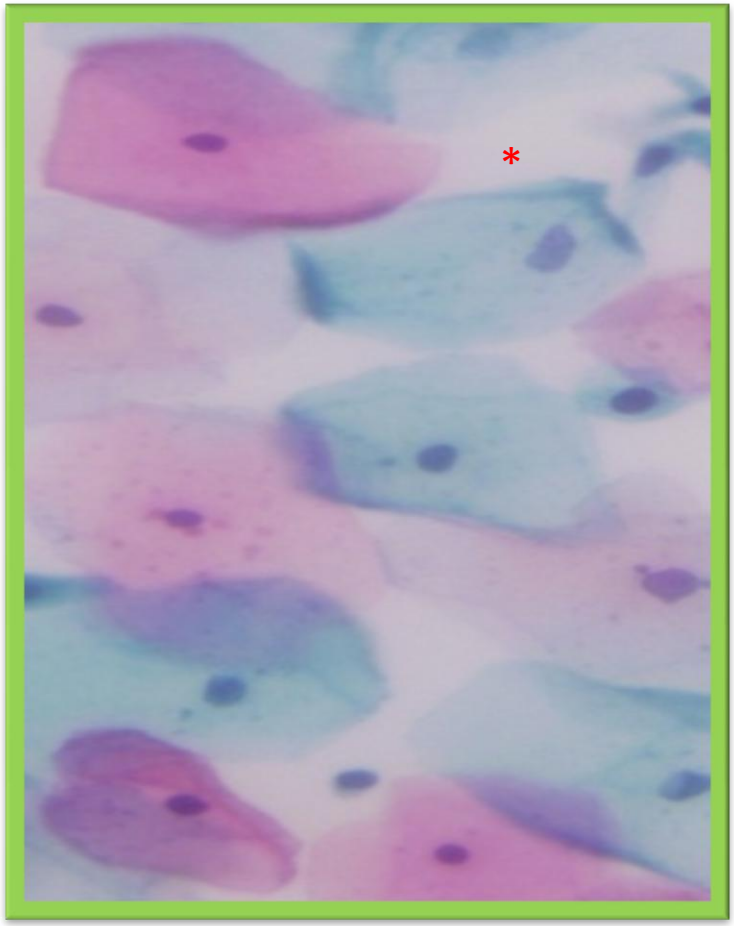
2- Specimen must be adequately fixed for optimal papanicolau staining, fixation in 95% ethanol, should be for at least 10 minutes prior to staining.

3- Gynecological smears that arrive spray – fixative, need to soak in 95% ethanol for at least 10 minutes to remove the spray fixative residues that may interfere with staining.

4- Any dipping of the slides in reagents/stain dishes must be gentle so not dislodge specimen; every effort be taken to minimize slide contamination.

5- The water rinse in immediately after hematoxylin stain should be in sink with gently running water to clear the background of excess hematoxylin. This step is important; and if neglegted or compromised, will lead to sub-optimal staining that is recognizable upon microscopic examination.

Smear taken from the buccal cavity



40 magnification

10magnification

Leukostat Diff – Quick staining

Use:

1- For cell and microorganism evaluation e.g. blood, body fluids and tissue aspirates

2- Demonstrate the cytoplasmic content.

***Summary and Explanation:**

Diff – Quick staining is a rapid method that stain air dried cytopreparations polychromatily within 4 sec. it is an ideal and permanent staining method allowing for immediate smear assessment for Non-Gyn or for FNA cases prior to patient discharge from the FNA clinic. This stain offers adequate differentiation of the various cellular components that are diagnostically important.

Reagents:

- **The first solution contains a fixative.**
- **The second contains a red stain.**
- **The third contains a blue counter-stain.**

Storage and Stability:

Store at 15°-30°C. Reagents are stable until expiration dates shown on the label.

***Warnings and Precautions:**

Staining reagents, xylene and alcohol are flammable, skin irritants and toxic. Keep away from sources of ignition. In case of contact with eyes, rinse immediately with water and seek medical advice. May be fatal or cause blindness if swallowed. Dispose of waste in accordance with applicable laws.

Procedure:

The staining time may differ depending on the cytopreparations stained and the stain results desired:

1. **Dip slide(s) with dried sample gently for few seconds in solution (1).**
2. **Rinse slide(s) in distilled water, and briefly allows excess stain and fluid to drain from the slide surface.**
3. **Dip slide(s) with dried sample gently for few seconds in solution (2).**
4. **Rinse slide(s) in distilled water, and briefly allows excess stain and fluid to drain from the slide surface.**
5. **Dip slide(s) with dried sample gently for few seconds in solution (3).**
6. **Rinse slide(s) the slide in distilled or tap water.**
7. **Air dries or blots the slide using care to avoid damaging the sample.**

Results:

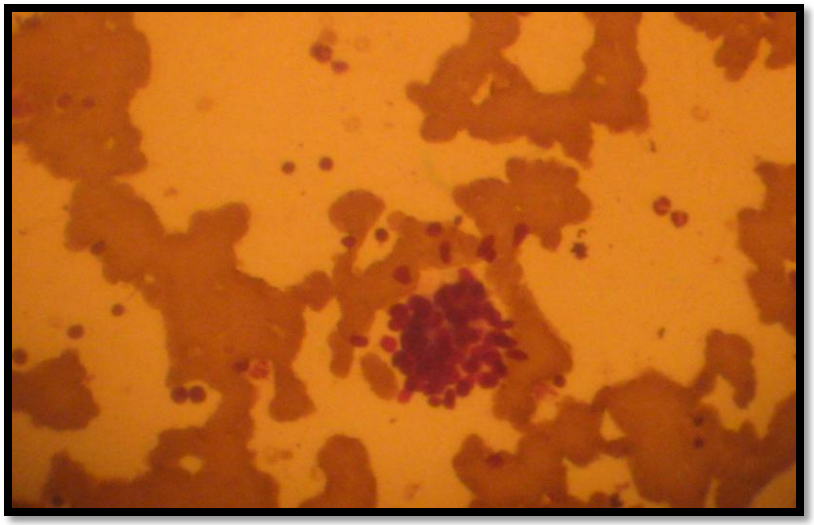
Nuclei are stained dark blue or violet while cytoplasm displays varying shades of red, pink and orange or blue and sky blue.

Limitations:

- **Smears must be completely Air-dried for adequate Diff-Quick staining.**
- **Smears must be thin for optimal stain penetration.**

*Quality control issue:

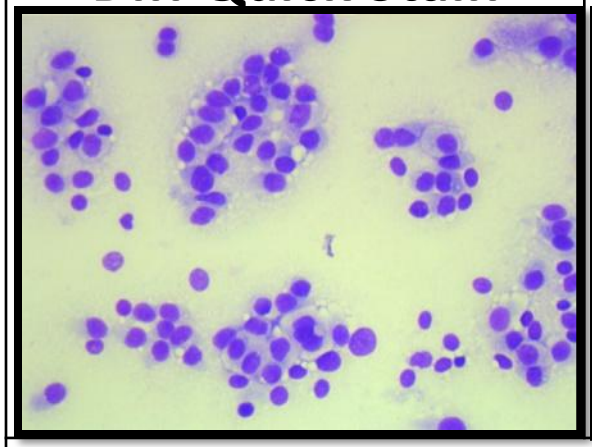
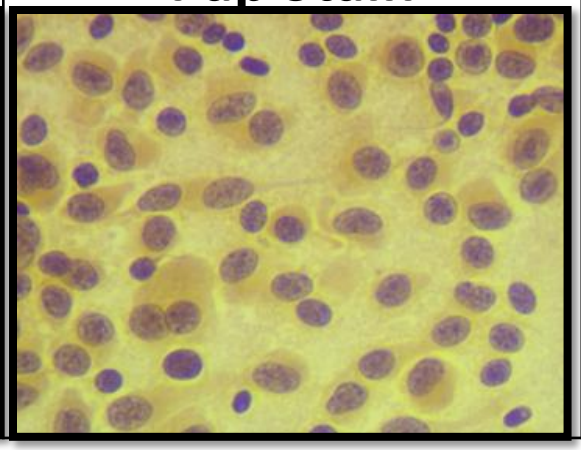
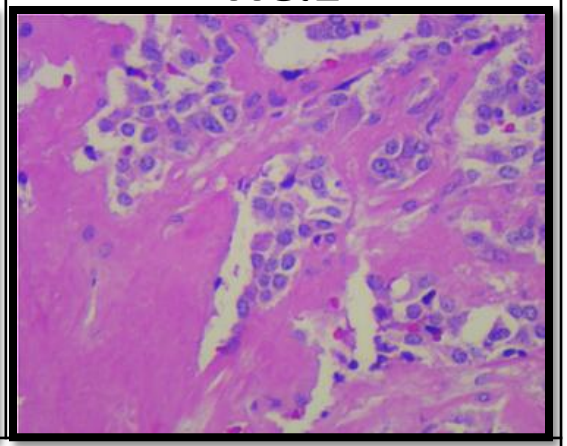
The Diff-Quick staining quality of Non-Gyn and FNA cyto-preparations is monitored daily and staining times are documented in the technical QC/QA schedule and log.



Breast FNA sample stained with Diff Quick Stain

H & E staining

- H & E staining is not perform in the cytology lab.
- Cytopreparations, for H & E staining are stain in the histology lab.
- Cell block sections are typically H & E stained.

Diff Quick Stain	Pap Stain	H&E
		
Medullary Carcinoma of Thyroid		

***Coverslippling procedure:**

For fixed slides:

- Wear protective gloves, work on clean, flat surface covered with towel.
- Coverslip should be clean, dust free.
- Place a drop of mounting medium on the coverslip, remove the slide from xylene and wipe excess xylene present under the slide with clean tissue or gauze.
- Gently place the slide (cellular material) over the coverslip at an angle, to allow for medium adherence.
- Excess medium that may ooze out of the slide edges can be wiped off with a xylene soaked gauze.
- Do not allow the cells to dry out at any stage.
- The end result should be a presentable slide with the coveslip centered over the cellular material, with no mountant on the coverslip surface, and no air bubbles.
- If air bubbles exist or if there is excessive amount of medium, remove the coversilp by soaking the coverslipped slide in xylene until the coverslip loosened. Repeat the procedure.
- Place coverslipped slides on the slide warmer for quick medium hardening.

Decolorizing slides for re-staining

- Cytopreparation requiring re-staining need to be decolorized without compromising cellular integrity.
- Reason for re-staining may be:

  a. inappropriate initial staining 2. suboptimal initial staining

  c. faded stain, or decolorization to re-stain slides with a different stain.
- Removing the coverslip properly, removing media and rehydrate the specimen are all important steps that need particular attention.
- Older slides with hardened mounting media require soaking in xylene for a long sufficient time for the coverslip to be removable.

***Decolorizing reagent 1% acid alcohol available in histology lab.**

Decolorizing methodology

- Remove coverslip by soaking in xylene,
- Rinse slide in xylene to remove all traces of mounting medium,
- Rinse slide in 3 changes of absolute alcohol to remove xylene,
- Rehydrate slides,
- Immerse slide in acid alcohol for several minutes, check microscopically ,
- Rinse the slide in distilled water once decolorized,
- Re-stain are required, re-coverslip.

Thank you