P is for Papanicolaou

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.

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Welcome to our A-Z of Staining series where we continue with the letter P for the Papanicolaou (Pap) stain. Developed in 1942 by George Papanicolaou, this polychromatic stain is one of the most widely used techniques in cytology. Employing several dyes, the stain is able to differentiate both acidophilic and basophilic components of cells. The method is commonly used for oral and cervical screening in asymptomatic populations, and the ability of it to detect cancer cells has secured its position as the most celebrated method in cervical screening programmes worldwide.

During his career, Papanicolaou published three modifications of his stain, with the classic form incorporating a number of dyes in three solutions. While the first solution contained the basic nuclear stain haematoxylin, the second included the acidic dye Orange G (OG-6) which stained the cytoplasm of mature and keratinized squamous cells. This solution also contained phosphotungstic acid (denoted by the number 6 in OG-6) which was added to adjust the pH and help optimize colour intensity. The third and final solution was Eosin Azure (EA), an alcoholic mixture containing the dyes Eosin, Light (or Fast) Green and Bismarck Brown. With this solution, the Eosin stained the cytoplasm of superficial epithelial squamous cells pink to red while the Light Green (another acid dye) stained the cytoplasm of intermediate squamous cells and other active cells green to blue (Figure 1).

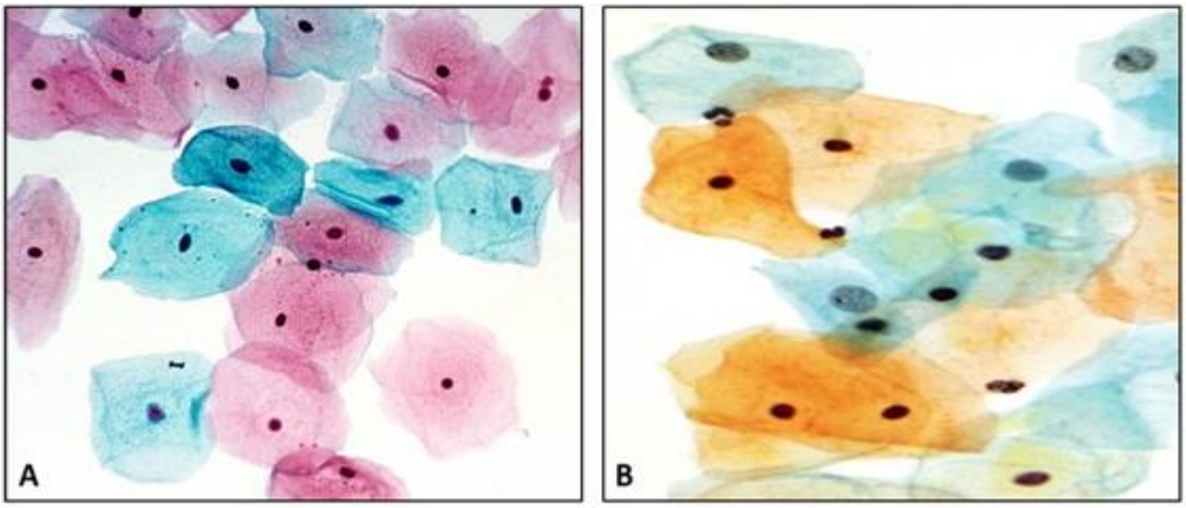

Figure 1. Pap stains showing red superficial and green to blue intermediate cells (A), with mature and keratinized squamous cells staining orange (B)

In solutions of EA, the quantity of Light Green varies between formulations. In EA-65 for instance, the concentration of the dye is half that of the EA-36/50 solution, the preferred formulation for cervical smears. Although EA-65 is able to differentiate endocervical adenocarcinoma cells from normal endometrium, this formulation is the desired solution for non-gynaecological cytology. Finally, the third component of the EA mixture is the diazo dye Bismarck Brown which is responsible for the differential staining of the Eosin and Light Green components. However, when Bismarck Brown is used in combination with phosphotungstic acid, it causes both to precipitate out of solution, thereby reducing the useful life of the formulation. Since Bismarck Brown does not actually stain anything, it is often omitted in current formulations. Although numerous modifications of the classic Pap stain are available, the sequence of events during staining of normal and abnormal smears remains the same: Haematoxylin to stain the nuclei blue, Orange G to stain the cytoplasm of keratinized squamous cells, and EA solution to stain the cytoplasm of koilocytic and superficial squamous cells pink to red, and intermediate squamous, parabasal and endocervical cells green to blue (Figure 2).

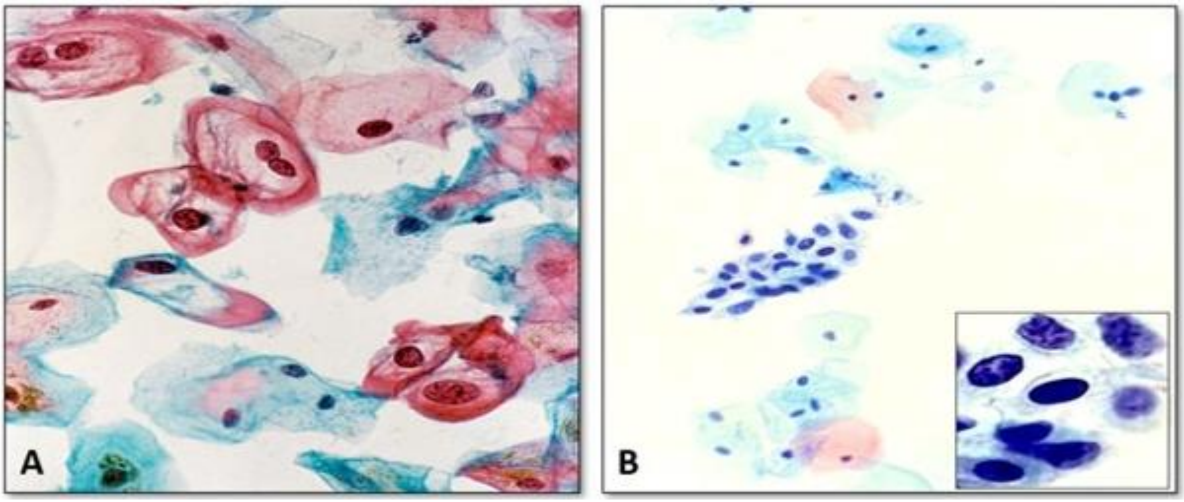

Figure 2. Pap stains with koilocytes showing perinuclear vacuolation and peripheral cytoplasmic condensation (A) and a group of dysplastic cells (B)

The Papanicolaou stain has undergone many changes since its first formulation in the 1940s. From the classical stain through to the rapid and ultrafast hybrid modifications offered as alternatives, the list is endless. While the Pap smear remains the most popular screening method for detecting cervical cancer and precancers, the uptake of conventional cervical screening programmes, particularly in developing countries, is poor. A recent global study revealed that there are more than one billion women of screening age who have never been screened for cervical cancer. With the World Health Organization proposing to eliminate the disease globally by 2030, they have recommended the use of vaginal self-sampling kits for home use to expedite the screening uptake. With the advent of other technologies such as liquid based cytology (LBC), HPV testing and computer-assisted systems for screening Pap stained smears, there is every likelihood that Papanicolaou will be with us for generations to come.

Further reading

1. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide (Bruni et al). The Lancet Global Health 2022;10(8): E1115-E1127. https://doi.org/10.1016/S2214-109X(22)00241-8

2. Evolution of Pap stain (Raju K). Biomedical Research and Therapy 2016;3(2):490-500. https://www.academia.edu/es/71512500/Evolution_of_Pap_Stain_Evolution_of_Pap_Stain

3. Nuances of the Papanicolaou stain (Sathawane P et al). CytoJournal 2022;19:43. https://dx.doi.org/10.25259/CMAS_03_18_2021

4. Pap smear-based cervical cancer detection using hybrid deep learning and performance evaluation (Kalbhor M et al). Computer Methods in Biomechanics and Biomedical Engineering: Imaging & Visualization 2023. https://doi.org/10.1080/21681163.2022.2163704

5. Rapid, economic, acetic acid Papanicolaou stain (REAP): An economical, rapid, and appropriate substitute to conventional Pap for staining cervical smears (Goel G et al). Journal of Cytology 2020;37(4):170-173. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7984519/

6. Ultra-fast Papanicolaou stain versus conventional Papanicolaou stain in oral cytology smears: A comparative study (Swetha RK et al). Journal of Oral Research and Review 2022;14(2):99-103. https://dx.doi.org/10.4103/jorr.jorr_70_21

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