e-ISSN: 3030-878X

The 4th International Conference on Medical Laboratory Technology (ICoMLT)

An Effect of Variations in Time of Using Harris Hematoxylin on Papanicolaou Staining

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ABSTRACT
Cytology is the science that studies the morphology of individual cells or cells from tissue fragments that are observed microscopically. This branch of science, especially cytopathology, is very dependent on the quality of the results of cytol-ogy preparations for the validity of the diagnosis. One of the stains used in cytol-ogy is the Papanicolaou stain, which is very important for the early detection of oral and cervical cancer. This stain used Harris hematoxylin, which is able to provide very clear nuclear details. The aim of this study was to identify the effect of variations in the time of use of Harris hematoxylin on Papanicolaou staining. The variable tested in this study was the staining time using Harris hematoxylin solution at intervals of 3 minutes, 5 minutes, 7 minutes, and 10 minutes. Obser-vations were made on the quality of the resulting staining, which was measured based on the clarity of the cytoplasm and cell nuclei. The method used is analyti-cal observation using a cross-sectional design. The population in this study were undergraduate students of applied medical laboratory technology, using 1 sample with a total of 24 repetitions. The results showed that variations in coloring time had a significant effect on the quality of Papanicolaou coloring. Based on re-search carried out within 7 minutes, the results showed that the cytoplasm was stained light pink and the dark blue cell nuclei were clearly visible. The conclu-sion is that there was an influence on the staining results on oral mucosa exami-nation with variations in the time of use of Harris hematoxylin for Papanicolaou staining.

Keywords: Harris Hematoxylin, Papanicolaou Staining, Time Variations

INTRODUCTION

Cytological examination aims to determine the morphology of cells, cell nuclei and cytoplasm, so as to provide a complete picture of the morphological state of the cells examined. Specimens used in cytology are urine, sputum, oral mucosa, pleura fluid, ascites, female genital mucosa, and tumor tissue aspiration. Oral cytology examina-tion is a microscopic examination of cells scraped from the mucosal surface of the oral cavity. The advantages of cytology examination are that it is easy, painless, fast, and does not cause bleeding. Cytological examination can determine the maturation index of epithelial cells and identify abnormal changes in epithelial cells ranging from mild dysplasia to more severe conditions such as carcinoma in situ. In cytology there are various kinds of coloring, namely, hematoxylin eosin, papanicolaou, immuno-histochemistry, and giemsa. The cytological stain used is papanicolaou. In various preparations for body secretions, this coloring is used to distinguish between cells. In addition, it is an early detection of oral cancer and cervical cancer that does

not show symptoms. Papanicolaou stain is one of the most commonly used stains during cyto-logical examination because it can color the cell nucleus very clearly, making it easier to see the cell nucleus in case of possible malignancy. This stain stains the cytoplasm using a highly contrasting comparator color, making it possible to see overlapping cells. The papanicolaou staining process is carried out in five stages, namely fixation, nuclear staining, cytoplasmic staining, clarification, and mounting. An important stage in making cytology preparations is staining. Staining aims to facilitate observa-tion using a microscope and distinguish morphological parts to be observed such as cell nuclei and cytoplasm. In this study the dye to be used is Harris hematoxylin. In the coloring stage, different times are used between one process and another. At the staining stage, the nuclear dye used is Harris hematoxylin. Harris hematoxylin is an alum hematoxylin that can be used for progressive staining of cytology specimens because it tends to provide very clear nuclear details with dark blue or purple cell nuclei staining. Staining stages can be influenced by the length of time of painting. The length of time in this painting process can determine the quality of the coloring results. Therefore, this research was carried out to clearly know the difference in vari-ations in the length of time the painting is done the results obtained are better or not so that it affects the quality of the coloring.

RESULT AND DISCUSSION

The results of the study of the quality of staining of oral mucosal smears with varia-tions in the time of use of Harris hematoxylin in Papanicolaou staining in the laboratory Cytohistotechnology of the Faculty of Health Sciences University of Muhammadi-yah Surabaya using 24 slides of oral mucosal smears with 4 different treatments. As show in Table 1.

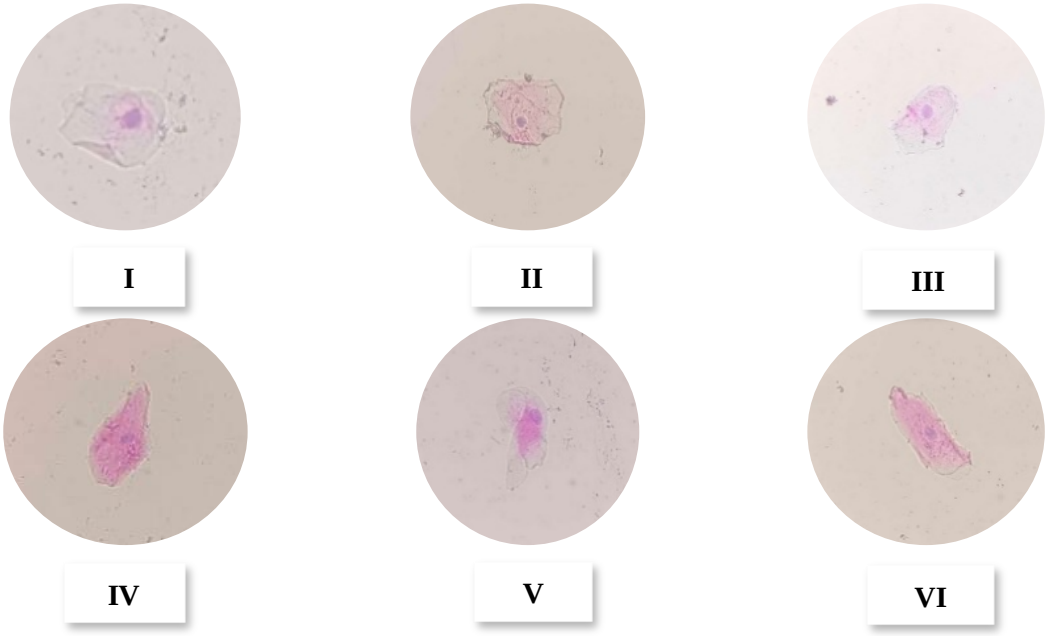
Table 1. Assessment of preparation quality in each treatment

No	Replication	Assessment score of oral mucosa staining results			
		3 minutes	5 minutes	7 minutes	10 minutes
1	I	2	2	3	3
2	II	1	1	2	3
3	III	2	2	3	2
4	IV	3	1	3	2
5	V	2	2	3	3
6	VI	3	2	3	2

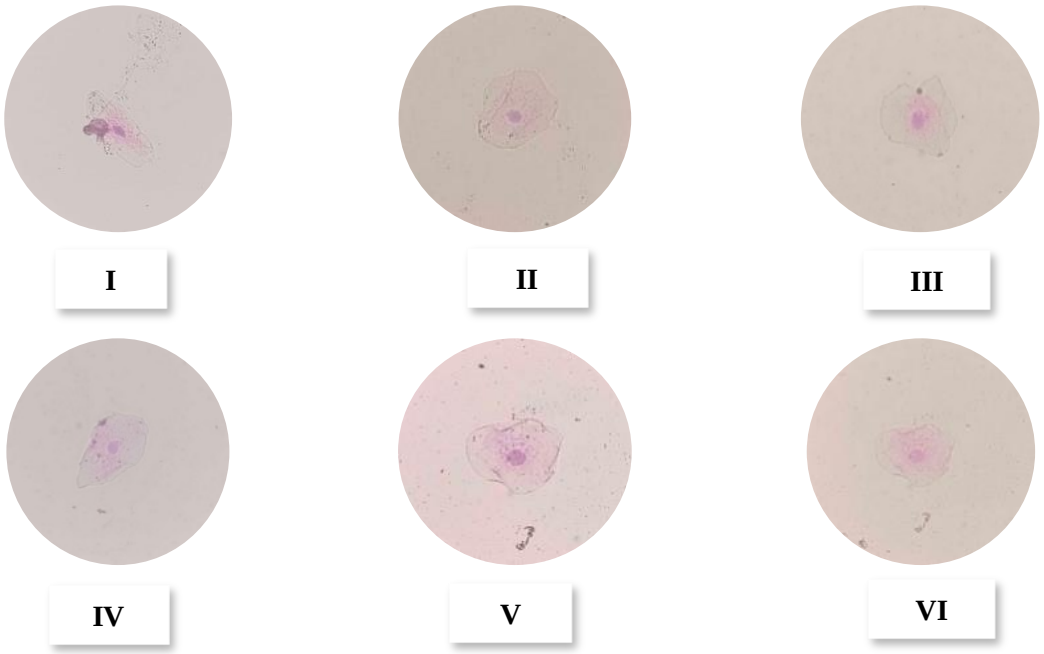
Description of assessment criteria based on an ordinal scale (Astuti, 2017):
Score 1: Cell shape is not clear, the intensity of the color of the cytoplasm is not clear, the intensity of the nucleus is not clear (not good).
Score 2: Cell shape is less clear, cytoplasm color intensity is less clear, intensity in the nucleus is less clear (not good).
Score 3: Clear cell shape, clear cytoplasmic color intensity, clear nucleus intensity (good).

A good preparation is that the shape of the flat epithelium is not faint, the intensity of the cytoplasm is pink and the dark blue cell nucleus is clearly visible. Based on the results of the study, it was found that at 3 minutes there were 4 unfavorable preparations and 2 good preparations found in numbers IV and VI, at 5 minutes 6 oral mucosa preparations had unfavorable criteria, at 7 minutes 5 preparations were obtained that met the good criteria while 1 preparation was not good at number II, and at 10 minutes 3 preparations were good and 3 preparations were not good at numbers III, IV, and VI.

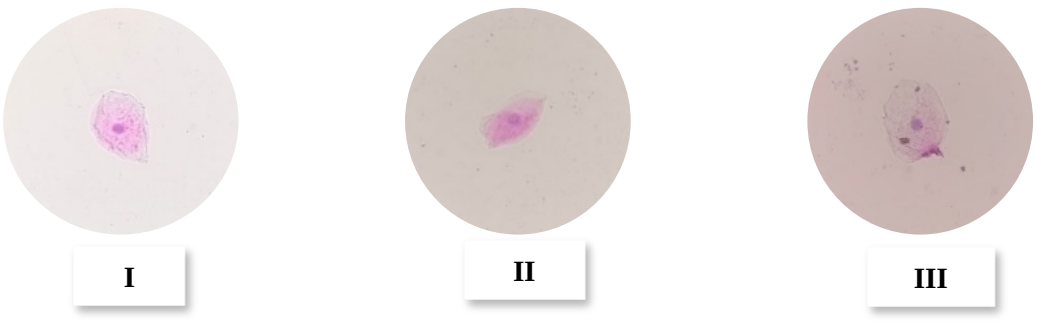
Coloring with 3 minutes



Coloring with 5 minutes



Coloring with 7 minutes



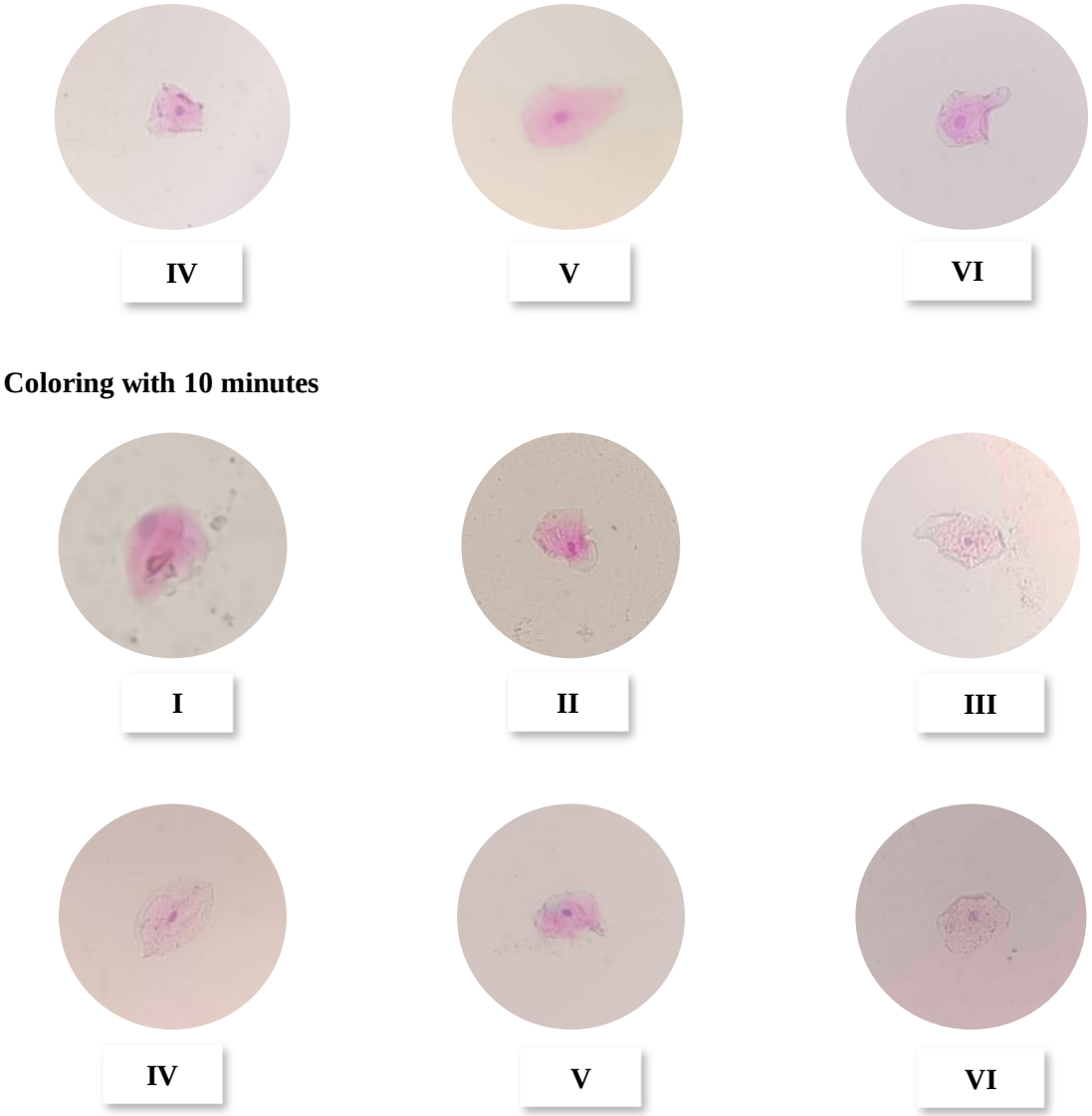


Figure 1. Results of Oral Mucosal Preparation Examination

In research on oral mucosal preparations, good accuracy from researchers is very important considering that this examination is a manual method examination. To minimize errors in this study, examination of the results of staining of oral muco-sal preparations in various time variations was carried out with 6 repetitions in 4 treatments. Several other factors that can affect the quality of the staining results of oral mucosal preparations include the technique of making oral mucosal prepara-tions, human resources (skills and accuracy of researchers), improper painting pro-cess, the quality of the reagents used does not meet the quality of good paint. Based on Figure 1, it shows that coloring time of 3 minutes there are 4 preparations that are not good because the color of the cytoplasm is not clear and the cell shape looks pale, coloring time of 5 minutes gives bad preparation results because the cytoplas-mic staining and cell shape are less clear, pale, and uneven, coloring 7 minutes there are 5 good preparations because the cell nucleus is purple and not pale and the cyto-plasm is pink or pink, coloring time of 10 minutes each 3 preparations are good and 3 preparations are not good resulting in optimal cell nucleus staining with minimal staining on the cytoplasm. The results of

this study are in accordance with existing theory that the results of coloring preparations using different times will give better results. Staining with the specified time there are 4, namely, 3 minutes, 5 minutes, 7 minutes, and 10 minutes so that when staining the cell shape in the preparation is clearly visible, but if the coloring time is too fast, the cell shape is not clearly visible and the color intensity in the nucleus is not how visible, and vice versa if the coloring time is too long it will make the preparation better because the cytoplasm is clear and the nucleus is visible, so that the staining produced at the time of microscopic exami-nation of the staining results is very good. Therefore, a time of 5 minutes is consid-ered unfavorable because it can decrease the contrast between the nucleus and cyto-plasm, making it difficult to distinguish between the two structures. While the time of 7 minutes according to research conducted by Iqbal (2019) shows a balance between staining the cell nucleus and cytoplasm resulting in optimal staining.

CONCLUSION

Based on the results of this study, it can be concluded that there is an effect of time variation on the results of oral mucosa preparations and the appropriate time for the use of Harris hematoxylin in Papanicolaou staining is 7 minutes. This research can be used as a source of information and learning in the field of cytology. Future research is expected to use variations in the coloring technique.

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