MASSON TRICHROM STAINING METHOD COMPARISON AND EVALUATION

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Introduction

The Masson trichrom staining is a three-colour staining used in histology.(1) It has been used for a long time and the protocol has changed over the years at the pathology department of Næstved. The staining has many specific uses but it is suitable to distinguish cells from connective tissue. Muscle tissue, connective tissue/collagen and cytoplasm all get a different colour with a dark nucleus. (2)

Objective

The purpose of this project is to make a comparison between eight protocols and their results. To make an overview on which protocol is the best staining method. With a second interest in seeing if mixing the dye and a heteropoly acid, phosphomolybdic acid (PMA), is beneficial.

Method

Three different protocols were used as the base of this experiment. (see table 1-3) First of all the currently used one, second is one from a book: "Histological and histochemical methods theory and practice"(3), and lastly the old one that was used in 2003. Based on these three another five were formed to come to a total of eight protocols. Every protocol was executed *in duplo* with a multiblock with five types of tissue on it: stomach, skin, colon, small intestine and just intestine.

References:

1) D. BJ. Bancroft's Theory adn practice of histological techniques. 7th ed. Kim SS, Christopher L, editors: churchill livingstone; 2012 1/06/2022. 560 p.
2) stingerfix. Masson trichrom vlek.
3) A. KJ. Histological and histochemical methods theory and practice 5ed. UK: scion; 20015. 554 p.

Table 1: currently used protocol

Deparaffinating	
Weigert-Lillies iron haematoxylin	8 min
Running water- thorough rinse	
1,2% watery picric acid	5 min
H_2O demi – Fast rinse	
1% ponceau BS in 1% acetic acid	10 min
1% watery PMA	10 min
2,5% methyl blue in 2% acetic acid	2 min
H_2O demi – Fast rinse	
1% watery PMA	2 min
Differentiating in 1% acetic acid	3 min
Dehydrate 3 x in 99% ethanol	
Histoclear ll	
Coverslip with mounting medium	

Table 2: protocol from the book

De-wax bring to 70% alcohol	
Aqueous picric acid at 55-60°C	60 min
Running water – thorough rinse	2 min
Weigert's or Lillies iron-hematoxylin	3 min
Running water	1 min
Ponceau 2R- acid fuchsine	4 min
Acidified water -rinse	
1% PMA	5 min
Fast green FCF	4 min
Immerse in 2 changes of acidified water	30 sec
Dehydrate 3x 99% ethanol	
Clear in xylene and cover slip	

Table 3: old protocol from 2003

Dewax bring to 70% alcohol	
Bouin solution at 54°C	60 min
Running water – thorough rinse	
Weigert-Lillies solution	5 min
Running water	5 min
Ponceau 2R- acid fuchsine solution	5 min
H_2O demi	
1% PMA	5 min
2,5% Methyl blue in 2% acetic acid	5 min
Differentiate	2 min
Dehydrate and cover slip	

Results

The results of this experiment are seen under the microscope. To compare the different methods they will be scored on four different pointers on a scale of five, with five being the best result and one being the worst (see table 4). They were graded based on the looks of figure 1+2.

Table 4: results of the staining based on the four pointers and a total score

Protocol	Contrast	Clarity	Environment and safety	Difficulty	Total
1. Current protocol	4	5	4	3	16/20
2. Protocol from the book	5	4	2	2	13/20
3. Old protocol from 2003	4	4	3	3	14/20
4. Alternative protocol mixing ponceau and methyl blue with PMA	1	3	4	4	12/20
5. Alternative protocol without differentiating	3	2	4	3	12/20
6. Alternative protocol with light green SF	1	1	3	3	8/20
7. Protocol from the book with methyl blue	5	5	3	2	15/20
8. Old protocol without the Bouin's solution step	1	3	4	4	12/20

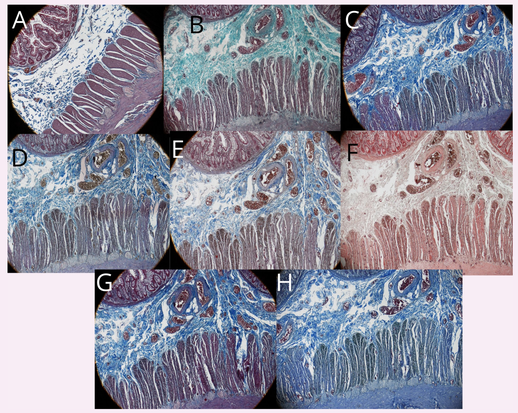

Figure 1: All the pictures are the colon stained on a x40 magnification. 1A) the current protocol. 1B)staining from the book. 1C) old protocol 1D) alternative protocol mixing dyes with PMA. 1E) alternative protocol without differentiating. 1F) alternative protocol with light green SF instead of methyl blue. 1G) protocol from the book with methyl blue instead of fast green FC. 1H) old protocol without Bouin's solution step

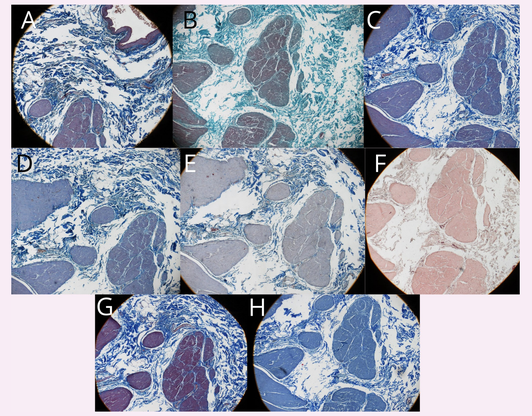

Figure 2: All pictures are the same muscle bundle in the stomach stained on a x40 magnification. 2A) current protocol 2B) protocol from the book 2C) old protocol from 2003. 2D) alternative protocol mixing dyes with PMA 2E) alternative protocol without differentiating 2F) alternative with light green SF instead of methyl blue 2G) protocol from the book with methyl blue instead of fast green SF. 2H) old protocol without Bouin's solution step

Conclusion: the staining method currently used at the department is the best protocol to use for this staining based on the outcome of this experiment. When something fails, the old protocol is a good alternative but the method from the book with methyl blue instead of green is a better replacement. Furthermore is it clear that mixing the dye and PMA is not beneficial for the stain.