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Comparison of Nathan alcohol formalin substitute fixative solution with 10% acetone-neutral buffered formalin in the preparation of cell blocks in ascitic fluid samples

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***Abstract*---**Cytology is one of the fields related to the study of the morphology of individual cells or cells derived from microscopically observed tissue fragments. Ascites is an abnormal collection of fluid in the peritoneal cavity. The peritoneal cavity contains 50-100 ml of serous fluid . In this study, the method used is the cell block method by doing a comparison between cell blocks fixed with NAFS and cell blocks fixed with acetone-NBF with an assessment of the quality of cytology preparations based on Hematoxylin-Eosin staining according to the criteria in the scoring table, namely scores 1 (not good), 2 (poor), and 3 (good). Good fixation will produce good quality preparations to be assessed by pathologists. The purpose of this study was to determine the comparison of fixative solution of nathan alcohol formalin substitute with 10% acetone-neutral buffered formalin in the manufacture of cell blocks in ascitic fluid samples. This type of research is quantitative research with experimental research design. The results showed the Wilcoxon test with a significance value ($p<0.05$), which means that there is a comparison of fixative solution of nathan alcohol formalin substitute with 10% acetone- neutral buffered formalin in the manufacture of cell blocks in ascitic fluid samples.

***Keywords*---**Cytology, a site, cell block, fixation.

Introduction

Cytology is one of the fields related to the study of the morphology of individual cells or cells derived from microscopically observed tissue fragments. Whether or

not a diagnosis is correct depends on the quality of the resulting cytological preparations. Meanwhile, to produce good cytological preparations, the quality of material preparation to be used as preparations must be known correctly (Erick & Dewi, 2017).

Ascites is an abnormal collection of fluid in the peritoneal cavity. The peritoneal cavity contains 50-100 ml of serous fluid. The peritoneum secretes serous fluid that lubricates the peritoneal surface and allows movement between the two layers of the peritoneum (Shikha, 2014). Ascites can cause symptoms such as abdominal distension, abdominal pain, early satiety, and respiratory, gastrointestinal and urinary system disorders (Ahmed & Stenvers, 2013).

The cell block method is a solid made from a concentrated specimen with the principle of gel or coagulation which is then followed by the process of tissue maturation and embedding and staining as in histology. In addition to cell assessment, cell blocks can also be used for other advanced cytological examinations such as immunocytochemistry and tumor molecules. The disadvantage of the cell block method is that the results of the diagnosis cannot be established in a short time when compared to conventional outlining because the work on the cell block takes longer than conventional outlining. But the method that is often chosen for cytological examination is cell block because it is easier to process and is good for use in samples with low cellularity, and it is possible to add sections without staining from cell blocks (Inderiati & Pratiwi, 2021).

Fixation is the basic step behind the study of pathology and is essential to prevent autolysis and degradation of tissues and cells and tissue components so that they can be observed both anatomically and microscopically. The main purpose of fixation is to keep cells and tissue components in a "life-like state". (Musyarifah & Agus, 2018). Fixative solutions in the manufacture of cell blocks are agar gel, nathan alcohol formalin substitute (NAFS), or plasma-thromboplastin. NAFS fixative solution consisted of 9 parts of 100% ethanol and 1 part of 40% formalin. NAFS is a fixative solution that is widely used for the manufacture of cell blocks because it is easier to obtain than the other two fixative solutions. In the anatomical pathology laboratory there are several fixative solutions that are widely available, one of which is neutral buffered formalin (NBF). 10%. NBF 10% is a fixative solution that is often used for tissue fixation and is widely available in anatomical pathology laboratories (Fajrina & Ariyadi, 2018). Another fixative solution is acetone, acetone has the same action as alcohol and has been used as a fixative and dehydrator in cell and tissue processing, especially manual rapid processing of small specimens. Acetone, also known as dimethyl ketone or 2 propanone, is an important compound of aliphatic ketones. Acetone was first produced by dry distillation of calcium acetate (Intani, 2012).

Method

The materials used in this study were ascitic fluid samples, acid alcohol, hematoxylin Harris, eosin phloxin, EA 50, OG 6, lithium carbonate, 0.4% HCl, entelan, paraffin, xylol, 70% alcohol, acetone, neutral buffered formalin. (NBF)

10% ready to use, 100% ethanol, 40% formalin, tube rack, centrifuge tube, centrifuge, tweezers, pipette, test tube, Waterbath, label, pencil, microtome, cover slip, slide, microscope, Excelsior AS, Histostar Embedding Module, base mould, Histostar Cold Module, cassette, hot plate.

This study uses quantitative research with an experimental research design where research is carried out on the manufacture of ascitic fluid cell blocks using nathan alcohol formalin substitute (NAFS) fixative. with acetone- neutral buffered formalin (NBF) 10%. The method of data collection in this study used a solution of primary data and microscopic readings on ascites cytology preparations. The results of the assessment are scored according to the criteria in the scoring table.

This research consists of five stages of research, namely:

Preparation of fixative solution

Preparation of the NAFS solution, namely 9 parts of ethanol, was put into a test tube. Then add 1 part of 40% formalin. Preparation of a ready-to-use 10% acetone-NBF solution, namely acetone and 10% NBF containing 4% formalin and 0.05 M phosphate buffer, was prepared, then 9 parts of acetone were put into a test tube. Then added 1 part NBF 10%.

Fixation process

The fixation process used NAFS, where the ascites preparation was put into a centrifuge tube, then a 1:1 NAFS solution was added and allowed to stand for 1 hour.

The fixation process used 10 % acetone-NBF, i.e. ascites preparation, into a centrifuge tube, then added a mixture of 10% 1:1 acetone-NBF, then allowed to stand for 1 hour. Centrifuged at 2500 rpm for 15 minutes and then the supernatant was discarded. 10% acetone-NBF mixture was added and allowed to stand for 1 day.

Cell block creation

The next step after the fixation process is the manufacture of cell blocks, using tweezers, the precipitate is taken and then placed on filter paper, folded and then inserted into a cassette that has been identified using a pencil. The cassette is inserted into the cassette rack, then the cassette rack is inserted into the chamber on the Excelsior AS tool for tissue maturation. For 4 hours 30 minutes, The device will automatically carry out the fixation process by immersing the cassette in 10% formalin buffer. Then for 6 hours the dehydration process was carried out by immersing the cassette in alcohol with a graded concentration of 70%, 80%, 96% and 100%, then the clearing process was continued by soaking the cassette in xylol for 3 hours, and the paraffin infiltration process by soaking the cassette into liquid paraffin. for 4 hours. After maturation is complete, the cassette rack is removed from the chamber and the cassette is removed from the cassette rack.

In the Histostar Embedding Module, the cassette is inserted into a tissue storage tank for the manufacture of paraffin blocks. The precipitate that has gone through the process of tissue maturation is removed from the filter paper and then transferred using tweezers to a base mould of appropriate size. The base mold containing the sediment is then filled with liquid paraffin using a wax dispenser, place the cassette lid on the base mold . Then move the base mold to a cold spot until the paraffin hardens. After the paraffin hardens, transfer the base mold to the coldplate on the Histostar Cold Module to cool the block. After the paraffin solidifies, remove the cassette from the base mold and place it on a cold plate until it is ready to be cut.

Paraffin block cutting

Paraffin blocks were cut using a microtome with a thickness of 3-5 microns, in order for the paraffin to expand, the results were placed in a water bath with a temperature of 48°C. Then it was taken using an object glass. For 15 minutes, place the slide on a hotplate at 70°C.

Slide coloring

The type of staining used in this study was Hematoxylin Eosin staining, carried out by immersing the preparation into xylol I for 5 minutes, then transferred to xylol II for 5 minutes, then the preparation was dried to dry. Furthermore, the preparation was immersed in ethanol for 5 minutes, transferred to 96% alcohol for 5 minutes, then into 70% alcohol for 5 minutes then rinsed with running water. Then soaked in hematoxylin dye (HE Harris) for 5-10 minutes. then rinse with running water for 5-10 minutes. Then the preparation was dipped into a 0.4% HCl solution as much as 2 dips. Furthermore, the preparation is immersed in lithium carbonate for 30 seconds, then rinsed with running water. Then soak the preparation in 70% alcohol and then 96% alcohol for 5 minutes each. After that, soak the preparation in eosin phloxine dye for 1-2 minutes. Then dip the preparation into ethanol I and ethanol II for 10 dips each. Then soak the preparation in xylol I and xylol II for 5 minutes each. Remove and drain the preparations to dry. Then drop the entelan and cover it with a cover slip. Furthermore, the preparation was carried out by a specialist in anatomical pathology (Inderiati & Pratiwi, 2021).

The statistical test in this study used the Wilcoxon test between cell block preparations with Nathan Alcohol Formalin Substitute fixative solution and cell block preparations with 10% acetone- Neutral Buffered Formalin fixative solution with a significance value ($P<0.05$). In analyzing the data obtained from the observation of the cytological form of ascites, I will use the criteria for evaluating the quality of the preparation based on the objective of observing the cytopathology of ascites, namely by describing the quality of the preparation.

Discussion

As research conducted by (Prativi et al., 2013) that to test a difference, statistical data analysis was carried out Non-parametric, namely the Wilcoxon test. According to research conducted by (Rudianto et al., 2020) the Wilcoxon test is

used to test conditions (variables) in paired samples or it can also be used for before and after research. The interpretation of the Wilcoxon test results is that if the significant value is more than 0.05 then Ho is accepted or there is no significant difference, and conversely if the significant value is less than 0.05 then Ho is rejected or there is a significant difference. The results obtained are 0.000 less than 0.05 which means there is a significant difference between the treatment and the assessment/score of the two preparations.

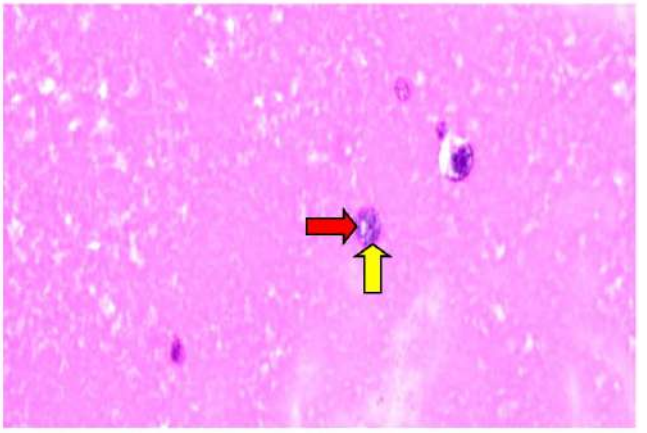

Figure 1. Microscopic view with fixation using 10% acetone-NBF at 400x magnification.

Information:

Figure 1: in the picture there is less blue in the cell nucleus (yellow arrow), red in the cytoplasm is lacking (red arrow), but from the picture it can still be diagnosed so that the manufacture of block cells using acetone-NBF 10 fixative solution This % gets a score of 2 with a poor ordinal scale.

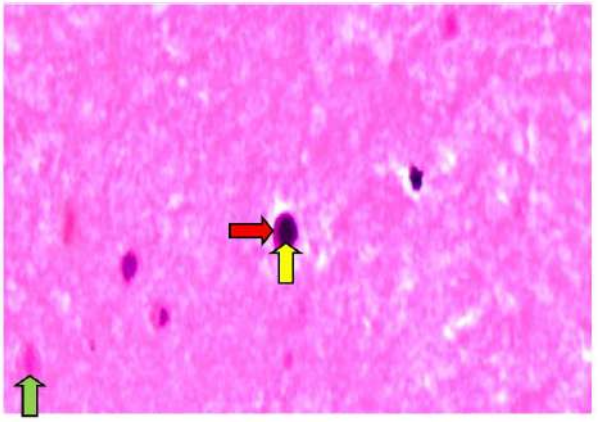

Figure 2. Microscopic view with fixation using 10% acetone-NBF with inflammatory cells 400x magnification.

Information:

Figure 2: Microscopic view with fixation of acetone-NBF, mesothelial cells appear, rounded nucleus with nuclear membrane regular (yellow arrows) with sufficient cytoplasm and well-defined cell membranes (red arrows), degenerated cells with indistinct nuclei and cytoplasm (green arrows).

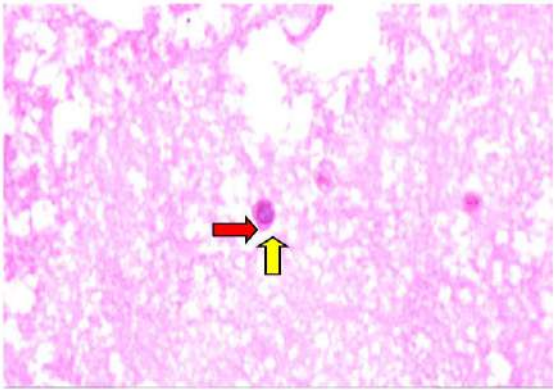

Figure 3. Microscopic view with fixation using NAFS with inflammatory cells 400x magnification

Information:

Figure 3: in the picture the blue color in the cell nucleus is not clear and mesothelial (yellow arrow), the red color in the cytoplasm is not clear (red arrow), and the preparation cannot be diagnosed so that the manufacture of block cells using this NAFS fixative solution gets a score of 1 with ordinal scale is not good.

Table 1
Wilcoxon test results for cell block preparations

RANKS				
		N	Mean Rank	Sum of Ranks
Aseton-NBF 10% - Nathan Alcohol Formalin Substitute	Negative Ranks	0[a]	.00	.00
	Positive Ranks	25[b]	13.00	325.00
	Ties	0[c]		
	Total	25		

Test Statistics[a]	
	Aseton-NBF 10% - Nathan Alcohol Formalin Substitute
Z	-5.000[b]
Asymp. Sig. (2-tailed)	.000
a. Wilcoxon Signed Ranks Test	
b. Based on negative ranks.	

Cell block preparations that were fixed using 10% acetone-NBF produced a lighter color than the cell block preparations fixed using NAFS so that they gave less blue color to the cell nucleus, less red color in the cytoplasm, but from the picture it can still be diagnosed. As research conducted by (Inderiati & Pratiwi, 2021) that cell block preparations fixed using NAFS resulted in lower scores than cell block preparations fixed using a solution mixed with 10% NBF. This is because acetone is a solvent that has been used as a fixative and NBF 10% itself is the fixation solution that is most often used so that the combination of these two fixation solutions produces better staining results. Meanwhile, in cell block preparations that were fixed using NAFS, the color was faded so that the blue color of the cell nucleus was not clear, the cytoplasm was not clear, and the preparation could not be diagnosed.

Table 2
Assessment of the quality of Hematoxylin-Eosin staining preparations based on (Ariyadi & Suryono, 2017)

No.	Description	Quality Ordinal Scale	Score
1.	The blue color of the cell nucleus is not clear, the red color (eosin) in the cytoplasm and connective tissue is not clear and the color of the preparations is not uniform. Preparation can not be diagnosed	Not good	1
2.	The blue color of the cell nucleus is lacking, the red color (eosin) in the cytoplasm and connective tissue is lacking, and the color uniformity of the preparations is lacking. Preparations can still be diagnosed	Poor	2
3.	Bright blue color in the cell nucleus, red color (eosin) in the cytoplasm and connective tissue and uniform color on preparations	Good	3

As research conducted by (Astuti et al., 2022) that can be said to be effective if the Wilcoxon test results Asymp value. The sig is less than 0.05. Interpretation of the results of the Wilcoxon Test from the SPSS output, it appears that the Asymp.sig (2-tailed) value is 0.000 less than 0.05 which means that there is a comparison between the fixative solution of Nathan Alcohol Formalin Substitute with 10% acetone-Neutral Buffered Formalin in the manufacture of cell blocks in ascitic fluid samples.

The results of observations with 10% acetone-NBF fixation showed that the cells were quite cellular, some of the cells appeared to be degenerating, which means that the cells fixed using the acetone-NBF fixation solution were not too many and not too few, so the resulting image resulted in a score of 2, namely not good, but still can be diagnosed. In contrast to the observations with NAFS fixation, it appears that the hypocellular cells and most of the cells undergo degeneration (changes), which means that the cells fixed using the NAFS fixation solution are too few, so the resulting image produces a score of 1, which is not good and cannot be diagnosed.

Conclusion

The 10% acetone-NBF fixative solution was better than the NAFS fixative solution with a score of 2 with the Wilcoxon test obtained a significance value of 0.000 less than 0.05. Then based on the quality of the assessment, it can be concluded that there is a comparison of fixative solution between NAFS and 10% acetone-NBF in the manufacture of cell blocks in ascitic fluid samples, so that 10% acetone-NBF fixative solution can be used as an alternative solution.

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