EFFECT OF IMMERSION TEMPERATURE VARIATION OF SODIUM LAURYL SULFATE 4% ASA DELIPIDATION AGENT ON QUALITY OF COLON TISSUE PREPARATIONS

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Abstract, Background: Adipose or fatty tissue will interfere with the maturation process of the tissue, causing the tissue to become hard, difficult to cut and interfere with the staining of the preparation. Therefore, the adipose tissue needs to be diluted through the delipidation stage. The purpose ofthis study was to determine the effect of variations in the immersion temperature of colonic tissueusing SLS 4% for 6 hours on the quality of the tissue preparations produced and the presence ofdifferences in the quality of the preparations produced.

Method: This research method uses a quasi-experimental type of research using data obtained from measuring the level of clarity and quality of tissue preparations and then processed statistically using the Paired Sample T-Test . The samples used in this study are the remaining samples in the form of colonic tissue that are no longer used by the Anatomical Pathology Laboratory of Cibabat Hospital. The colonic tissues used is as many 15 units. The parameters used are a 4% SLS clarity rate, and the quality of colonic tissues preparations that are observed microscopically and using ImageJ software.

Result: The results of the Paired Sample T-Test obtained a value of t = -1.740, df = 14 and gave a p-value much smaller than 0.05. The results of the study showed that there was an influence of temperature variations on the quality of colonic tissue preparations and there were differences in the quality of the colonic tissue preparations produced.

Conclusion: Colonic tissues that are immersed using a 4% SLS at 60°C provide a more optimal quality preparation compared to colonic tissue at 4% room temperature SLS immersion and tissues that do not go through delipidation.The research suggestion is to conduct further research to obtain optimal time, temperature and concentration in SLS as a delipidation agent so that good quality preparations are obtained but do not eliminate fat completely.

Keywords: Sodium Lauryl Sulfate, Delipidation, Temperature Variation, Quality of Tissue Preparations

Background

Adipose tissue (fat) is a special type of connective tissue composed of fat cells [1]. Adipose tissue is spread in various parts of the body including located under the skin (subcutaneous fat), around internal organs including the colon (visceral fat), bone marrow, intramuscular (muscle system), and breast tissue [2,3].

Adipose tissue will interfere with histopathological examination, including the tissue will become hard and difficult to cut. In addition, adipose tissue will interfere with the staining of the preparation because the dye cannot enter the tissue to color the components of cells covered by fat [4].

Therefore, fat needs to be sloughed off so as not to interfere with histopathological examination. The process of fat loss in the tissue is in the delipidation stage using xylol. Long-term use of xylol has toxic and carcinogenic effects that are quite dangerous for health [5].

So in this study the authors used SLS as a delipidation agent which is safer to use in the delipidation stage [6]. The success of the delipidation stage can be determined by assessing the quality of the tissue preparations produced by

observing the quality of the staining carried out on the intensity of the color of the cell nucleus and cytoplasm, the uniformity and clarity of the stainingof the resulting tissue [7].

The reagents commonly used in the delipidation step are xylol, toluene, benzol or chloroform [8]. The use of xylol in the delipidation stage can be replaced by a solution containing a mixture of surfactants, one of which is Sodium Lauryl Sulfate(SLS). Sodium Lauryl Sulfate is one of the delipidating agents that can be used as a substitutefor xylol in the delipidation stage. In general, delipidation is carried out at room temperature or 37°C, but requires a longer incubation time. Delipidation by heating can create random molecular movements that can accelerate the diffusion of chemical reagents into biological materials. The SLS concentration to be used is 4%with a minimum immersion time of 6 hours at roomtemperature and 60°C. The control in this study was colonic tissue which was not delipidated using 4% SLS. Good staining quality will be obtained if the tissue produced at this stage shows clear or transparent results [9].

The purpose of this study was to determine theeffect of variations in the temperature of colonic tissue immersion using SLS 4% for 6 hours on the quality of the tissue preparations produced and whether or not there were differences in the qualityof the preparations produced. The results provide valuable information for future studies on sodium lauryl sulfate 4% in the sloughing of adipose tissue for medical laboratory technologist and improve knowledge in the field of Cytohistotechnology, especially regarding delipidation.

Method

The type of research used is quasi- experimental, namely to see the effect of immersion using 4% SLS as a delipidation agent atroom temperature and 60°C on the quality of the resulting colonic tissue preparations.

The population used in this study was colonic tissue. While the samples used were colon tissue units that were taken representatively, namely the right, left and middle parts. In addition,the colonic tissue used for the sample in this study must contain fat. The number of samples used were 15 tissue units consisting of 5 right colon tissue units, 5 left colon tissue units and 5 middle colon tissue units.

Sample preparation

The sample used in this study is the remaining sample in the form of colonic tissue that is no

longer used by the Anatomical Pathology Laboratory of Cibabat Hospital. The number ofcolon tissue used is 15 tissue units. The colonic tissue used must meet several requirements including not autolysis, contain fat and canrepresent the population/representative, namely by taking the right, left and middle parts of the colonic tissue.

Neutral Fixation Buffer Formalin 10%

Put the colon tissue into a beaker containing 10% Neutral Buffer Formalin then soak for 24 hours. The ratio of the volume of the fixative solution used was 1:20. After fixation, the colonic tissue was cut first and then the adipose decay (delipidation) process was carried out by immersion using 4% SLS.

Delipidation using Sodium Lauryl Sulfate 4%

Prepare 30 beakers consisting of 15 beakers for room temperature and 15 beakers for 60°C. Labeleach beaker. Then fill each beaker with 20 mL of 4% SLS. After that, the colonic tissue in the cassette that had been fixed using Neutral Buffer Formalin 10% was put into each beaker and soaked according to the time and temperatureaccording to the label on each beaker. Meanwhile, for control, colonic tissue did not go through the immersion stage using 4% SLS, but instead carried out the tissue processing stage.

Tissue Processing

Tissue processing starts from the fixation process using 10% Neutral Buffer Formalin,followed by dehydration using graded alcohol concentration. Then the clearing stage uses xylol, after that it is followed by infiltration and embedding using paraffin. Then ended with trimming using a microtome. The process of tissue maturation will produce a block of tissue called blank preparations or unstaining preparations. The process of tissue maturation is important, and success at this stage depends on the tissue maturation procedure performed and the reagents used.

Hematoxylin Eosin (HE) Staining

Tissue staining aims to color the cell nucleus and cytoplasm with contrasting colors so that it is easy to distinguish cellular components of the tissue. Commonly used tissue coloring is Hematoxylin Eosin. The procedure for staining Hematoxylin Eosin can be seen in Table 1.

Table 1. Hematoxylin Eosin Stain Procedure

Stages	Substance used	Time
Deparaffinization (process remove residual paraffin is on the tissue)	Xylol I	2 minutes
	Xylol II	2 minutes
Rehydration (The process of adding water into the tissue via alcohol immersion from high to low concentration)	Etanol 100%	2 minutes
	Etanol 100%	2 minutes
	Alkohol 95%	2 minutes
Washing	Tap Water	2 minutes
Hematoxylin Stain (In principle, the cell nucleus that is acid will attract dye alkaline hematoxylin so the cell nucleus will be colored blue)	Hematoxylin	3 minutes
Washing	Tap Water	1 minutes
Differentiation (Process for reduce the blue color in the cell nucleus)	HCl Alkohol	1 minutes
Washing	Tap Water	1 minutes
Bluing (Color strengthening process faded blue in the cell nucleus)	Litium Karbonat 0,5%	1 minutes
Washing	Tap Water	1 minutes
Eosin Staining (Principles alkaline cytoplasm will attracts the dye eosin which acidic so that the cytoplasm will be red)	Eosin	45 seconds
Washing	Tap Water	1 minutes
Dehydration (process of removing/ draw the water inside tissue through immersion low to high concentration alcohol)	Alkohol 95%	1 minutes
	Alkohol 100%	1 minutes
	Alkohol 100%	1 minutes
Clearing	Xylol I	2 minutes
	Xylol II	2 minutes
Mounting (Closing process process the tissue between the cover glass and glass object by entelan)	Entelan	-

Tissue Staining Quality Observation

The stained tissue preparations were then observed using a microscope with a magnification of 400x to see the quality of the resulting tissue staining. Parameters observed were color intensity of nucleus and cytoplasm, cellular uniformity and clarity of staining. For each tissue preparation, digital images were taken 3 times with different fields of view. Then, the results of the digital image are entered into the ImageJ software to get the value of the color intensity of the nucleus and cytoplasm [10].

Observations of cellular uniformity and clarity of staining were carried out microscopically by an Anatomical Pathologist. The clarity of the staining is said to be good, if the entire tissue can be observed without any blurry or faint parts. Preparations that have uniformity of staining can be said to be good, if the intensity of the color produced is evenly distributed throughout the field of view. Then record all the observational data obtained and perform statistical data processing using SPSS.

Measurement of Clarity Level of Sodium Lauryl Sulfate 4%

The clarity level of SLS 4% can be used as an indicator of the ability to shed fat in colonic tissue. The 4% SLS clarity level serves to see the difference in clarity before and after tissue immersion. The higher the 4% SLS turbidity level used, the better the fat sloughing ability that occurs in the colonic tissue. The turbidity standard used is the Mc standard. Farland whose turbidity level is known in NTU (Nephelometric Turbidity Unit) units. The following is a standard Mc.Farland image used in measuring the clarity level of SLS 4%.

The Mc.Farland standard above is a turbidity standard that has been measured turbidimetrically and the level of turbidity is known in NTU units. The lowest scale is 0.5 with a turbidity level of 73 NTU and the highest scale is 9 with a turbidity level of 691 NTU.

Data Analysis

In this study, to determine whether there is a significant effect caused by immersion using a 4% SLS clearing agent can seen from the clarity level of SLS 4% used and the quality of the preparations the resulting tissue.

These three indicators will provide an overview of adipose sloughing in colonic tissue. The data obtained will be Statistical tests were carried out, namely using the normality test and the difference test.

Result and Discussion

Result

Colonic tissue samples used in this study came from the remaining tissue samples that were no longer used in the Anatomical Pathology Laboratory of Cibabat Hospital. The colonic tissue received was 15 units with the condition that it had been fixed for more than 24 hours and had a solid consistency, yellowish brown in color with fat. The colonic tissue was cut with a volume size of 3 x 2 x 0.3 cm as shown in Figure 1.

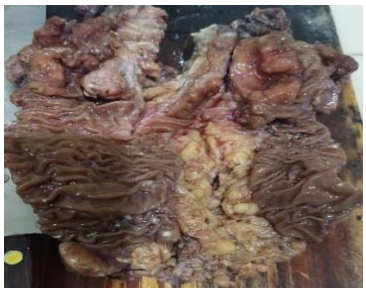

Figure 1. Human Colon Tissue Samples

The first parameter, which is seen in this study, is the SLS clarity level of 4%. This measurement of clarity level aims to see the ability of SLS 4% in shedding fat contained in the colonic tissue. In addition, the measurement of the clarity level of SLS 4% was used to see the difference in clarity before and after immersion. This measurementwas carried out before the tissue was put into 4% SLS and after immersion for 6 hours. Data clarity level SLS 4% can be seen in table 2.

Table 2. Measurement Data Clarity Level of SLS 4%

Clarity Level of SLS 4%			
Treatment	Sample Number	Observation Result	
		Before Immersion Tissue	After Immersion Tissue
Room Temperature	L1	0,5	1
	L2	0,5	1
	L3	0,5	1
	L4	0,5	1
	L5	0,5	0,5
	M1	0,5	1
	M2	0,5	1
	M3	0,5	0,5
	M4	0,5	0,5
	M5	0,5	1
	R1	0,5	1
	R2	0,5	0,5
	R3	0,5	1
	R4	0,5	0,5
	R5	0,5	1
Temperature 60°C	L6	0,5	3
	L7	0,5	3
	L8	0,5	3
	L9	0,5	5
	L10	0,5	5
	M6	0,5	5
	M7	0,5	5
	M8	0,5	7
	M9	0,5	9
	M10	0,5	5
	R6	0,5	1
	R7	0,5	3
	R8	0,5	7
	R9	0,5	3
	R10	0,5	9

Furthermore, the data was processed statistically using the normality test and the difference test. Based on the results of the statistical test of clarity level above, colonic tissue that was soaked using SLS 4% at 60°C had a better ability to slough off fat compared to soaking at room temperature. This can be seen from the average value of the 60°C temperature clarity level which is greater than the average room temperature value.

The next parameter that was observed was the quality of the resulting colonic tissue preparations. Assessment of the quality of tissue preparations was carried out by observing the quality of the staining carried out on the color intensity of the cell nucleus and cytoplasm, the uniformity and clarity of the resulting tissue staining. This assessment was carried out using a microscope at 400x magnification and then observed using imageJ to see the color intensity of the cell nucleus and cytoplasm and was assessed by a specialist in Anatomical Pathology. The following is an overview of colonic tissue preparations observed using a microscope at 400x magnification as shown in Figure 2.

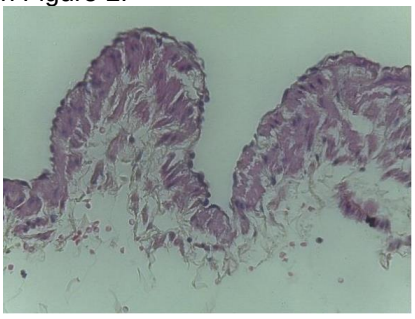

Figure 2. Microscopic Overview of Colonic Tissue Preparations 400x magnification

Based on the results of observations using a microscope, the data obtained morphological picture of colonic tissue preparations as shown in table 3.

Table 3. Morphological Observation Data of Colonic Tissue Preparations Microscopically

Morphology	Control	Room Temperature	Temperature 60°C
Cell Nucleus	On the edge	On the edge	On the edge
Cytoplasm	Clear	Clear	Clear
Cell Membrane	Unclear	Clear	Clear
Fat	More	More	Less

After observing the morphology of the preparations, colonic tissue, then an assessment of the quality of colonic tissue preparations is carried out using a microscope at 400xmagnification. Here are the results assessmentand observation of the quality of colonic tissue preparations. The data are listed in table 4.

Table 4. Data on Quality Assessment Tissue Preparations of SLS 4%

Quality of Colonic Tissue Preparations		
Treatment	**Sample Number**	**Rating Result Total Score**
Room Temperature	L1	3
	L2	3
	L3	2
	L4	3
	L5	2
	M1	2
	M2	3
	M3	2
	M4	2
	M5	3
	R1	2
	R2	2
	R3	2
	R4	3
	R5	2
Temperature 60°C	L6	3
	L7	2
	L8	3
	L9	3
	L10	2
	M6	2
	M7	3
	M8	2
	M9	3
	M10	3
	R6	3
	R7	3
	R8	3
	R9	3
	R10	2
Control	C1	2
	C2	2
	C3	2

The data is processed statistically with normality test, descriptive test, and different test. When viewed from all the statistical test results above, the tissue preparations that were immersed using SLS 4% at 60°C had better quality than at room temperature and had a significant difference.

The last parameter that is seen is the quality of the color intensity of the nucleus and cytoplasm using ImageJ. Colonic tissue preparations that have been stained using dyes hematoxylin eosin, digital images were taken using camera microscope, then proceed with quantitative measurements by entering the results of the visual field documentation into the software ImageJ. Inspection of the quality of the color intensity of the core and cytoplasm of the device ImageJ software uses the Color Deconvolution feature, namely the core structure and cytoplasm can be separated and observed independently. Then, each part of the cytoplasm and nucleus was measured for color intensity using the option histogram and obtained the mean (mean) of color intensity so that data from each stock.

In the ImageJ software, the Color Deconvolution menu using hematoxylin eosin dye can decipher the drawn image inputs are separated, each representing a different color concentration used. In this case, separate the original image into the background hematoxylinand eosin color. The data for measuring the intensity of the core color and The cytoplasm is shown in the table 5.

Table 5 Measurement Data Quality Intensity of Cell Nuclear and Cytoplasmic Color

Cell Core Color Intensity (OD Value)			
		Measurement results	
Treatment	**Sample Number**	**Cell Core OD Value**	**Cytoplasmic OD Value**
Control	C1	164,427	163,714
		152,209	164,913
		155,022	214,033
	C2	151,634	176,407
		152,052	193,014
		156,527	212,744
	C3	166,758	180,292
		157,590	189,602
		157,002	193,103
Room Temperature	L1	171,527	164,878
		153,570	197,055
		146,772	215,644
	L2	157,290	194,237
		157,966	191,521
		156,597	218,203
	L3	163,861	171,126
		148,333	201,529
		154,558	182,597
	L4	148,576	209,019
		163,721	167,741
		156,552	196,331
	L5	163,157	168,219
		152,587	178,970
		147,319	214,444
	M1	163,848	161,199
		146,821	169,038
		165,997	209,644
	M2	170,694	190,143
		166,144	201,617

		149,426	188,084
	M3	165,089	187,166
		155,771	193,966
		156,072	182,596
	M4	138,049	182,779
		143,818	177,775
		169,188	169,458
	M5	161,724	184,051
		154,802	184,357
		156,194	192,245
	R1	161,504	197,068
		155,830	153,423
		157,313	195,916
	R2	168,551	164,545
		165,377	192,110
		158,076	189,594
	R3	158,243	171,184
		171,967	184,712
		169,959	183,628
	R4	174,538	166,668
		154,442	181,309
		179,296	188,182
	R5	173,679	208,341
		153,730	183,213
		160,453	172,482
	L6	182,094	173,079
		154,058	182,624
		146,937	216,393
	L7	161,001	180,862
		142,183	176,081
		148,304	182,113
	L8	160,518	181,432
		144,053	204,342
		152,662	205,593
	L9	148,076	214,078
		165,174	165,806
		154,136	206,600
	L10	147,681	217,315
		163,825	189,326
		166,455	187,051
	M6	158,983	186,350
		163,718	204,585
		165,840	180,884
	M7	174,902	195,243
		166,744	206.855
		173,793	183,141
Temperature 60ºC	M8	165,272	200,558
		166,558	190,369
		162,413	195,378
	M9	161,167	202,230
		170,685	196,479
		164,391	196,450
	M10	150,369	202,099
		153,212	177,322
		166,211	205,351
	R6	167,360	192,861
		176,356	167,367
		168,649	155,710
	R7	172,768	199,886
		179,342	189,502
		167,883	197,455
	R8	185,831	173,935
		178,011	199,308
		174,180	198,435
	R9	164,007	189,542
		178,091	193,387
		163,323	204,530
	R10	176,066	208,719
		172,948	204,489
		179,129	196,978

Furthermore, on the measurement data of the color intensity of the cell nucleus and cytoplasm, normality tests and different tests were carried out. If seen from all the statistical test results above, the tissue preparations used were through immersion using 4% SLS at a temperature of 60°C has the quality the color intensity of the cell nucleus and cytoplasm is better than at temperature room OD value of the cell nucleus and cytoplasm of the sample is close to or exceeds the OD value control and have a significant difference.

Discussion

Accurate macroscopic examination of the tissue can provide the necessary clues in establishing the final diagnosis [11]. In this study, the sample used was colonic tissue that was not autolysed, contained fat and could represent the population/representative by taking the right, left and middle sections of the colonic tissue.

The colon is one part of the body that contains adipose tissue or fat. Colon containing adipose tissue or fat will interfere with the tissue maturation process, including causing the tissue to become hard, difficult to cut and will interfere with the staining of the preparation because the dye cannot enter the tissue to color the components of cells covered by fat [12]. Therefore, the adipose tissue needs to be shed to remove the disturbing adipose tissue by carrying out the delipidation stage so that when observed only the cells are found not covered by adipose tissue or fat [13].

The delipidation process can be carried out at high temperatures because it can create random molecular movements that can accelerate the diffusion of chemical reagents into biological materials. It is generally carried out at room temperature or 37°C, but requires a longer incubation time, to shorten the time, the temperature can be increased [14].

In the colonic tissue delipidation process using 4% SLS, there were differences in the quality of the preparations produced by immersion at room temperature and 60°C. Based on the clarity level of SLS used for immersing colonic tissue in the delipidation stage, SLS at 60°C had better delipidation ability than room temperature. This can be seen from the measurement results of SLS 4% which is used for immersion of colonic tissue at a temperature of 60°C which has the highest level of clarity, namely 691 Nephelometric Turbidity Units (NTU).

The level of clarity is related to the ability of SLS 4% in shedding fat (delipidation) in colonic tissue. This will affect the process of maturation and tissue coloring. Based on the results of research that has been carried out, colonic tissue that has been

delipidated using 4% SLS at room temperature and 60°C has the same thing that is easy to cut. This happens because the tissue maturation process is carried out correctly, resulting in a paraffin block that is easy to cut. However, there were differences in the quality of the tissue preparations that underwent delipidation using SLS 4% at room temperature and at 60°C.

The differences in the quality of these preparations included the intensity of the color of the cytoplasmic cell nucleus, the clarity of the cell membrane, and the condition of fat in the colonic tissue preparations. In delipidation using SLS 4% at 60°C, the color intensity of the cell nucleus and cytoplasm was good, the membrane was clear when observed using small magnification, and there was less fat in the tissue preparations because it was reduced which was indicated by the destruction of the structure of the fat tissue. In delipidation using SLS 4% at room temperature, the color intensity of the cell nucleus and cytoplasm is not good, the membrane is clear when observed using a small magnification, and fat in the tissue preparations is more. Whereas in the control, the tissue without going through 4% SLS immersion had a fairly good color intensity of the cell nucleus and cytoplasm, the membrane was not clear when observed at small magnification and found more fat. The process of delipidation and perfect tissue maturation will facilitate the entry of dye into the tissue at the staining stage so that good quality preparations are obtained and can be diagnosed by Anatomical Pathology Doctors [15].

Staining the nucleus and cytoplasm generally uses hematoxylin and eosin dyes. Good staining quality is indicated by the color of the cell nucleus and cytoplasm. Nuclear staining is said to be good if the cell nucleus is stained by hematoxylin to blue and the cytoplasm is stained by eosin to red [16].

Measurement of the color intensity of the cell nucleus and cytoplasm in this study using digital images by converting the tissue into a numerical form, dividing it into very small areas, namely image elements or pixels. Image analysis enables automatic morphometry and stereology aimed at understanding arrays based on slice analysis showing two-dimensional information, measuring morphological structures in an unbiased, appropriate and uniform way from random sampling of sections. Through the correct use of stereology, quantitative studies can be carried out with still good accuracy [17].

Based on the results of this study, it was shown that the use of SLS 4% as a delipidation agent at room temperature, 60°C and control gave the color intensity of the nucleus and cytoplasm which did not differ significantly. However, in the overall quality of the preparations, there were some differences, including the clarity of cell membranes and the condition of fat in the resulting colonic tissue preparations.

Measurement of the color intensity of the cell nucleus and cytoplasm using ImageJ software is influenced by the quality of the tissue staining performed. During the staining stage, hematoxylin-eosin dyes allow for stains which are quite difficult to use in image analysis. Light microscopy images depend on the absorption of light by the dye used to color the tissue preparation. While the eye can easily detect the difference between the blue-purple hematoxylin and the red eosin. This is due to the lack of differentiation, i.e. the absorption curves of hematoxylin and eosin overlap in most of the visible spectrum [18].

Conclusion

Based on the results of the research that has been done, it can be concluded that the variation of the temperature of the colonic tissue immersion using SLS 4% for 6 hours affects the quality of the tissue preparations produced. Colonic tissue that was soaked using 4% SLS at 60o C gave a more optimal quality of preparations compared to colonic tissue that was not soaked using 4% SLS.

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