Study Of Romanowsky Stains Which One Is Better For Surface Epithelial Cells

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ABSTRACT

Our sole purpose in conducting this entire research was to determine which stain was most effective for surface epithelial cells. And for this, anyone who will do future research on surface epithelial cells should be able to easily stain the cells and perform microscopy very well. As a method, we had to apply different methods here because there are many types of Romanowsky stains , so we tried to do almost all of the Romanowsky stains that scientists used to do. After doing all the Romanowsky stains well, we saw that Diff-Quik gave the best results and the other stains gave but compared to them, the Diff-Quik results were the best. So after doing these alll stains,, we found that Diff-Quik staining stained the cytoplasm and nucleus of the cells the best. So finally we reached the point that Diff-Quik or Giemsa Stain is the best stain for surface epithelium cells.

Objective :- Here we have tested different types of Romanowsky stains on surface epithelial cells at LNCT University located on Kolar Road, Bhopal to see which one is more effective for surface epithelial cells and which of the stained slides looks best under the microscope. Here we have used different types of Romanowsky stains such as Diff Quick stain, May-Grunwald, Giemsa's, Jenner's, Leishman's, and Wright's stain. Because these trains are most effective for different types of epithelial cells. Among them, the Diff Quick stain were stained surface cells well.

Introduction :- Romanowsky staining is an early staining technique that served as the basis for several closely related stains now commonly used in hematology and cytopathology. The staining technique is named after the Russian physician Dmitri Leonidovich Romanowsky (1861–1921), who was one of the first to recognize its potential for use as a blood stain.

Different types of Romanowsky stains :-

Wright stain:- Wright's stain, often used on its own or in combination with Giemsa stain—forming what is known as the Wright-Giemsa stain—is named after James Homer Wright, who in 1902 developed a method involving heat to create polychromed methylene blue. This compound is mixed with eosin Y, and the resulting eosinate precipitate is redissolved in methanol. When Giemsa is added to Wright's stain, it enhances the brightness of the cytoplasmic granules' "reddish-purple" coloration. Both Wright's and Wright-Giemsa stains belong to the Romanowsky-type family of stains commonly employed in the United States, primarily for examining blood and bone marrow smears.

Jenner stain:- Jenner's stain, a dark blue dye, is used in microscopy to stain blood smears, producing clearly visible and well-defined nuclei.

Giemsa stain:- Giemsa stain consists of a combination of eosin Y and "Azure II," dissolved in methanol and glycerol. "Azure II" is believed to be a blend of methylene blue and azure B—referred to by Giemsa as "azure I"—though the precise makeup of "azure I" remains a proprietary secret. Similar formulations using well-characterized dyes have been published and are available commercially. Giemsa stain is widely recognized as the standard method for detecting and identifying malaria parasites.

May-Grunwald stain:- The May-Grünwald-Giemsa stain is employed in histopathology laboratories to stain slides prepared from fine-needle aspiration samples for the diagnosis of tumor cells.

Pappenheim stain:- This technique combines the May-Grünwald and Giemsa staining methods.

Leishman stain:- In 1901, William Leishman developed a stain similar to that of Louis Jenner, substituting pure methylene blue with polychromed methylene blue. Leishman's stain is made from the eosinate of polychromed methylene blue and eosin Y, with methanol serving as the solvent.
Fields stain:- Field stain is utilized for staining thick blood smears to detect malarial parasites.
Diff-Quik stain:- DQ stain, or Diff-Quik, is a commercially available Romanowsky-type stain commonly used for the rapid staining and differentiation of cells in various pathology samples, especially blood and cytological smears. Diff-Quik is an adapted form of the Wright-Giemsa stain that provides a quicker staining procedure and allows control over staining intensity by altering the duration the smear remains in the staining solutions.
We did this research for three months and while doing this research we found that among all the types of Romanowsky stains available, Diff-Quik stain is the most effective for surface epithelial cells.
Method and materials :- We did the full research at LNCT University and the stains we took for the research are listed below:-
At the very beginning of our research, we collected samples, then stained the epithelial cells with wright stain and then did microscopy, the results of which I have given here. Then all the staining were done one by one and finally the results came out. Some stains stained the cells very well, while some stains did not stain as well as others, but there were a couple of stains that stained the cells very well. Of all the stains, Wright-Giemsa and Leishman stains stained the cells the best.

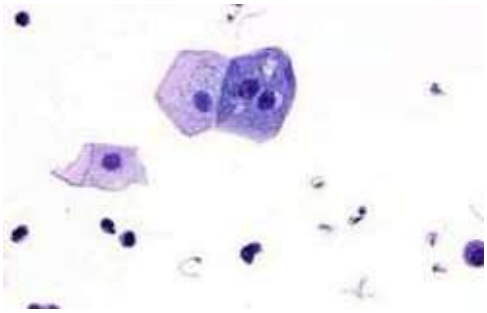

Fig:- Wright-Giemsa stain on epithelial cells and blood film.

Along with tissue cells, we have also tested some samples of blood cells, among which, no doubt, Leishman stain is the best, but when we studied tissue cells, Leishman stain stained better, but Giemsa is better then Leishman stain, if we compare the two, then Giemsa stains better. If we say which stain is most effective for surface epithelium, then according to our research, Giemsa is better.
Result:- We are perform different Romanowsky stains on epithelial cells for this research, like Giemsa, Wright, Leishman, and May-Grünwald stains. These stains are all based on a combination of eosin (an acidic dye) and methylene blue or its derivatives (basic dyes), allowing them to differentially stain cellular components.
In this staining competition, the results of Giemsa stain were the best, so we can say that Giemsa is the best among the Romanowsky stains for epithelial cells.

Perform all Romanowsky stains manually and results:-
Wright stain:-
Principle:- Wright stain is a Romanowsky-type stain, primarily used in hematology and cytology. Wright's stain is a polychromatic stain used to differentiate and visualize cells. It contains a combination of eosin (an acidic dye) and methylene blue (a basic dye).Eosin (acidic dye) stains basic (alkaline) components, such as the cytoplasm and hemoglobin, in shades of pink to red. Methylene blue (basic dye) stains acidic components, such as nuclei and RNA, in shades of blue to purple.

Reagents:-
Wright's Stain:-A commercially available solution containing a mixture of dyes, including methylene blue and eosin Y, which stains the cell components.
Buffered Water/Phosphate Buffer (pH 6.4-6.8):- Used to dilute the stain and adjust the pH for optimal staining.
Methanol (Fixative):- Used to fix the smear, preventing cell damage and ensuring proper staining.
Distilled/Deionized Water:- Used for rinsing the stained slide.

Procedure:-

1. Smear Preparation: Create a thin smear of cell on a clean glass slide and let it air dry.
2. Fixation: Briefly fix the dried smear in methanol to preserve cell structure.
3. Staining: Apply Wright's stain to cover the smear and let it sit for about 1–3 minutes.
4. Dilution and Mixing: Add an equal amount of buffered water to the stain and gently mix by blowing across the slide.
5. Staining Time: Allow the mixture to remain on the slide for an additional 5 minutes to complete the staining process.
6. Rinsing: Gently rinse the slide with distilled or deionized water to remove excess stain.
7. Drying: Leave the slide to air dry thoroughly.
8. Observation: View the stained smear under a microscope, starting with low power and progressing to high power magnification.

Result:-

- Nuclei (rich in DNA) :- take up the methylene blue and appear blue to purple.
- Cytoplasm:- may take on shades from light blue to pink, depending on the cell type and maturity

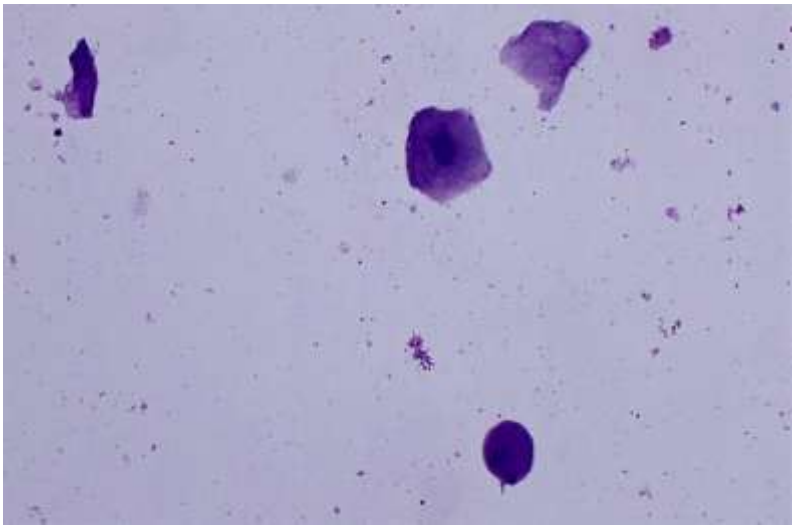

Fig:- Wright's stain on epithelium cells

Giemsa stain:-

Principle:- Giemsa stain is a Romanowsky-type stain, which contains a mixture of methylene blue, eosin, and azure B. These dyes interact with cellular components based on their chemical nature. Methylene blue and azure B are basic dyes that bind to acidic structures (like nucleic acids in the nucleus).Eosin is an acidic dye that binds to basic components (like cytoplasmic proteins).

Reagents:-

1. Methylene blue
2. Azure B
3. Eosin
4. Methanol
5. D/W

Procedure:-

1. Fix the air-dried smear in methanol for 3–5 minutes.
2. Place the fixed slide in the diluted Giemsa stain.Stain for 15–20 minutes at room temperature.
3. Gently rinse the slide with buffered water or distilled water until excess stain is removed.
4. Allow the slide to air dry completely in a vertical position.
5. Then observe on microscope.

Result:-

- Nucleus: Dark blue to purple

- Cytoplasm: Pale blue to pink
- Keratinized cells: Pinkish cytoplasm

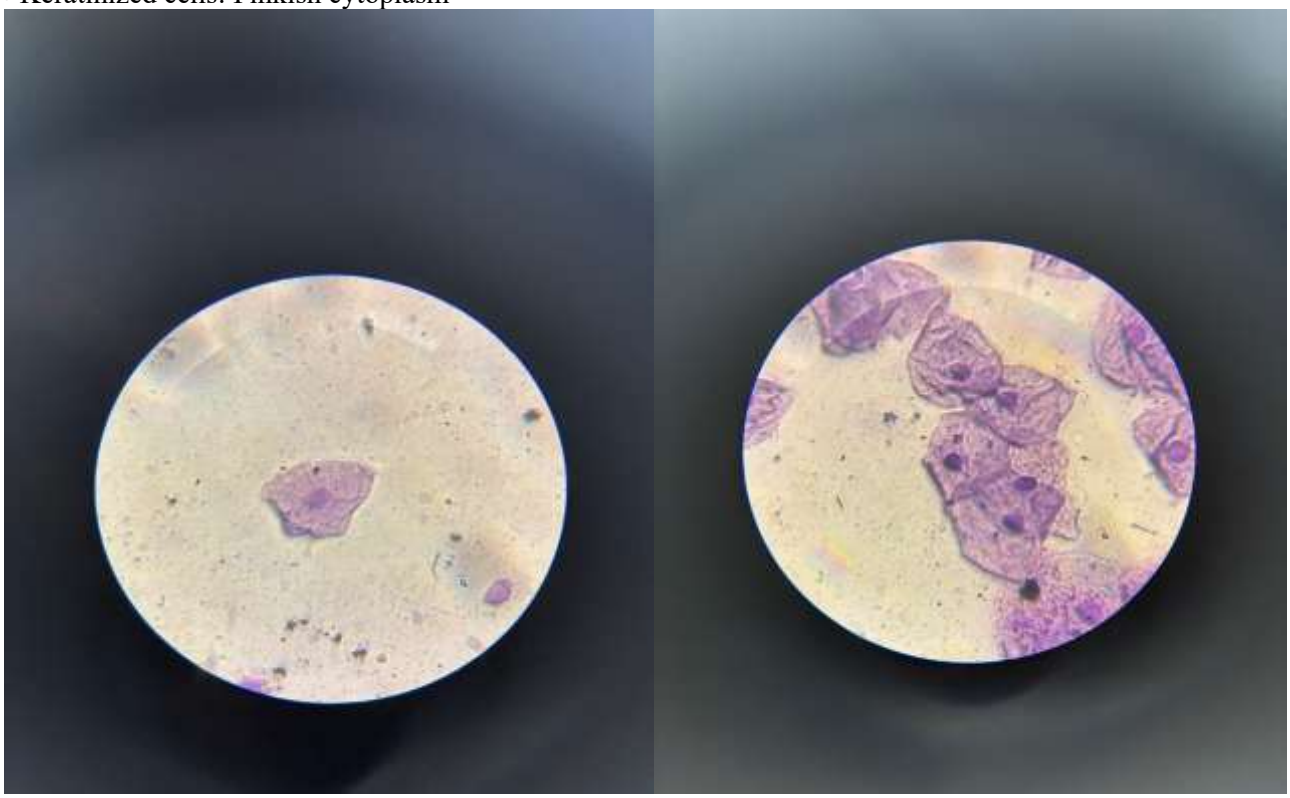

Fig:- Giemsa stain on epithelial cell.

Leishman stain:-

Principle:- Leishman stain is a Romanowsky-type stain, similar to Giemsa, and it is primarily used for blood smears but can also be applied to epithelial cells (e.g., buccal smears) for nuclear and cytoplasmic differentiation.

Reagents:-

1. Methylene blue
2. Eosin Y
3. Methanol (100% alcohol)

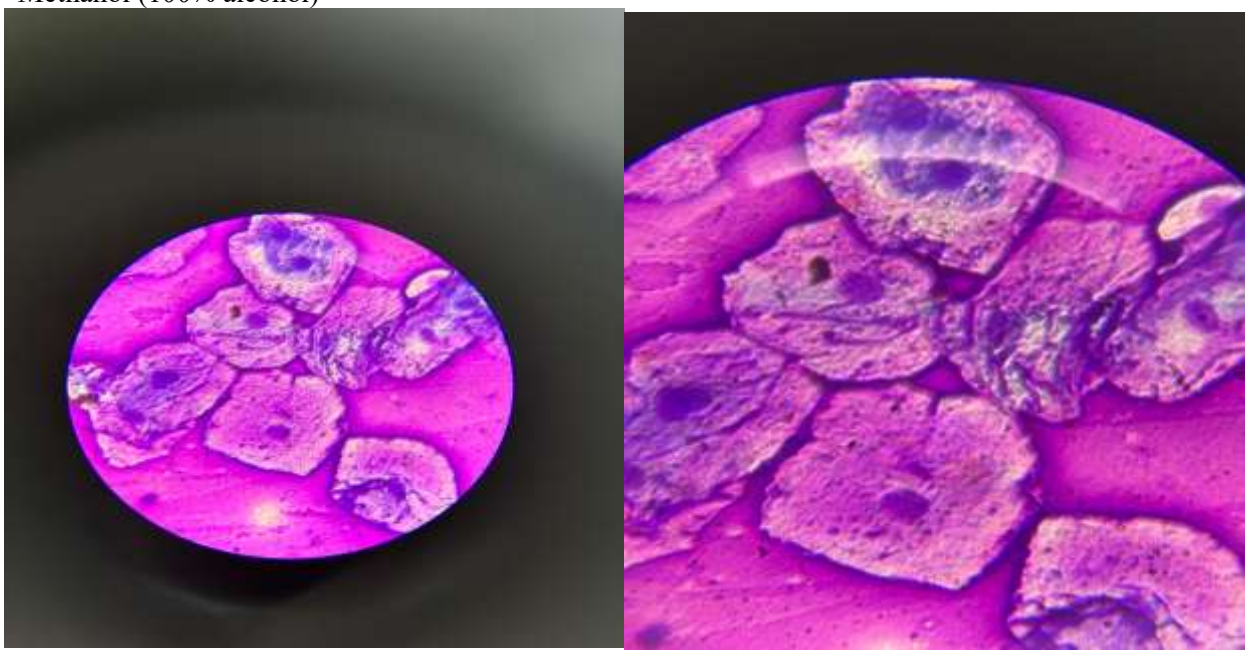

Fig:- Leishman stain on epithelial cell

Procedure and result:- air dry the smear and then stain on leishman stain for 4 to 5 minutes and then add 100% alcohol for 10 minutes. Then air dry and seen on light microscope. Nucleus purple and cytoplasm pink.

Comparison summary:-

Feature	Giemsa stain/ Diff Quik stain	Wright/Leishman stain	Jenner stain	Fields stain
Nuclear detail	Excellent	Better	Good	Good
Cytoplasmic clarity	High	Moderate	Moderate	Slightly moderate
Use in epithelial cytology	Preferred	Less preferred	Less preferred	Less preferred
Time for staining	Longer (20 min)	Shorter (2-5 min)	Shorter (2-5 min)	Shorter (1-2min)
Diagnostic utility	Higher in non-hematologic	Primary hematologic	Primary hematologic	Primary hematologic

After performing all the stains, we did very fine microscopy and in the microscopy we saw that in all the stains we did, the nuclei and cytoplasm of the giemsa stained cells were very clearly visible and could be identified well. So for this reason we have identified Giemsa stain as the best of all the stains are perform here.

Discussion:-

Giemsa stain has superior nuclear detail and chromatin pattern definition, which is essential for distinguishing different epithelial cell types and identifying atypia or malignancy.Epithelial cell cytoplasm stains a characteristic light blue to grey-blue, and the nucleus stains dark purple, allowing clear contrast.Giemsa can highlight glycogen or mucopolysaccharide-rich cytoplasm in epithelial cells better than Wright or Leishman.This is helpful in distinguishing superficial squamous cells (which have a clear or vacuolated cytoplasm) from intermediate or parabasal cells.While Wright and Leishman are faster and used more commonly in hematology (blood films), Giemsa provides higher fidelity staining at the cost of time. For diagnostic cytology, especially when surface epithelium is being examined, quality is preferred.

So for these features, giemsa stain colored the epithelial cells very well and stained the nucleus and cytoplasm parts of the cells very well and clearly, which is why we have come to the conclusion that Giemsa is the best stain among all the Romanowsky stains available.

Conclusion:-

Giemsa stain is the preferred Romanowsky-type stain for identifying surface epithelial cells, due to its superior nuclear detail, cytoplasmic differentiation, and ability to highlight diagnostic features in cytology. While other Romanowsky stains like Wright or Leishman are quicker and more common in hematology, they lack the fine resolution required for epithelial cell analysis.

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