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PAPANICOLAOU STAIN - REVIEW

Pathology

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ABSTRACT

Pap stain was developed by George Nicholas Papanicolaou , the father of cytopathology in 1947. Pap stain is a polychromatic stain containing multiple dyes to differentiate cells in smear preparations of various bodily secretions. It is used for cervical cancer screening programme in gynaecology to detect precancerous and cancerous lesions of the cervix, the entire procedure is known as pap smear. It is also used in oral cancer screening programmes. Since the time of its invention, it has undergone various modifications from conventional method from regressive to progressive. It has undergone further modifications to rapid, UFP(ultra fast pap stain), MUFP(modified ultra fast pap stain), Enviro-pap(environmentally friendly, inexpensive pap stain) and REAP(Rapid, Economic, Acetic acid, PAP Stain). Each method has its own advantages and disadvantages. Each laboratory has its own staining protocol which should be standardized.

KEYWORDS

Conventional pap stain, Pap smear, Rapid pap stain, RAEP, UFP, MUFP, Enviro pap.

INTRODUCTION

Papanicolaou stain is a multichromatic staining cytological technique developed by George Nicholas Papanicolaou, the father of cytopathology. Since the time of its invention by papanicolaou, pap stain undergoes several modifications.[1] Pap stain is the most important stain utilized in the practice of cytopathology. It is a polychromatic stain containing multiple dyes to differentiate cells in smear preparations of various bodily secretions. The specimens can be gynaecological smears, sputum, brushings, washings, urine, cerebrospinal fluid, ascitic fluid, pleural fluid, synovial fluid, seminal fluid, fine needle aspiration material, or other materials containing cells.

Pap staining is a very reliable technique, it is used for cervical cancer screening programme . The entire procedure is known as pap smear. Pap smear test has decreased the incidence of cervical cancer by 70% in the developed countries. It is also used in oral cancer screening programmes.

DEVELOPMENT OF PAP STAIN

Until 1933, papanicolaou analyzed squamous epithelial cell morphology initially in guinea pigs and later in human vaginal fluid samples using haematoxylin and eosin staining, correlating cellular morphology with endocrinology and histology. Development of the pap stain method followed two salient historical phases.

The first was 1933-1942, during which alcohol ether fixation and aqueous water blue staining was introduced to make cells transparent, aiding the distinction between cervical cancer cells and benign cells. The second was 1942-1960, when progressively refined alcoholic cytoplasmic counterstaining variations using OG and EA(light green, Bismarck brown, eosin, and PTA) improved cellular transparency, producing polychromasia and optimizing cell type differentiation.

OBJECTIVES OF PAP STAIN

Papanicolaou described three chief objectives for staining of cytological smears:

- Definition of nuclear details : Good staining of nucleus is primarily important because of the widespread nuclear abnormalities of cancer cells and their diagnostic significance.
- Transparency of cytoplasm: This is important because of the varying thickness and frequent overlapping of cells.
- Differentiation of cells: Differences in the staining reaction such as that between acidophilic and basophilic cells help greatly in the identification of certain cell types.

PRINCIPLE OF PAP STAIN

Papanicolaou stain includes both acidic and basic dyes. Acidic dye stains the basic components of the cell and basic dye stain the acidic components of the cell. The classic form of pap stain involves five dyes in three solutions.[2]

- A nuclear stain, haematoxylin, is used to stain cell nuclei. The unmordant haematin may be responsible for the yellow colour of glycogen.
- 0G-6 counterstain (-6 denotes the used phosphotungstic acid concentration) stains keratin.
- EA (Eosin azure) counterstain, comprising three dyes: Eosin y, Light green and Bismarck brown y. eg: EA-36, EA 50, EA65 (number denotes the proportion of the dyes).

i. Eosin Y stains the superficial epithelial squamous cells, nucleoli, cilia and red blood cells.
ii. Light green SF yellowish stains the cytoplasm of other cells, including non keratinizing squamous cells like intermediate squamous cells, parabasal cells, leukocytes undifferentiated carcinoma cells and cells from adenocarcinoma.
iii. Bismark brown Y stains nothing . it precipitates phosphotungstic acid, the ingredients are responsible for differential staining by light green and eosin Y.

EA 36/50 has two times more light green than EA 65, hence EA36/50 is preferred for gynaecological smears and EA 65 is used for non gynaecological smears.When performed properly, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, intermediate and parabasal cells are green to blue.

PAP STAINING PROCEDURE

Both progressive and regressive nuclear staining techniques can be used in pap stain. Before staining, wet fixation immediately with cytology spray fixative 96% ethanol is required. In progressive method , the nucleus is stained with haematoxylin to a intensity desired. The intensity of nuclear staining is controlled by the immersion of slide into a blueing agent. Most commonly used blueing agent is scott's tap water. Mayors or Gills haematoxylin is used. In regressive staining method, the nucleus is deliberately overstained with a non- acidified haematoxylin (Harris haematoxylin). The excess stain is removed with dilute hydrochloric acid solution. The progressive method is the most commonly used method in the laboratories.

Conventional pap staining procedure:

It takes 20-30 minutes. Harris haematoxylin without acetic acid is preferred. the conventional pap stain procedure has 24 solutions, in which three are acid solutions, six water, one is blueing agent, eight 95% alcohol, three absolute alcohol and three xylene.[3,4] The steps are as follows:

1. Fixation of the slide – 10-20 minutes
2. 95% ethanol – 15 seconds
3. 80% ethanol – 15 seconds
4. 70% ethanol – 15 seconds
5. 50% ethanol – 15 seconds
6. 0.5% HCL solution – 1-2 dips
7. Distilled water – 10 dips
8. Distilled water – 10 dips
9. Harris haematoxylin – 6 minutes
10. Distilled water – 15 seconds
11. Distilled water – 15 seconds

12. Bluing in tap water – 15 seconds
13. 50% Ethanol – 15 seconds
14. 70% ethanol – 15 seconds
15. 80% ethanol – 15 seconds
16. 95% ethanol – 15 seconds
17. 0G 6 – 2-3 minutes
18. 95% Ethanol – 10 dips
19. 95% Ethanol – 10 dips
20. EA 36/50 – 2-3 minutes
21. 95% Ethanol – 10 dips
22. 95% Ethanol – 10 dips
23. 100% Ethanol – 10 dips
24. Alcohol : xylene (1:1) – 10 dips
25. Xylene – 10 dips
26. Xylene – 10 dips
27. Mount with DPX.

RAPID PAP STAIN

The conventional pap stain procedure is a time taking procedure, requiring atleast 30 minutes. To cut down the time, the rapid pap stains were developed by Kline, Tao and Sato with respective staining time of 4 minutes, 5 minutes and 90 seconds with fixation time of 1-2 minutes.[1]

However the quality of rapid stains is usually not as satisfactory , as they show sub-optimal cell morphology and still require wet fixation which is time consuming and requires substantial amounts of ethanol which is expensive and need license and renewal .[3] Pap stain is expensive. In addition alcohol and xylene are inflammable solutions and has to be stored and dispose safely.

ULTRA FAST PAPANICOLAOU SATIN(UFP)

To overcome the problems of conventional and rapid pap staining procedures, Yang and Alvarez developed Ultra fast Pap stain which is a hybrid of the technique by Romanowsky and conventional pap stain, to reduce the staining time to 90 seconds.[5,6,7]

Three modifications were made : first modification - rehydration of air dried smears with normal saline which restored the transparency of the cells and hemolyzed red blood cells. Second- use of a 4% formaldehyde/65% ethanol fixative which reduced the time for proper fixation and staining from minutes to seconds. Third – use of Richard-Allan haematoxylin 2 and cyto satin which simplified the procedure. Richard Allan cytostain consists of alcoholic mixture of orange G, Eosin Y, light green and aniline blue.

The advantages of UFP are, it gives cytoplasmic transparency, crisp nuclear details, clear background, and it avoids cell loss as in wet fixation.[6,7] UFP stain especially helpful in diagnosing tumours with distinctive nuclear features, like thyroid tumours, malignaNt lymphoma. It is also preferred for intra-operative cytology.[6,7]

The disadvantages of UFP stain are Richard-Allan haematoxylin and cytostain are not available universally. 95% ethanol is expensive. To overcome these problems modified UFP is developed.

MODIFIED ULTRA FAST PAPANICOLAOU STAIN (MUFP)

Kamal et al. from india further modified the UFP stain to overcome the problems like shortage supply of Richard Allan haematoxylin, cytostain and 95% ethanol in Indian set up.[6,7] He used gills haematoxylin, modified EA (alcoholic mixture of Eosin Y, Light green, Phosphotungstic acid and glacial acetic acid without orange G) and isopropyl alcohol. This method takes 130 seconds respectively. OG is omitted because it causes nuclear, cytoplasm and background orange discolouration. The advantages of MUFP are staining solution can be prepared from locally available reagents, no wet fixation, no cell loss as in wet fixation, no drying artefacts, short turn around time, clear background, cells appear large with crisp morphological features, no deleterious effect on immunophenotyping. Hence it is preferred for FNAC smears(thyroid and lymph node pathology) and for intra-operative cytology.

The disadvantages of MUFP are cytoplasmic keratinisation cannot be interpretated due to lack of Orange G, inadequate air drying causes suboptimal results and solution is storage sensitive. Universal standardization is recommended, as the locally available solutions may influence the results.

Kamal from india further modified the MUFP by replacing the gills haematoxylin with Harris haematoxylin which is easily available in India. It does not alter the staining quality and give equally good results.[6,7]

ENVIRO-PAP

Enviro-pap is an environmentally friendly, inexpensive pap stain developed in 1995. Enviro-pap stain uses water instead of 95% ethanol to remove carbowax from spray-fixed slides, plain tap water for bluing, xylene indefinetly with water scavenging beads and daily filtration. It doesn't use graded alcohols. Therefore enviro-pap is eliminating eight 95% ethanol baths, store-bought bluing agents and xylene disposal. It can also be easily implemented in other cytology labs and even can be extend to histopathjology lab. It decreases the lab annual staining reagent purchase. These cost savings will vary fromlab to lab, depends on its local pap stain practices staining volume and reagent purchase.[3,,8,9]The only disadvantage of enviro-pap is overstaining in OG, which can be easily prevented by carefully limiting the staining time.

REAP STAIN

Rapid Economic Acetic acid Papanicolaou technique (RAEP) was introduced by S.B Dighe in 2005. As the name implies it is a rapid technique(staining time is 3-4 minutes), economical, acetic acid is used as a dehydrant and colour preservation. Hence the REAP staining technique is superior than that of standard PAP.[1,10]

In REAP preheated haematoxylin was used, acid differentiation step was discarded and 1% acetic acid acts as a nuclear fixative and also intensifies the intensity of staining. Therefore nuclear staining in REAP was better than standard Pap stain.

REAP staining has many advantages like excellent cytoplasmic and nuclear staining intensity, cost effective, long term colour preservation and rapid method. The disadvantages of REAP are , it gives sub-optimal staining in thick smears and inferior staining quality with poor preservation for longer periods.

CONCLUSION

The pap stain was first developed in 1942 and since then it has undergone various modifications from conventional method to rapid, UFP, MUFP, Enviro-pap and RAEP. Each method has its own advantages and disadvantages.

REFERENCES

1. Asthana A, S.A.(2014). Comparision of routine Papanicolaou staining technique with the rapid, economic, acetic acid, papanicolaou technique (REAP). IJMDS 3(2), 484-489.
2. Carson , Freida L; Hladik, Christa(2009). Histotechnology: A Self-instructional Text (3 ed.). Hong Kong: American Society for Clinical Pathology Press. Pp.361-3363. ISBN 978-0-89189-581-7.
3. Gill , G.W.(2006). Enviro-pap: an environmentally, friendly, economical, and effective pap stain. Laboratory medicine 37, 105-108.
4. Catherine M Keebler M.F.(2008). CYTOPREPARATORY TECHNIQUES. IN: Masluce Bibbo, David C Wilbur (Eds). Comprehensive cytopathology, 3rd edition (Philadelphia: saunders (elsevier))
5. Yang, G.C., Papellas, J., Wu, H.C., and Waisman , J. (2001). Application of UFP stain to body fluid cytology. Acta cytological 45, 180-185.
6. Choudhary P., Sudhamani, S., Pandit,A., and Kiri , V.(2012). Comparision of modified ultra fast papanicolaou stain with the standard rapid papanicolaou stain in cytology of various organs. Journal of cytology 29,241.
7. Kamal, M., Bodele, A., Munshi, M.,Bobhate, S., and Kher, A.(2000). Efficacy of a modified ultra Fast Papanicolaou stain for breast aspirates. Indian journal of pathology & microbiology 43, 417-421.
8. Bales, C.E,(2006). Laboratory techniques, in: Leopold G koss, Myron R Melamed (Eds). Koss" Diagnostic cytology and its histopathologic basis, vol 2,5 th edition.(Philadelphia: Lippincott Williams& Wilkins).
9. Izhar , S., Kaur, R., and Masih, K.(2014). Efficacy of rapid, economical, acetic acid, papanicolaou stain in cervical smears as an alternative to conventional papanicolaou stain. Journal of cytology/ Indian academy of cytologists 31,154.
10. Roy biswas , R., Paral, C.C., Dey, R, and Biswas, S.C.(2008). REAP – Is it suitable alternative to standard PAP stain..