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DIFFERENCES IN *HEMATOXYLIN EOSIN* (HE) STAINING RESULTS ON KIDNEY, SKIN AND COLON HISTOLOGY OF MICE *(Mus musculus)* BASED ON CUTTING THICKNESS OF 3 µm, 6 µm and 9 µm)

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ABSTRACT

Influencing factors absorption coloring *Hematoxylin Eosin* (HE) is one of them size thickness cutting network, cuts are not appropriate and on time coloring that is not appropriate causes the absorption process color No perfect so that moment observation microscopic view of the cell nucleus and cytoplasm seen more pale and faint . Research objectives This is For now difference results coloring *Hematoxylin Eosin* (HE) on histology kidneys , skin , and colon mice (*Mus musculus*) based on thickness piece 3 µm, 6 µm, and 9 µm microtomes . Research methods This use method Experimental with nine groups treatment that is thickness cutting 3 µm , 6 µm and 9 µm microtome, then preparation done HE staining and observed quality microscopic includes the cell nucleus, cytoplasm and uniformity color. Data collection uses primary data, reading field done with 400x magnification (40x objective). Data processing uses statistical tests Mann Whitney . The results of the *Mann Whitney* test show the preparation kidney ($p<0.05$) that there is difference in a way significant, preparation skin cuts of 3 µm and 6 µm ($p > 0.05$) that No There is difference significant cuts of 6 µm and 9 µm, 3 µm and 9 µm ($p< 0.05$) that there is difference in a way significant, colon preparation ($p<0.05$) that there is difference in a way significant. Conclusion of study This is in the kidneys cutting 3 µm microtome results preparation with quality the best coloring compared to with cutting 6 µm and 9 µm microtome, on skin and colon organs cutting 6 µm microtome results preparation with quality the best coloring compared to with cutting 3 µm and 9 µm microtome.

I. INTRODUCTION

Histotechnics is a method or process of preparing organs, tissues or tissue parts through a series until they become preparations that are ready to be observed or analyzed. The process of making histological preparations has several stages, namely fixation , dehydration , clearing , embedding , blocking , sectioning , staining , mounting , and labeling [1],[2]. Cutting is one of the stages in microtechniques which is very important to produce good preparations. Cutting tissue with a microtome will produce results in the form of thin bands that are translucent to light so that thin slices can help in producing accurate diagnostic results [3].

Of the factors that influence the absorption of *Hematoxylin-eosin* staining is the thickness of the tissue cut. Improper cutting, such as a cut that is too thin and the staining time is not according to the procedure, will cause the color absorption process to be incomplete so that when microscopically observed the cytoplasm looks pale, faint and the boundaries between cells are blurred. Cutting too thick can cause the color intensity to increase, because it is caused by the effect of the optical density of eosin dye which is greater than *hematoxylin* [4],[5]. The absorption process in HE staining is based on an acid-base reaction where the acidic cell nucleus will attract substances that have basic properties which will be blue in color from *hematoxylin*, while eosin has acidic properties

which function to bind protein molecules with a positive charge in connective tissue and cytoplasm [6].

Previous research conducted by [7] stated that the thickness of the light microtome used could vary between 1-10 µm. Shield (2019) explains that the standard thickness of histological tissue sections is 6 µm, which shows good quality results [7],[8]. Explained that cutting histological tissue with a cutting thickness of 6 µm with HE staining showed good quality results with clear cell nuclei and cytoplasm and uniform color [8].

In this study, the organs that can be used to make tissue preparations are the kidneys, skin and colon. Researchers use mice in research because mice have a short life cycle, are easy to breed, and have an anatomical, genetic and physiological structure similar to humans. The criteria for mice that can be used as research objects are mice that are healthy, male, 1-3 months old and have a body weight of around 20-30 grams [9].

Researchers found gaps related to microtome settings for histological tissue cutting because the thickness used in each journal was still different. So the researchers wanted to find differences in HE staining results with microtome cutting thicknesses of 3 µm, 6 µm, 9 µm on histological preparations of the kidneys, skin and colons of mice.

II. THEORETICAL REFERENCE

II.1 SUBTITLE

Cutting (sectioning) is part of one of the stages that must be passed before *Hematoxylin Eosin* (HE) staining. The advantage of using a microtome as a tool for cutting biological samples is that it can cut into thin segments for microscopic examination, and is transparent. The disadvantage of a microtome is that if the thickness used is not correct, what will happen is diffusion and poor light penetration by the light microscope [7].

The tissue staining method that is often used routinely is a dye that can stain the nucleus and cytoplasm as well as connecting tissue, namely *Hematoxylin Eosin* (HE) staining. *Hematoxylin* works as a basic dye and Eosin works as an acid dye. One of the factors that influence the absorption of *Hematoxylin-eosin* staining is the thickness of the tissue cut. Improper cutting, such as a cut that is too thin and the staining time is not according to the procedure, will cause the color absorption process to be incomplete so that when microscopically observed the cytoplasm looks pale, faint and the boundaries between cells are blurred. Cutting too thick can cause the color intensity to increase, because it is caused by the effect of the optical density of eosin dye which is greater than *Hematoxylin* [4],[5]. The absorption process in HE staining is based on an acid-base reaction where the acidic cell nucleus will attract substances that have basic properties which will be blue in color from *hematoxylin*, while eosin has acidic properties which function to bind protein molecules with a positive charge in connective tissue and cytoplasm [5],[6].

Previous research states that for light microscopes, the standard cutting used ranges from 1-10 µm [7]. According to [10], histological tissue cutting with a cutting thickness of 3-4 µm with HE staining showed good preparation quality results, the cell nuclei were visible blue, the cytoplasm with connective tissue appeared pink and the color uniformity of the preparations appeared uniform. For explained that cutting histological tissue with a cutting thickness of 6 µm with HE staining showed good quality results with clear cell nuclei and cytoplasm visible and uniform color [7],[8],[10].

III. MATERIALS AND METHODS

This study analyzed the quality of the results of kidney, skin and colon preparations from mice that were stained with *Hematoxylin-eosin* based on a microtome cutting thickness of 3 µm, 6 µm and 9 µm. This type of research is experimental with a true experimental post test only control group design . The research was conducted from August 2022 to April 2023. This research was carried out at the Animal Testing Laboratory, Faculty of Medicine, Diponegoro University. This research population used kidney, skin and colon preparations from mice (*Mus musculus*) and the samples used were kidney, colon and skin preparations from mice (*Mus musculus)* involving 9 treatment groups, namely microtome cutting thickness of 3 µm, 6 µm and 9 µm. The number of sample preparations used was 15 preparations from kidney, skin and colon from mice determined using the Federer formula.

The tools used in this research were a microtome, glass jar, cutting board, scalpel, tweezers, pin, tissue cassette, base mold, pencil, label paper, ruler, surgical scissors, microtome knife, 1 set of painting containers, jar with lid, microscope, object glass, deck glass, water bath, stopwatch . The materials used in this research were kidney organs, skin and colons of mice, paraffin, xylol, graded alcohol (70%, 80%, 96%, 100%), distilled water , NaCl 0.9%, NBF 10%, chloroform, paper filter, cotton, *Hematoxylin Eosin* (HE) dye solution , immersion oil, entelan (Canadian balsam).

IV. RESULTS AND DISCUSSIONS

IV.1RESULTS

This research uses samples kidney, skin, colon originating from experimental mice (*Mus musculus*), which were then dissected and continued with microtechnical stages until it became a ready-to-read preparation for further microscopic observation by the validator and author. Each reader assessed 5 visual fields in each preparation.

IV.1.1 Field of View Assessment Results Data

Table 1:Tabulation of Data from Field of View Assessment Results of Kidney, Skin and Colon Tissue Preparations from Mice (*Mus musculus*) Assessment criteria preparation kidney with a 3 µm cut getting an average total yield of 8.97%, a 6 µm.

Variable	Code	Preparations Preparation								
		Kidney			Skin			Colon		
		Average Score	Total Average	Quality Preparations	Average Score	Total Average	Quality Preparations	Average Score	Total Average	Quality Preparations
3µm cutting	3A	9	8.97%	GOOD	5.2	5.26%	NOT GOOD	7.2	7.02%	GOOD
	3B	9		GOOD	5		NOT GOOD	7		GOOD
	3C	8.8		GOOD	5,6		NOT GOOD	7.2		GOOD
	3D	9		GOOD	5,6		NOT GOOD	6,8		NOT BAD
	3E	9		GOOD	5.2		NOT GOOD	6.4		NOT BAD

Variable	Code	Preparations Preparation								
		Kidney			Skin			Colon		
		Average Score	Total Average	Quality Preparations	Average Score	Total Average	Quality Preparations	Average Score	Total Average	Quality Preparations
	3F	9		GOOD	5,6		NOT GOOD	8		GOOD
	3G	9		GOOD	5.2		NOT GOOD	6.4		NOT BAD
	3H	9		GOOD	5		NOT GOOD	7		GOOD
	3I	9		GOOD	5		NOT GOOD	7.2		GOOD
6 µm cutting	6A	7.5		GOOD	5		NOT GOOD	8.8		GOOD
	6B	7.5		GOOD	5		NOT GOOD	8.6		GOOD
	6C	8		GOOD	5,6		NOT GOOD	8.6		GOOD
	6D	8.2		GOOD	5		NOT GOOD	8.8		GOOD
	6E	8.1	8.07%	GOOD	5,6	5.57%	NOT GOOD	8.8	8.73%	GOOD
	6F	8.7		GOOD	5.4		NOT GOOD	9		GOOD
	6G	8		GOOD	6		NOT GOOD	8.8		GOOD
	6H	8		GOOD	7		GOOD	8.8		GOOD
	6I	8.7		GOOD	5,6		NOT GOOD	8.4		GOOD
9 µm cutting	9A	4.9		NOT BAD	5		NOT GOOD	5,6		NOT BAD
	9B	4		NOT BAD	5		NOT GOOD	5		NOT BAD
	9C	4		NOT BAD	5		NOT GOOD	6.2		NOT BAD
	9D	4		NOT BAD	5		NOT GOOD	5.2		NOT BAD
	9E	5	4.65 %	NOT BAD	5	5.0%	NOT GOOD	5	5.22%	NOT BAD
	9F	5		NOT BAD	5		NOT GOOD	5		NOT BAD
	9G	5		NOT BAD	5		NOT GOOD	5		NOT BAD
	9H	5		NOT BAD	5		NOT GOOD	5		NOT BAD
	9I	5		NOT BAD	5		NOT GOOD	5		NOT BAD

Source: Authors, (2024).

cut getting an average total yield of 8.07% while a 9 µm cut gets an average total yield of 4.65%. Assessment criteria preparation skin with a 3 µm cut getting an average total yield of 5.26%, a 6 µm cut getting an average total yield of 5.57% while a 9 µm cut gets an average total yield of 5.0%. Assessment criteria preparation colon with a 3 µm cut getting an average total yield of 7.02%, a 6 µm cut getting an average total yield of 8.73% while a 9 µm cut gets an average total yield of 5.22%.

IV.1.2 Group Data Differences in Quality of Preparations Cutting Preparations at 3 µm, 6 µm and 9 µm.

Table 2: Group Data on Differences in Quality of Preparations for 3 µm , 6 µm and 9 µm cutting preparation.

Organ	Variable	Score	Quality Preparations		
			3 µm	6 µm	9 µm
			n (%)	n (%)	n (%)
Kidney	Bad	1	0 (0%)	0 (0%)	0 (0%)
		2	0 (0%)	0 (0%)	0 (0%)
		3	0 (0%)	0 (0%)	0 (0%)
	Not Bad	4	0 (0%)	0 (0%)	**4 (44.5%)**
		5	0 (0%)	0 (0%)	**5 (55.5%)**
		6	0 (0%)	0 (0%)	0 (0%)
	Good	7	0 (0%)	**2 (22.2%)**	0 (0%)
		8	**1 (11.1 %)**	**7 (77.8%)**	0 (0%)
		9	**8 (88.9 %)**	0 (0%)	0 (0%)
Total			**9 (100%)**	**9(100%)**	**9 (100%)**
Skin	Bad	1	0 (0%)	0 (0%)	0 (0%)
		2	0 (0%)	0 (0%)	0 (0%)
		3	0 (0%)	0 (0%)	0 (0%)
	Not Bad	4	0 (0%)	0 (0%)	0 (0%)
		5	**9 (10 0%)**	**7 (77.8 %)**	**9 (10 0%)**
		6	0 (0%)	**1 (11.1 %)**	0 (0%)
	Good	7	0 (0%)	**1 (11.1 %)**	0 (0%)
		8	0 (0%)	0 (0%)	0 (0%)
		9	0 (0%)	0 (0%)	0 (0%)
Total			**9 (100%)**	**9(100%)**	**9 (100%)**
Colon	Bad	1	0 (0%)	0 (0%)	0 (0%)
		2	0 (0%)	0 (0%)	0 (0%)
		3	0 (0%)	0 (0%)	0 (0%)
	Not Bad	4	0 (0%)	0 (0%)	0 (0%)
		5	0 (0%)	0 (0%)	**8 (88.9 %)**
		6	**3 (33.3 %)**	0 (0%)	**1 (11.1 %)**

Organ	Variable	Score	Quality Preparations		
			3 µm	6 µm	9 µm
			n (%)	n (%)	n (%)
	Good	7	**5 (55.5 %)**	0 (0%)	0 (0%)
		8	**1 (11.1 %)**	**8 (88.9 %)**	0 (0%)
		9	0 (0%)	**1 (11.1 %)**	0 (0%)
Total			**9 (100%)**	**9(100%)**	**9 (100%)**

Source: Authors, (2024).

The quality of the kidney preparations in the 3 µm cutting group showed good quality with a score of 8 totaling 1 preparation (11.1%) and a score of 9 totaling 8 preparations (88.9 %). The quality results of the preparations from the 6 µm cutting group showed that the quality was good, with a score of 7 totaling 2 preparations (22.2%) and a score of 8 totaling 7 preparations (77.8%). The quality of the preparations for the 9 µm cutting group was found to be of poor quality with a score of 5 totaling 5 preparations (55.5%) and a poor quality score of 4 totaling 4 preparations (44.5%).

The quality of the skin preparations in the 3 µm cutting group was found to be of poor quality with a score of 9 totaling 9 preparations (100%). The results of the quality of the preparations for the 6 µm cutting group showed that the good quality score was 7 totaling 1 preparation (11.1 %) and the poor quality score 5 was 7 preparations (77.8 %) and the score 6 was 1 preparation (11.1%) . The quality of the preparations from the 9 µm cutting group was found to be of poor quality with a score of 5 totaling 9 preparations (100 %).

The quality of the colon preparations in the 3 µm cutting group was good quality with score 6 totaling 3 preparations (33.3%), score 7 totaling 5 preparations (55.5 %) and score 8 totaling 1 preparation (11.1%). The results of the quality of the preparations from the 6 µm cutting group showed good quality with a score of 8 totaling 8 preparations (88.9 %) and a score of 9 totaling 1 preparation (11.1 %). The quality of the preparations for the 9 µm cutting group was found to be of poor quality with a score of 5 totaling 8 preparations (88.9 %) and a poor quality score of 6 totaling 1 preparation (11.1 %).

IV.2 Data Normality Test Using the Shapiro-Wilk Test.

The data normality test uses the Shapiro-Wilk test because the variables are <50 and the following data is obtained:

Table 3: Data Normality Test Results.

Organ	Variable	*Shapiro-Wilk*
		Sig.
Kidney	3 µm cutoff	0,000
	6 µm cutoff	
	9 µm cutoff	
Skin	3 µm cutoff	0,000
	6 µm cutoff	
	9 µm cutoff	
Colon	3 µm cutoff	0,000
	6 µm cutoff	
	9 µm cutoff	

Source: Authors, (2024).

Shapiro-Wilk test on the three qualities of preparations for each organ obtained significant results for both p = 0.000, which means that all variables have an abnormal data distribution (p<0.05), so continue with and for comparing results between groups followed by Mann Whitney hypothesis testing.

IV.2.1 Mann Whitney Non-Parametric Hypothesis Test.

Mann Whitney non-parametric hypothesis test was used to determine whether there were significant differences between the two treatment groups with the following results:

Table 4: *Man Whitney* Hypothesis Test Results.

Organ	Variable	*p*	Sig.
Kidney	3 µm	0.000	*p*<0.005
	6 µm		
	3 µm	0.000	*p*<0.005
	9 µm		
	6 µm	0.000	*p*<0.005
	9 µm		
Skin	3 µm	0.412	*p*>0.005
	6 µm		
	3 µm	0.004	*p*<0.005
	9 µm		
	6 µm	0.004	*p*<0.005
	9 µm		
Colon	3 µm	0.004	*p*<0.005
	6 µm		
	3 µm	0.001	*p*<0.005
	9 µm		
	6 µm	0.001	*p*<0.005
	9 µm		

Source: Authors, (2024).

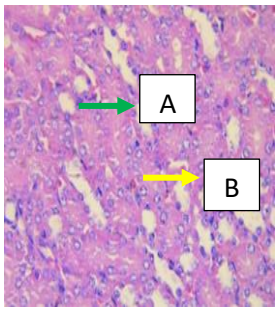
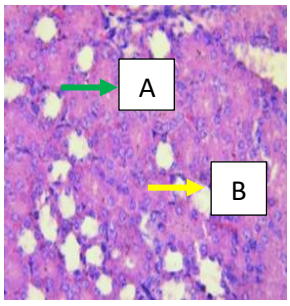
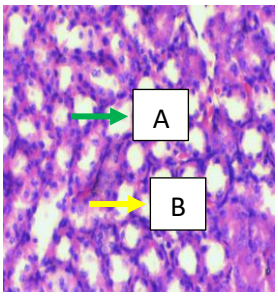
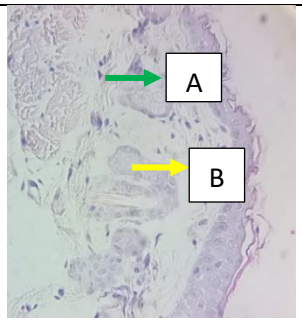
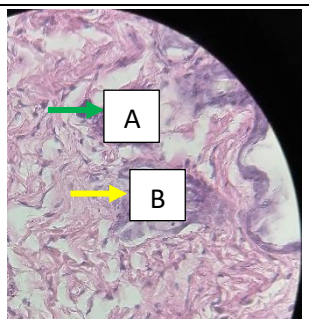
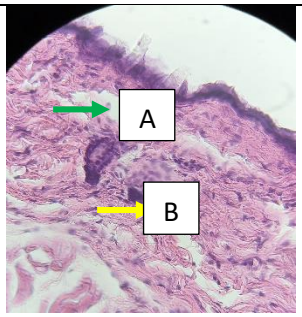
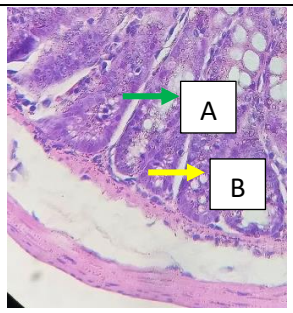
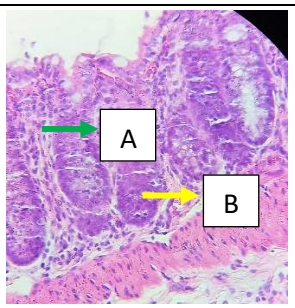
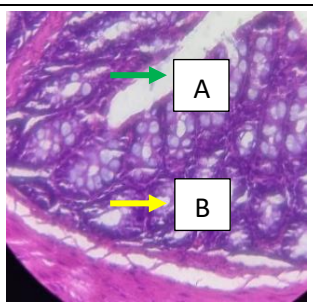
Mann Whitney statistical results on the kidney organs of mice in each group, namely microtome cutting at 3 µm and 6 µm, 6 µm and 9 µm, 3 µm and 9 µm results were obtained significant p<0.005 , then It can be interpreted that there is a significant difference in results between the groups staining the kidney preparations from mice (Mus musculus) .

Mann Whitney statistical results In the skin organs of mice in the 3 µm and 6 µm microtome cutting groups, significant results were obtained at p > 0.005 , so it could be interpreted that there was no significant difference. Cutting groups 6 µm and 9 µm, 3 µm and 9 µm results were obtained significant p <0.05 , so it can be interpreted that there is a significant difference in results between the groups staining the mouse (*Mus musculus*) skin preparations .

Mann Whitney statistical results on the colon organs of mice in each group, namely microtome cuts at 3 µm and 6 µm, 6 µm and 9 µm, 3 µm and 9 µm results were obtained significant p

<0.00 5, it can be interpreted that there is a significant difference in results between the groups staining the mouse (*Mus musculus*) colon preparations.

Table 5: Differences in *Hematoxylin Eosin* Staining Results in Histology of Kidney, Skin and Colon Tissue Preparations from Mice (*Mus musculus*) Based on Cutting 3 µm, 6 µm and 9 µm.

Organ	Cutting 3 µm	Cutting 6 µm	Cutting 9 µm
Kidney	 (A) Cell nucleus = clearly visible purplish blue color (B) Cytoplasm = clearly visible pink color	 (A) Cell nucleus = less clear purplish blue color (B) Cytoplasm = appears less clearly pink	 (A) Cell nucleus = less clear purplish blue color (B) Cytoplasm = appears less clearly pink
Skin	 (A) Cell nucleus = less clear purplish blue color (B) Cytoplasm = appears less clearly pink	 (A) Cell nucleus = clearly visible purplish blue color (B) Cytoplasm = appears clearly pink	 (A) Cell nucleus = less clear purplish blue color (B) Cytoplasm = appears less clearly pink
Colon	 (A) Cell nucleus = less clear purplish blue color (B) Cytoplasm = appears less clearly pink	 (A) Cell nucleus = clearly visible purplish blue color (B) Cytoplasm = appears clearly pink	 (A) Cell nucleus = appears unclear purplish blue color (B) Cytoplasm = appears unclear pink

Source: Authors, (2024).

V. DISCUSSIONS

The results obtained from tables 4.1 and 4. 2 show that kidney preparations at 3 µm cutting have good results compared to 6 µm and 9 µm cutting. This can be seen from the average value of the nucleus, cytoplasm and color uniformity of the 3 µm cutting which obtained a higher value than the 6 µm and 9 µm cutting preparations. This is in accordance with research by Brilian (2021) explaining that kidney preparations were cut with a thickness of 3-5 µm which showed good quality results, namely bright blue color in the cell nucleus, red (eosin) in the cytoplasm and uniform color on the preparations [11]. Meanwhile, in skin and colon preparations, cutting 6 µm obtained better results compared to cutting 3 µm and 9 µm. This is in accordance with research conducted by Akyun, Fajariyah & Mahriani (2019), who conducted research on mouse (*Mus musculus*) skin tissue which was cut at 6 µm resulting in observations of the histological picture of the skin with clearly visible nuclei and cytoplasm [12]. As well as research by [13] that a cutting size of 6 µm is the standard for cutting histological tissue which shows the maximum score on the microscopic quality of cell nuclei and cytoplasm.

Poor cutting results can be influenced by microtome calibration (lack of knife sharpness and consistency in cutting speed can cause artifacts in the tissue band) [14]. In addition, the temperature of the tissue block must be cool to make cutting easier. Microtome cuts that are too thick cause creases in the paraffin bands of the kidney, skin, colon due to heat from friction between the tissue block and the microtome blade when cuts are made more than once. As well as an embedding process that is not optimal (the tissue is not yet free from the clearing fluid), it will result in crystallization and make it easier for the tissue to tear [15].

Unfavorable results can also be caused by the *Hematoxylin Eosin* (HE) staining factor which acts as a basic dye [16]. Staining of tissue components occurs due to the acid-base reaction process. The acidic cell nucleus will attract alkaline substances, so the nucleus will have a purplish blue color from *Hematoxylin*. While the alkaline cytoplasm will attract acidic substances, the cytoplasm will be pink from eosin [6].

In this study, the cell nucleus parameters in kidney preparations in the 3 µm microtome cutting group obtained the best results compared to 6 µm and 9 µm microtome cutting. The nuclei appear purplish blue at 3 µm microtome sectioning. Meanwhile, when cutting with a microtome at 6 µm and 9 µm, the cell nuclei were seen to be on top of each other, which was caused by poor absorption due to cutting that was too thick. This is in line with research conducted by [11] that cutting 3-5 µm produces good preparations with a percentage of 100% good cell nucleic. In skin and colon preparations, the cell nucleus parameters of 6 µm cuts obtained the best results compared to the 3 µm and 9 µm cuts groups. The visible nuclei appear purplish blue in color and do not overlap in the 6 µm cutting group. This is in line with research conducted by [12] that when cutting the skin on the backs of mice (*Mus musculus*), cuts of 6 µm of cell nuclei were clearly visible. Meanwhile, the cell nuclei in the 3 µm cut group looked faint or did not appear purplish blue and the 9 µm cut cell nuclei appeared stacked so that the purplish blue color was not visible. The intensity of staining of cell nuclei appeared to increase with greater section thickness [17].

In this study, the best results were obtained for cytoplasmic parameters in kidney preparations from the 3 µm microtome cutting group compared to 6 µm and 9 µm microtome cutting. The cytoplasm appeared clear and pink in the 3 µm microtome cutting group, whereas in the 6 µm and 9 µm microtome cutting groups the cytoplasm could not be clearly observed because the intercellular boundaries were less clear. This is in line with research conducted by [18] that cutting 3-4 µm produces good preparations with 100% cytoplasm and good cell nuclei. Meanwhile, the 6 µm cutting skin and colon preparations obtained the highest value compared to the 3 µm and 9 µm cutting groups. This is in line with research by [8] that the cytoplasm in the skin and colon of mice (*Mus musculus*) cutting 6 µm is clearly visible [8].

The color uniformity parameters in the 3 µm and 6 µm microtome cut kidney preparations obtained the best results because they had color uniformity with even color intensity throughout the field of view compared to the 9 µm cut group. According to [3], poor color uniformity can be caused by a lack of time at the deparaffinization stage to remove paraffin using xylol from the tissue, causing artifacts on the preparation and the presence of sediment in the reagent because before the coloring process was carried out, the paint was not filtered completely. Meanwhile, skin and colon preparations cut at 3 µm, 6 µm and 9 µm obtained good average results. This is in line with research by Trianto, Ilmiawan, Pratiwi & Suprianto (2019), that preparations that have good color uniformity show even color intensity throughout the field of view. Color uniformity is influenced by the tissue fixation process, so that adequate fixation will produce an even color throughout the field of view, and is influenced by the cleanliness of the water in the water bath so that it does not cause artifacts in the tissue bands [19].

The results of all visual field scores were then tested for normality of the data, based on table 4.3, the results showed that the data (p=0.000) was not normally distributed. If the data distribution is not normal, it is continued with a non-parametric statistical test, namely the Man Whitney test , to compare the results between the 2 cutting groups.

Table 4 shows that the results of the Man Whitney test with kidney preparations obtained a significant p = 0.000 (p<0.05), meaning that there was a difference in the results of the staining quality of mice kidney preparations (*Mus musculus*) between the 2 treatment groups (3 µm and 6 µm , 3 µm and 9 µm, 6 µm and 9 µm). In this study it can be said that there are differences in the results of *Hematoxylin Eosin* (HE) staining on mouse kidney histology based on microtome thicknesses of 3 µm, 6 µm, and 9 µm with the best size found at 3 µm cuts.

The results of the Man Whitney test with skin preparations obtained a significance value for the 3 µm and 6 µm cutting groups, namely 0.412 (p>0.05), which statistically means there is no significant difference in results. In the 6 µm and 9 µm, 3 µm and 9 µm cutting groups, a significance value of 0.004 (p<0.05) was obtained, which can be interpreted as a significant difference in results between these groups. In this study, it can be said that in the histology of mouse skin, *Hematoxylin Eosin* (HE) staining was based on microtome thicknesses of 3 µm, 6 µm, and 9 µm with the best size found at 6 µm cuts.

The results of the Man Whitney test with colon preparations obtained a significance value for the 3 µm and 6 µm cutting groups, namely 0.000 (p<0.05), which statistically means there is a significant difference in results. In the 6 µm and 9 µm, 3 µm and 9 µm cutting groups, a significance value of 0.004 (p<0.05) was obtained, which can be interpreted as a significant difference in results between these groups. In this study it can be said that there are differences in the results of *Hematoxylin Eosin* (HE) staining on the histology of mouse colons based on microtome thicknesses of 3 µm, 6 µm, and 9 µm with the best size being found at 6 µm

V. CONCLUSIONS

The conclusion of this research is that the quality of staining of cell nuclei, cytoplasm and color uniformity of kidney preparations *Hematoxylin Eosin* (HE) staining based on microtome cutting thicknesses of 3 µm, 6 µm and 9 µm is the best at 3 µm cutting, skin and colon preparations are the best at 6 µm cutting.

VI. AUTHOR'S CONTRIBUTION

Conceptualization: Eko Naning, Rihanesa, Tristania. Utami.
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Investigation: Eko Naning, Rihanesa, Tristania.
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