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COMPARISON OF 10% BUFFERED FORMALIN NEUTRAL FIXATION SOLUTION WITH BOUIN ON MICROSCOPIC IMAGES OF CHICKEN HEPAR AND CEREBRUM WITH HEMATOXYLIN EOSIN (HE) STAINING

Robestri Natalia Sitanggang*[1]

[1]Magister of Immunology, Postgraduate School, Airlangga University, Indonesia
*E-mail : robestri.natalia.sitanggang-2022@pasca.unair.ac.id

Abstract

The fixation process is an important stage in the manufacture of histopathology preparations. Fixation aims to prevent autolysis and tissue degradation, so that the results can be observed both anatomically and microscopically. Fixation solutions that are often used in routine histopathological examinations are 10% Formalin Buffer Neutral and Bouin. This study was used to determine the results of the comparison of 10% NBF fixation solution with Bouin on the microscopic picture of hepar and cerebrum with hematoxylin eosin staining. The type of research used is a cross sectional approach with a qualitative descriptive design. Sampling was performed on chicken animals. The samples used were hepar and cerebrum fixed with 10% NBF and 10% Bouin for 12 hours, stained with Hematoxylin Eosin and observed microscopically. Then give an assessment with good, less good, or not good categories based on assessment indicators based on cell color and shape. The results showed a microscopic picture of hepatic and cerebrum tissue as much as 6 preparations fixed with 10% NBF liquid showed good results. While hepatic and cerebrum tissue fixed with Bouin liquid as many as 6 preparations showed poor results. The conclusion is that there are differences in the microscopic results of hepatic and cerebrum tissue fixed with 10% NBF solution and Bouin. Good microscopic observation results based on the assessment indicator criteria are tissues fixed with 10% NBF liquid.

Keywords: Bouin, HE staining, NBF 10%, microscopy

Abstrak

Proses fiksasi merupakan tahapan penting dalam pembuatan sediaan histopatologi. Fiksasi bertujuan untuk mencegah autolisis serta degradasi jaringan, sehingga hasilnya dapat diamati baik secara anatomis maupun mikroskopis. Larutan fiksasi yang sering digunakan dalam pemeriksaan histopatologi rutin adalah Netral Buffer Formalin 10% dan Bouin. Penelitian ini digunakan untuk mengetahui hasil perbandingan larutan fiksasi NBF 10% dengan Bouin terhadap gambaran mikroskopis hepar dan serebrum dengan pewarnaan hematoksilin eosin. Jenis penelitian yang digunakan adalah pendekatan cross sectional dengan desain deskriptif kualitatif. Pengambilan sampel dilakukan pada hewan ayam. Sampel yang digunakan adalah hepar dan serebrum yang difiksasi dengan NBF 10% dan Bouin 10% selama 12 jam, diwarnai dengan Hematoksilin Eosin dan diamati secara mikroskopis. Kemudian memberikan penilaian dengan kategori baik, kurang baik, atau tidak baik berdasarkan indikator penilaian berdasarkan warna dan bentuk sel. Hasil penelitian menunjukkan gambaran mikroskopis jaringan hepar dan serebrum sebanyak 6 preparat yang difiksasi dengan cairan NBF 10% menunjukkan hasil yang baik. Sedangkan jaringan hepar dan serebrum yang difiksasi dengan cairan bouin sebanyak 6 preparat menunjukkan hasil yang kurang baik. Kesimpulannya yaitu terdapat perbedaan hasil mikroskopis jaringan hepar dan serebrum yang difiksasi dengan larutan NBF 10% dan Bouin. Hasil pengamatan mikroskopis yang baik berdasarkan kriteria indikator penilaian adalah jaringan yang difiksasi dengan cairan NBF 10%.

***Kata Kunci**: Bouin, pewarnaan HE, NBF 10%, mikroskopis*

1. INTRODUCTION

Histology is one of the branches of biological science that studies the tissue structure, especially on thinly cut tissue samples, and the results can be seen under a microscope. Histology is invaluable in studying the physiological functions of cells in the human, animal and plant body,

as well as histopathology, which is useful in establishing disease diagnosis including changes in physiological function and organ deformation (Kemenkes RI, 2015).

The histotechnical method is a process that involves a series of steps to produce histological preparations from certain specimens that are ready to be viewed and evaluated. One of the important stages in the histotechnical method is fixation. Because fixation plays a role in preventing autolysis and degradation of tissues and tissue components, the results can be seen anatomically and microscopically (Alwi, 2016).

Failure to do so at this stage will result in an unfavorable picture of the histopathology preparation. Therefore, the selection of a good fixation solution is very important in tissue processing. A 10% Neutral Buffered Formalin fixation solution is often used to preserve tissues in routine histopathology examinations. NBF 10% has a pH of 7, is easier to use, inexpensive, and can be used to preserve tissues for a long time. The disadvantage is that the fixation time is longer, ranging from 12 to 24 hours (Sriwahyunizah, 2018).

In addition to 10% NBF, bouin solution is also often used for fixation of certain tissues. This solution has the advantage of being able to penetrate more quickly into the tissue in the nucleus, and the connective tissue will be well exposed. However, if the fixation time used is too long, the tissue becomes destroyed and difficult to slice. Bouin liquid can be stored for a long time and can be used at any time (Rusmiatik, 2011). Hepar and cerebrum are two organs that can be examined microscopically using tissue histology preparations. Hepar is one of the largest organs in the digestive system and consists of 70-80% liver cells/hepatocytes (Trianto et al., 2015).

While the cerebrum is a vital organ that functions to organize and coordinate all normal functions of the body and store memory (Yustisia et al., 2020). Both organs require adequate preparation and staining in the histopathological process to reveal the necessary cells or structures clearly (Djuwita et al., 2012). Hematoxylin Eosin is a routine stain often used in histopathology. Hematoxylin stains the cell nucleus blue and eosin stains the cytoplasm pink. (Musumeci, 2014).

The purpose of this study was to determine the microscopic picture of tissues fixed with 10% Neutral Buffer Formalin and Bouin solution and analyze the results of a good microscopic picture between tissues fixed with 10% NBF and Bouin with Hematoxylin Eosin staining.

2. RESEARCH METHOD

The research was carried out in March-June 2021 at the Regional General Hospital Dr. Soeratno Gemolong Sragen, Central Java. The type of design used is descriptive qualitative research design (cross sectional study) by comparing the fixation fluid between NBF 10% and Bouin on the quality of comparing fixation fluids between 10% NBF and Bouin on the quality of microscopic images on tissue with hematoxylin eosin (HE) staining at the Regional General Hospital dr. (HE) staining at the Regional General Hospital Dr. Soeratno Gemolong Sragen, Central Java. Central Java.

4. RESULTS AND DISCUSSION

In this study, hepatic and cerebrum tissues taken from chicken organs. Chickens have been dissected and then taken cerebrum and liver organs which are immediately fixed using 10% NBF and Bouin, through tissue processing and processed into 4 paraffin blocks. Paraffin blocks were identified and cut using a microtome with a size of 4 µ. From 1 paraffin block, it was cut into 3 preparations, making a total of 12 preparations consisting of 6 hepatic tissue preparations (3 Bouin fixation preparations, 3 10% NBF fixation preparations) and 6 cerebrum tissue.

Table 1. Microscopic observation of hepatic tissue fixed with 10% NBF and Bouin on hematoxylin eosin staining

Sample	Microscopic Assessment	
	NBF 10%	**Bouin**
Slide 1	Good	Less Good
Slide 2	Good	Less Good
Slide 3	Good	Less Good

Based on the recapitulation of the table above, it shows that of the 3 hepatic organ preparations that are fixed using 10% NBF fixative liquid obtained results in the good category and 3 preparations of hepatic organs that were fixed with Bouin obtained

results in the category of less good. Overview of microscopic results of hepatic tissue fixed with NBF 10% can be seen in the picture below.

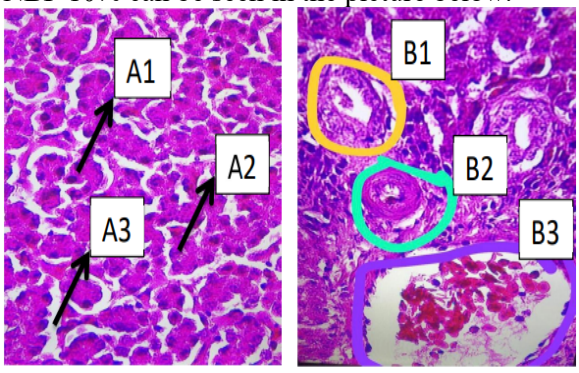

Figure 1. Microscopic Results of hepar fixed with 10% NBF on HE staining. Description: Cell Nucleus (A1), Cytoplasm (A2), Sinusoid (A3), In Figure B, namely Kiernan triangle consists of artery (B1), ductus (B2), vein (B3)

The figure above shows that the hepatic tissue fixed with 10% NBF obtained results with a good category because of the bright blue color in the cell nucleus, red color (eosin) in the cytoplasm and connective tissue and uniform color in the preparation. Then in addition to these assessment indicators, other cells such as the Kiernan Triangle, namely arteries, ducts, and veins are also clearly visible during microscopic measurements.

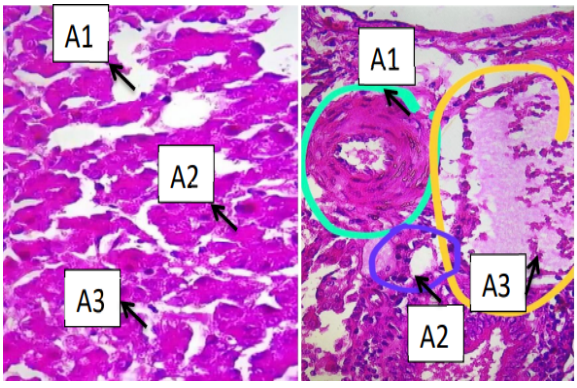

Figure 2. Overview of Microscopic Results of Hepar Tissue fixed with Bouin on HE staining. Description: Cell Nucleus (A1), Cytoplasm (A2), Sinusoid (A3), In Figure B, namely Kiernan triangle consists of artery (B1), ductus (B2), vein (B3)

The picture above shows that the hepatic tissue fixed with Bouin obtained results in the poor category, namely the blue color in the cell nucleus is less, the red color is eosin in the cytoplasm and connective tissue is less, and the color uniformity in the preparation is less. But can still be diagnosed.

Table 2. Microscopic Observation of Cerebrum Tissue fixed with 10% NBF and Bouin on Hematoxylin Eosin Staining

Sample	Microscopic Assessment	
	NBF 10%	Bouin
Slide 1	Good	Less Good
Slide 2	Good	Less Good
Slide 3	Good	Less Good

Based on the recapitulation of the table above, it shows that of the 3 preparations of cerebrum organs that were fixed using NBF fixative fluid 10% obtained results in the good category and 3 preparations of cerebrum organ which are fixed with Bouin, the results are in the poor category. Overview of the microscopic results of cerebrum tissue fixed with NBF 10% with 10% NBF can be seen in the figure below.

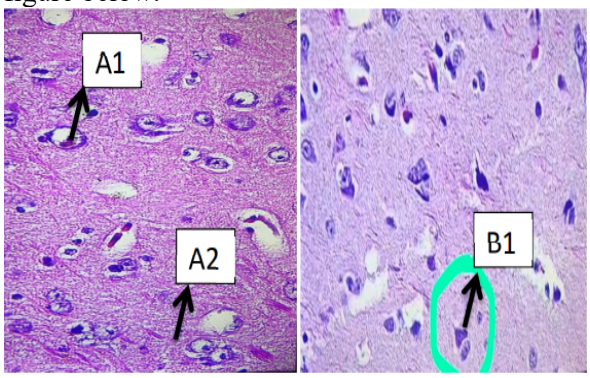

Figure 3. Microscopic results of cerebrum tissue fixed with 10% NBF on HE staining. Description: Cell Nucleus (A1) Cytoplasm (A2), Stela Cell (B1)

Microscopic results of cerebrum fixed with 10% NBF showed good results because of the bright blue color in the cell nucleus, red color (eosin) in the cytoplasm and connective tissue and uniform color in the preparation. As well as a clear stela cell shape during microscopic observation.

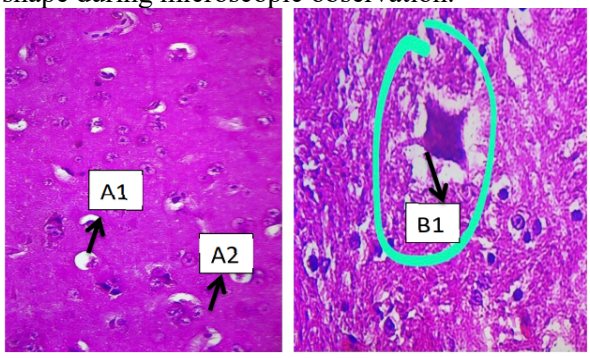

Figure 4. Overview of Microscopic Results of Cerebrum Tissue Speciated with Bouin.

Description: Cell nucleus (A1), Cytoplasm (A2), Stela Cell (B1)

The figure above is the microscopic result of cerebrum preparations fixed with Bouin. The same as the hepatic tissue above, it can be seen that the cerebrum tissue fixed with Bouin obtained staining results with poor categories, namely the blue color in the cell nucleus is less, the red color eosin in the cytoplasm and connective tissue is less, and the color uniformity in the preparation is less. But it can still be diagnosed. In addition, it can be seen that the cell shape is irregularly shrunken, broken but the stela cells can be seen clearly.

In this study, the fixation action is a stage that greatly determines the success of the readability indicators of microscopic preparations of a tissue. In addition, the coloring process used in this study has the principle of affinity between the tissue and the coloring material. The affinity is acidic will attract substances that are alkaline so that the color will be blue in the cell nucleus, while alkaline affinity will attract acidic substances so that the color becomes red in the cytoplasm (Setiawan, 2016).

The use of NBF fixation solution is often used because formalin buffer solution is a common solution for tissue fixation in many histology and histopathology studies, and the results show that the use of formalin buffer solution fixation fluid still provides good cell interpretation results. Because the buffer is a buffer solution, the acidity of the tissue pH remains consistent with the neutral pH in the presence of the solution in the solution, maintaining the properties and structure of the tissue. Neutral Buffer Formalin also has benefits in terms of ease of use, availability as a fixative in histology laboratories, and lack of explosive potential (Khristian & Inderiati, 2017).

According to Howan and Wilson (2014), the penetration power using Bouin's solution is faster and good for almost every type of tissue except mammalian kidney. However, tissues fixed with Bouin's solution require several rinses with 70% alcohol to remove picric acid and prevent disturbed staining results. So that it becomes a disadvantage of using Bouin's solution, which is very time consuming for the act of washing with alcohol. In addition, one of the compositions of the bouin solution, namely picric acid, carries additional risks in every handling and disposal, because it has mutagenic potential and explosive properties. In this study, the poor microscopic results on bouin-fixed tissues were due to the fact that after fixation, 70% alcohol was not rinsed (Howat & Wilson, 2014).

5. CONCLUSIONS AND SUGGESTIONS

Based on the results of the research and discussion conducted, it can be concluded that there are differences in the microscopic results of hepatic and cerebrum tissues fixed with 10% NBF liquid and Bouin. Good microscopic observation results based on the assessment indicator criteria are tissues fixed with 10% Neutral Buffer Formalin liquid. Suggestions for further researchers are to conduct research on comparison of fixation solutions by differentiating fixation time and different types of solutions.

BIBLIOGRAPHY

Alwi, M. A. (2016). Fiksasi 2 minggu pada gambaran histologi organ ginjal, hepar, dan pankreas tikus *Sparague Dawley* dengan pewarnaan hematoxylin-eosin.

Djuwita, I., Riyacumala, V., Mohamad, K., Prasetyaningtijas, W., & Nurhidayat, N. (2012). Pertumbuhan dan sekresi protein hasil kultur primer sel-sel serebrum anak tikus. *Jurnal Veteriner, 13*(2), 125–135.

Howat, W. J., & Wilson, B. A. (2014). Tissue fixation and the effect of molecular fixatives on downstream staining procedures. *Methods, 70*(1), 12–19. https://doi.org/10.1016/j.ymeth.2014.01.022

Kemenkes RI. (2015). *Histologi dan anatomi fisiologi manusia*. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 53*(9), 1689–1699. [Note: URL unclear and possibly incorrect; suggest verifying source.]

Khristian, E., & Inderiati, D. (2017). *Bahan ajar teknologi laboratorium medis (TLM) sitohistoteknologi*. Pusat Pendidikan Sumber Daya Manusia Kesehatan. [Note: Duplicate/irrelevant URLs removed; recommend verifying publisher details.]

Rusmiatik. (2011). Perbandingan fiksasi larutan Bouin dan formalin pada sediaan preparat histologi testis marmut. *Jurnal Bio Educatio, 3*(5), 1–5.

Setiawan. (2016). Optimalisasi metode automatic slide stainer untuk pewarnaan jaringan menggunakan haematoksilin-eosin. *Praktikum Kimia Analitik Penetapan Kadar, 1*. [Note: Title resembles a student manual or report; verify if it's a journal or institutional publication.]

Trianto, H. F., In, M., Pratiwi, S. E., & Suprianto, A. (2015). Perbandingan kualitas pewarnaan histologis jaringan testis dan hepar menggunakan fiksasi formalin metode intravital dan konvensional. *Jurnal Kesehatan Khatulistiwa, 1*, 2–15.

Yustisia, A., Winaya, I. B. O., Berata, I. K., & Samsuri, S. (2020). White rats brain histopathology changes in the form of congestion and perivascular edema due to tape yeast supplementation in feed. *Indonesia Medicus Veterinus, 9*(6), 910–919. https://doi.org/10.19087/imv.2020.9.6.910