

Comparison of Pap Smear Preparation Quality Containing Orange G and Without Orange G on the Results of the Papanicoulou Painting

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ABSTRACT

Examination procedure for pap smear preparation is influenced by fixation, drying and painting, and interpretation capability of examination. The Papanicolaou painting uses Harris Hematoxylin, Eosin Azure (EA) -50, and orange G. Orange G colors the orange matured and keratin cells Keratinizing-pink/orange cells. Chromatin in the nucleus will bind alkaline paint (hematoxylin) and cytoplasm protein will bind acidic paint (Orange G) and nucleus in the core will bind acid paint (EA 50) so that the cell will turn into pink with a blue core. The coloring stage according to procedure is using Orange G, but some PA laboratories do not use Orange G. The research goal is to know the comparison of pap smear preparation quality which contains orange G and without orange G towards papanicoulou painting result. The research type is experimental; the sample was 16 samples with two treatments namely coloring which using Orange G and without Orange G. The preparation quality which contains Orange G obtained moderate result of 12.5%, and good 87.5%. The preparation quality without Orange G was not good result of 6.3%, moderate 18.8%, and good 75%. The statistical test result of Chi Square and Kappa showed that the result was $p > 0.05$ which means that comparison result of preparation quality which contains orange G and without orange G there was no significant difference.

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INTRODUCTION

Pap smear is a clinical procedure for examining cells from the cervix (Maria, Zahra, & Sara, 2013). The main purpose of a Pap smear examination is to assess abnormal cell changes which may originate from cervical cancer of before it develops into cancer (pre-cancerous lesions). (Ackerson, Pohl, & Low, 2008). This examination requires special skills from the examiner and examination material taken from the endocervix and actocervix (Manuaba, 2005).

Pap smear test provides a classification of cell cytology that is useful to find out the extent of infection of Human Papillomavirus (HPV) (Danico & Sugiharto, 2019) so that it is beneficial to determine treatment or further medical treatment. When conducted with a very good procedure, the accuracy of pap smear test sensitivity is between 76% - 94%. Pap smear examination should be done every year. Women aging 30 years old or more with the normal results of the examination as many as three times may do the test again every 2-3 years, however women with high risk have to do the test every year. (Barut, Kale, & Kuyumcuoğlu, 2015)

The accuracy of the pap smear is influenced by human resources, procedures of taking inspection materials, procedures of examination, and women or patients who should undergo the screening. Procedure of examination is influenced by the way of fixation, drying and painting, and inspection interpretation capabilities. (Richardson, Tota, & Franco, 2011). Some things that must be considered in making cytological preparations include the number of cells in the specimen, the viscosity of the specimen, and the technique for making cytological preparations (Mach, Adeyiga, & Di Carlo, 2013).

Papanicolaou reagents consist of Harris Hematoxylin, Eosin Azure (EA) -50, and orange G. Harris Hematoxylin used for staining cell nuclei in black or dark blue. Eosin Azure (EA) -50 used for coloring the cytoplasm of squamous superficial cells into pink (non keratinizing squamous cells will be blue/green). Orange G colors mature cells and orange keratin,

Keratinizing cells – pink / orange. The chromatin in the nucleus will bind to alkaline paint (hematoxylin) and the cytoplasm protein will bind to acidic paint (Orange G) and the nucleus in the nucleus will bind to acidic paint (EA 50) so that the cell will be pink with a blue core. According to the procedure the coloring phase with Papanicolaou uses Orange G, but some Anatomical Pathology laboratories do not use it (Tan, Tatsumura, & Papanicolaou 2015; Choudhary, 2012).

Anatomical Pathology Laboratory of Rumah Sakit Umum Daerah (RSUD) Kardinah, Tegal received pap smear examination from Kardinah Hospital, private laboratories, as well as from midwives. Samples in the form of wet preparations with 96% alcohol fixation. Pap smear examination using Papanicolaou staining helps differentiate and analyze morphology and pathology in cervical mass samples. Painting according to the procedure using Orange G is expected to check the quality of a good pap smear preparation. (Asthana, 2014). Painting without Orange G is often forced to be done because the reagent runs out while the doctor asks for an examination to be done immediately. This encourages the authors to conduct research that aims to determine the comparison of the quality of the pap smear preparations containing orange G and without orange G on the results of Papanicolou painting.

MATERIALS AND METHODS

Inspection material is taken from cervical / vaginal smear. The sample is a wet preparation with 96% alcohol fixation as many as 16 sample. All samples came from the Kardinah Regional Hospital, Private Clinical Laboratories, and Midwives. The researcher got permission from the head of the laboratory installation in the Kardinah Tegal Regional Hospital. The material is made into preparation with Papanicolou painting using Orange G and without Orange G. Papanicolaou reagents consist of Harris Hematoxylin, Eosin Azure (EA) -50, and orange G. The difference in

the quality of the paps smear using orange G and without orange G was analyzed using the chi square test.

RESULT AND DISCUSSION

Research conducted on 16 pap smear preparations, each sample received two painting treatments, using Orange G and without using Orange G. Quality preparations are categorized as bad, moderate, and good. The criteria to

determine a “Bad”, “Moderate”, and “Good” quality of examination results based on readings by an anatomical pathologist, is said to be bad if gray/pale superficial cells, gray / pale cytoplasm, and gray or pale cell nuclei. Moderate quality if intermediate cells, pink/purple cytoplasm disguised, and light blue cell nucleus. Good quality if blue parabasal cells, cytoplasm of bright pink/purple, and dark blue cell nucleus. The results of the study are presented in the following Table 1 and Figure 1.

Table 1. Description of the Quality of Pap Smear Preparations Using Orange G and without Orange G Against Papanicolaou Painting.

Variabel	Quality of Examination Result						p value
	Bad		Moderate		Good		
	N	%	N	%	N	%	
Using Orange G	0	0	2	12,5	14	87,5	0,12
Without Using Orange G	1	6,3	3	18,8	12	75	

The table above states that the examination results of pap smear preparations for Papanicolaou painting with good quality were with Orange G 87.5%, and without Orange G 75%. Moderate Quality using Orange G was 12.5% of the samples, and without Orange G was 18.8%. Bad quality was found 6.3% in painting without Orange G . Using Orange G provide more results of good quality than those of painting without Orange G. Moderate and not good quality is more common in painting without Orange G. Chi square statistical test obtained p value of 0.12.

The following pictures are the results of research on preparations of good, moderate and not good quality. Figure 1 shows the good quality preparations, characterized by among others (1) blue parabasal cells, (2) cytoplasm of bright pink/purple, (3) dark blue cell nucleus. Figure 2 shows moderate quality preparations characterized by among others (1) intermediate cells, (2) pink/purple cytoplasm disguised, and (3) light blue cell nucleus. Figure 3 shows the poor quality preparations, characterized by among others (1) gray/pale superficial cells, (2) gray/pale cytoplasm,

(3) gray or pale cell nuclei.

Papanicolaou staining is a multicromatic histological staining technique developed by George Papanikolaou. Papanicolaou staining is used to distinguish cells in smear preparations from gynecological smears (Pap smears), sputum, urine, cerebrospinal fluid, pleural fluid, synovial fluid, seminal fluid, aspiration material, tumor touch samples, or other materials containing cells. Papanicolaou staining technique known as Pap smear is a very reliable technique for screening cervical cancer in gynecology. (Suvarna et al., 2015; Pawliszyn, 2012).

The ingredients used for the coloring of Papanicolaou are haematoxylin, Orange G, Eosin Azure counterstain, Eosin Y stains, Light Green SF yellowish. All the ingredients contained in Papanicolaou coloring have different functions. Orange G functions to color keratin, coloring small cells of keratinized squamous cell carcinoma. In well prepared specimens, Orange G will dye the high content of keratin to yellow and also glycogen to yellow. (Aldrich, 2020; Izhar et al., 2014)

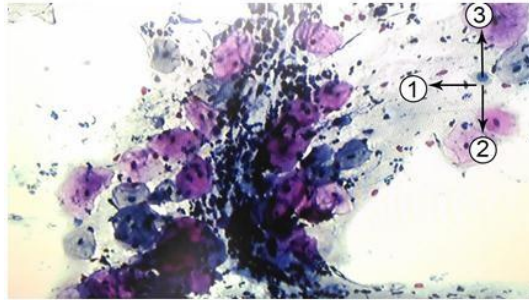


Figure 1. Sample of Good Quality Preparation

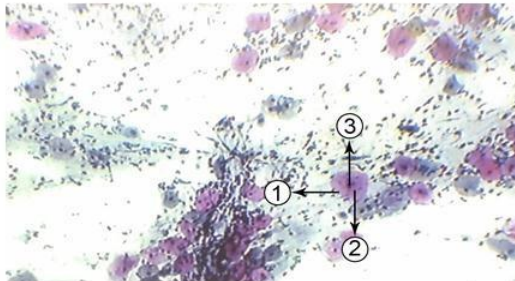


Figure 2. Sample of Moderate Quality Preparation

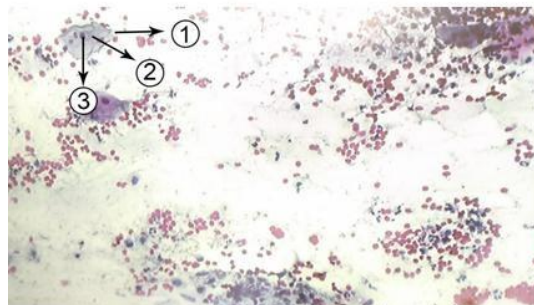


Figure 3. Sample of Poor Quality Preparation

The ingredients used for the coloring of Papanicolaou are haematoxylin, Orange G, Eosin Azure counterstain, Eosin Y stains, Light Green SF yellowish. All the ingredients contained in Papanicolaou coloring have different functions. Orange G functions to color keratin, coloring small cells of keratinized squamous cell carcinoma. In well prepared specimens, Orange G will dye the high content of keratin to yellow and also glycogen to yellow. (Aldrich, 2020; Izhar. et al., 2014)

The results of a comparative study of the quality of pap smear preparations showed the results of good quality were found more on the preparations using Orange G than those without using Orange G. The statistical test results concluded that there was no meaningful comparison of the quality of preparations using Orange G and without using Orange G. Different assessment of the quality of preparations occurred in preparation number 12, and preparation number 14. Good quality on

preparation number 12 was shown using Orange G, but without Orange G the quality of the preparation is not good. Good quality on preparation number 14 was shown when using Orange G, but without using Orange G the quality of preparation is medium.

The existence of one poor quality preparation of pap smear without orange G can be caused by many influencing factors. These factors include fixation, drying, painting, and the examiner's ability to interpret the results. Errors during fixation include smears that have dried before fixation. Orange G is acidic opponent paint. The use of Orange G in making preparations serves to brighten so that the cells will be seen clearly (Raju, 2016).

CONCLUSION

The research on the comparison of Pap smear preparations quality on the results of Papanicolaou painting were concluded as follow; quality of preparations containing Orange G obtained was moderate yield of 12.5%, and good yield 87.5%, quality preparations without Orange G. obtained was poor results 6.3%, moderate results 18.8%, and good results 75%, the results of statistical tests on the quality of Pap smear preparations containing Orange G and without Orange G using Chi Square and Kappa showed $p > 0,05$ which means that the comparison results between the two variable differences are not significant.

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