

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service Available Online at:www.ijdsir.com

Volume – 7, Issue – 5, September – 2024, Page No. : 42 - 46

Efficacy of Various Postfixative Substitutes for Masson's Trichrome Staining

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Citation of this Article: Dr Rupa Barman, Dr Paramjit Singh, Dr Sanjeet Singh, Dr Nishant Singh, Dr Kanika Sharma, "Efficacy of Various Postfixative Substitutes for Masson's Trichrome Staining", IJDSIR- September – 2024, Volume –7, Issue - 5, P. No. 42 – 46.

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Postfixation is a method with the aim of preserving the structural and functional components in the tissue and cells as closely as possible in a state similar to that of a living state. For decades bouins fixative has been the dominating chemical solution when postfixation is required, making it the golden standard for postfixation around the world. Unfortunately, bouins fixative is toxic, causing regular skin irritations and is considered to be carcinogenic. The purpose of this study was to find a less toxic postfixation solution that could yield the same adequate results of preserving the cell morphology as bouins fixative. For that purpose, citrate buffer, gram's iodine, Coles hematoxylline, picromercuric alcohol postfixatives were chosen to be tested as alternative postfixatives and the results were compared to bouins fixated paraffin embedded tissues. The results obtained from tissues postfixated with bouins fixative and gram's iodine have excellent quality in the chosen criteria. Tissues postfixated in citrate buffer fixative did not yield adequate results compared to gram's iodine. We therefore conclude that gram's iodine is a possible postfixation alternative and could in some aspects replaces bouins fixative.

Keywords: Masson Trichrome, Fixative, Bouins, Grams iodine, Post Fixation

Introduction

The word "pathology" comes from two words "pathos" meaning suffering and "logos" meaning study of suffering respectively. It is a major field in the modern

diagnosis and thus for overall treatment. In simple words, it can be referred as study of disease. Almost all the hospital, in India and the world depend upon accurate pathological reports. It basically acts as a junction between medicine and science, using the cutting edge technology of genetics for better diagnosis, screening and treatment. It is done primarily for the benefit of the patient and also to make the treatment procedure much smoother for doctors, nurses and other healthcare professionals as well. Several people actually believe that pathology is restricted to autopsy or more commonly known as post mortem and thus applicable for only the ones present in the morgue. However, this notion is absolutely wrong, pathological screenings are carried out for disease which include, cough, blood, urine tests to biopsy for Cancer detection. Pathology is a very wide branch and over 19 types of specializations coexist and all obviously pathologists doesn't have the same work. Depending on their particular specializations, skill and interest, they work either in laboratories, hospitals, clinics or pathology centres. Often they provide valuable advice to the doctor and alongside decide for the best possible treatment in case of complicated diseases.¹"Trichrome" stains (Masson) are used for distinguishing collagen from muscle tissue. In general, they consist of nuclear, collagenous and cytoplasmic dyes in mordants such as phosphotungstic or phosphomolybdic acid. The replacement of muscle with fibrotic tissue is a hallmark of muscles from patients and mouse models. Since The Masson Trichrome staining procedure stains the collagen-rich fibrotic regions in blue, it is especially suited to assess and visualize the extent of fibrosis in dystrophic skeletal muscle on transverse muscle sections. In addition, and similar as with the H&E staining, Masson Trichrome stained sections also reveal adipose tissue, the variation

in muscle fiber diameters, the presence of small or rounded fibers (de-/regeneration) as well as centralized nuclei (indicative of regeneration).⁴

Aims

To compare the efficacy of post fixatives in masson trichrome staining

Objectives

- To evaluate the post fixative properties of Bouin's fixative, Citrate buffer, coles hematoxylin, grams iodine and picromercuric alcohol.
- To compare efficacy of post fixation between the above mentioned solutions in terms of nuclear and cytoplasmic staining characteristics and its applicability as best fixative in masson trichrome.
- To determine the best post fixative in masson trichrome staining.

Method

The study comprises of fresh goat liver, skin, heart, and artery which will be collected from a local slaughter houses. 150 tissue samples measuring 4 microns will be obtained from the same.

Sample	BF	CB	GI	CH/CB	PMA	TOTAL
OSMF	30	30	30	30	30	150

Table 1:BF [Bouin's fixative] CB [citrate buffer] GI[gram's iodine] CH[coles hematoxylline] CB[Celestineblue] PMA[picromercuric alcohol]

The tissues will be transferred to 10% normal buffered formalin solution for overnight fixation at room temperature. Dehydration of the tissues will be done using the graded concentrations of Propan-2-ol followed by their immersion in the clearing agent, Xylene. The samples will be embedded in paraffin wax and 4 micrometre sections will be obtatained. Then the samples will be heat fixed in a conventional oven at 60 degree celcius for 35 min. The slides will be deparaffinized and hydrated to distilled water. Postfix in

one of the above solutions for 1 hour at 60-degree C and then stained with masson trichrome using standard protocol. The prepared slides will be then subjected to scoring by two oral pathologists individually for the histological assessment of fixation to minimize the subjectivity using the following scoring system with little modification to assert if the tissues procured were sufficient or insufficient for the clinical diagnosis with / without any problems.

Cellular Quality among the Groups

In the Group A the cellular activity was excellent in nil subjects, ggod in 50% of the subjects and average in 50% of the subjects. In the Group B the cellular activity was average in 40% of the subjects and good in 40% of subjects. In the Group C the cellular activity was good in 80% of the subjects and in the Group D the cellular activity was average in 50% of the subjects and good in 50% of the subjects. In the Group E the cellular activity was average in 30% of the subjects, good in 50% of the subjects and excellent in 20% of the subjects. When the Chi-Square test was used to compare the four groups, it was found to be not statistically significant.

Table 2:

	Poor	Average	Good	Excellent	Mean	SD	
Group	0	5	5	0	2.50	0.527	
А	0%	50.0%	50.0%	.0%	2.50		
Group	0	4	4	2	2.90	0.788	
В	0%	40.0%	40.0%	20.0%	2.80		
Group	0	0	8	2	3 20	0.421	
С	0%	.0%	80.0%	20.0%	5.20	0.421	
Group	0	5	5	0	2.50	0.527	
D	0%	50.0%	50.0%	.0%	2.50	0.327	
Group	0	3	5	2	2 90	0.737	
Е	0%	30.0%	50.0%	20.0%	2.00	0.727	
Chi Square test - 10.716							
P VALUE- 0.261 (Non-Sig)							

Nuclear Quality among the Groups

In the Group A the nuclear activity was excellent in nil subjects, good in 30% of the subjects and average in 70% of the subjects. In the Group B the nuclear activity was average in 10% of the subjects, good in 40% of subjects and excellent in 50% of the subjects. In the Group C the nuclear activity was good in 60% of the subjects and in the Group D the nuclear activity was average in 60% of the subjects and poor in 40% of the subjects. In the Group E the nuclear activity was average in 20% of the subjects, good in 50% of the subjects and excellent in 30% of the subjects. Statistical analysis using the Chi-Square test revealed a meaningful intergroup comparison among the four categories. Table 3:

	Poor	Average	Good	Excellent	Mean	SD
Group A	0	7	3	0	2.30	0.483
Group IX	.0%	70.0%	30.0%	.0%		
Group B	0	1	4	5	3.40	0.699
Group D	.0%	10.0%	40.0%	50.0%		
Group C	0	3	6	1	2.80	0.632
r -	.0%	30.0%	60.0%	10.0%		
Group D	4	6	0	0	1.60	0.516
Group D	40.0%	60.0%	.0%	.0%		
Group E	0	2	5	3	3 10	0.737
	.0%	20.0%	50.0%	30.0%	5.10	0.757
Chi Square test – 38.041						
P VALUE- 0.001 (Sig)						

Graph 1:



Graph 2: Graph 3:



Staining Quality among the Groups

In the Group A the Staining Quality was excellent in nil subjects, good in 60% of the subjects and average in 40% of the subjects. In the Group B the Staining Quality was average in 60% of the subjects, good in 20% of subjects and excellent in 20% of the subjects. In the Group C the Staining Quality was good in 100% of the subjects and in the Group D the Staining Quality was average in 30% of the subjects and good in 70% of the subjects. In the Group E the Staining Quality was average in 40% of the subjects, good in 40% of the subjects and excellent in 20% of the subjects. The results of the Chi-Square test showed that there was a statistically significant difference in the four groups when compared to each other.

Table 4:

	Poor	Average	Good	Excellent	Mean	SD	
Group	0	4	6	0	3.60	0.516	
А	.0%	40.0%	60.0%	.0%			
Group	2	6	2	2	3.00	0.666	
В	20.0%	60.0%	20.0%	20.0%			
Group	0	0	10	0	4.00	0.001	
С	.0%	.0%	100.0%	.0%			
Group	0	3	7	0	3.70	0.483	
D	.0%	30.0%	70.0%	.0%			
Group	2	4	4	2	3 20	0 788	
Е	20.0%	40.0%	40.0%	20.0%	5.20	0.700	
Chi Square test – 17.921							
P VALUE- 0.023 (Sig)							





Overall Quality among the Groups

In the Group A the Overall Quality was excellent in nil subjects, good in 70% of the subjects and average in 30% of the subjects. In the Group B the Overall Quality was average in 60% of the subjects, good in 10% of subjects and excellent in 30% of the subjects. In the Group C the Overall Quality was good in 90% of the subjects and in the Group D the Overall Quality was average in 40% of the subjects and good in 60% of the subjects. In the Group E the Overall Quality was average in 40% of the subjects, good in 50% of the subjects. Statistical analysis using the Chi-Square test revealed a meaningful intergroup comparison among the four categories.

Table 5:

	Poor	Average	Good	Excellent	Mean	SD	
Group	0	3	7	0	3.70	0.483	
А	.0%	30.0%	70.0%	.0%			
Group	3	6	1	3	2.80	0.632	
В	30.0%	60.0%	10.0%	30.0%			
Group	0	1	9	0	3.90	0.316	
С	.0%	10.0%	90.0%	.0%			
Group	0	4	6	0	3.60	0.516	
D	.0%	40.0%	60.0%	.0%			
Group	1	4	5	1	3.40	0.699	
Е	10.0%	40.0%	50.0%	10.0%	5.40	0.077	
Chi Square test – 19.146							
P VALUE- 0.012 (Sig)							

Conclusion

Since bouins fixative is known to cause cancer in humans, it is imperative that histopathology laboratories switch to less harmful postfixatives. This research shows that several postfixatives may be made in a lab and are good for histopathology of common surgical samples. The histomorphological characteristics of sections dyed with Masson Trichrome have been the exclusive focus of investigation. Particular this histochemical and immunohistochemistry procedures did not account for tissue properties. Nonetheless, these post fixatives are just as effective as traditional bouins fixation for routine histopathology utilizing diagnostic the Masson Trichrome stain, and they significantly lower formalin vapor density while improving laboratory air quality.

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