Masson 染色实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
脱水机	常州市中威电子仪器有限公司	TSJ-SD
包埋机	常州市中威电子仪器有限公司	BMJ-A
病理切片机	赛默飞世尔科技有限公司	SHANDON FINESSE 325
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	常州市中威电子仪器有限公司	PHY-III
防脱载玻片	湖北百奥斯生物科技有限公司	BP0510
正置显微镜	奥林巴斯有限公司	CX-31
成像系统	日本滨松光子学株式会社	NanoZoomer®S360

2、主要试剂及货号

名称	厂家	型号
无水乙醇	国药集团化学试剂有限公司	100092683
环保透明剂	同声科技	
环保封片剂	同声科技	
Bouin 固定液	湖北百奥斯生物科技有限公司	BP0140
Masson 三色套盒	湖北百奥斯生物科技有限公司	BP0310

二、实验步骤

1、组织切片常规脱蜡至水，将脱好水的切片浸泡于 Bouin 氏液或 Zenker 氏液过夜，流水冲洗干净。

2、Harris 苏木素或铁苏木素染色 5-10 分钟，流水稍洗。

3、用 0.8%-1%盐酸酒精分化，流水冲洗数分钟；碳酸锂返蓝数秒，流水冲洗。

4、丽春红酸性品红染液染 5-10 分钟，流水稍冲洗。

5、磷钼酸溶液处理约 5 分钟，不用水洗，直接用苯胺蓝染液复染 5 分钟。

6、1%冰醋酸处理 1 分钟，95%酒精脱水多次。

7、无水酒精脱水，二甲苯透明，中性树胶封固。

三、结果判读

胶原纤维呈蓝色（用苯胺蓝液复染）或绿色（用亮绿复染）。胞质、肌纤维和红细胞呈红色。细胞核呈蓝褐色。

四、注意事项

1、组织用 Bouin 氏液或 Zenker 氏液固定为佳。若已用 10%甲醛液固定，切片可在脱蜡至水后，再放入 Bouin 氏液常温作用一晚或置 37℃温箱内 1-2 小时，然后流水冲洗切片至黄色消失再进行染色。

2、磷钼酸处理时需要镜下控制，见肌纤维呈红色，胶原纤维呈淡红色即可。

3、冰醋酸分化过程中应严格控制时间，若分化过度，胶原蓝色太浅；若分化不足，易与红色叠加呈紫蓝色。

Experimental Report on Masson Staining

1. Instruments and key reagents

1.1 Instruments

Instrument	Manufacture	Specifications/Model
Tissue processor	Changzhou Zhongwei Electronics Co., Ltd	TSJ-SD
Tissue embedder	Changzhou Zhongwei Electronics Co., Ltd	BMJ-A
Microtome	ThermoFisher Scientific	SHANDON FINESSE 325
Freezing table	Wuhan Junjie Electronics Co., Ltd	JB-L5
Water bath - Slide drier	Changzhou Zhongwei Electronics Co., Ltd	PHY-III
Slide	Hubei BIOSSCI Biotech Co., Ltd	BP0510
Upright microscope	Olympus	CX-31
Digital scanner	HAMAMATSU PHOTONICS	NanoZoomer®S360

1.2 Key reagents

Reagent	Manufacture	Specifications/Model
Ethanol	Sinopharm	100092683
Clearer	Wuhan Tongsheng Technology Development Co., Ltd	
Neutral balsam	Wuhan Tongsheng Technology Development Co., Ltd	
Bouin’s fix solution	Hubei BIOSSCI Biotech Co., Ltd	BP0140
Masson staining kit	Hubei BIOSSCI Biotech Co., Ltd	BP0310

2. Procedures

2.1 Deparaffinization and rehydration.

2.1.1 Tissue sections were immersed in clearer for 10min. Repeat this step two times, gently shaking off excess liquid between each step.

2.1.2 Tissue sections were immersed in progressively more dilute ethanol solutions and ultimately immersed in distilled water to rehydrate the tissue: Absolute ethanol for 5min, 95% ethanol for 5min, 85% ethanol for 5min, 75% ethanol for 5min. Rinsing with distilled water for 1min.

2.1.3 The dehydrated tissue sections were immersed in Bouin's solution or Zenker's solution overnight, then, rinsed with running water.

2.2 Sections were stained with hematoxylin solution (Harris) or iron hematoxylin for 5-10 minutes and slightly washed with running water.

2.3 Sections were differentiated with 0.8% - 1% hydrochloric acid alcohol and washed with running water for several minutes. Sections can also be treated with lithium carbonate solution to be bluer and washed with running water.

2.4 Sections were stained with ponceau acid fuchsin solution for 5-10 minutes and washed with running water.

2.5 Sections were treated with phosphomolybidic acid solution for about 5 minutes and then stained with aniline blue solution for 5 minutes without washing.

2.6 Sections were treated with 1% glacial acetic acid for 1 minute and dehydrated with 95% alcohol for several times.

2.7 The tissue sections were dehydrated with absolute alcohol and transparent with xylene, then mounted with neutral balsam. Examination with microscope.

3. Results

Collagen fibers were blue (counterstained with aniline blue solution) or green (counterstained with bright green solution). The cytoplasm, muscle fibers and red blood cells are red. Nuclei are blue brown.

4. Attentions

4.1 It is better to fix the tissue with Bouin's solution or Zenker's solution. If it has been fixed with 10% formaldehyde solution, the tissue sections can be dewaxed to water, and then put into Bouin's solution overnight at room temperature or put into a 37℃ incubator for 1-2 hours. Then, washed sections with running water until the yellow disappeared before staining.

4.2 It need to be controlled under microscope during treatment of phosphomolybdic acid. It can be seen that muscle fibers are red and collagen fibers are light red.

4.3 It is important to control the time of differentiation with glacial acetic acid, if the differentiation is excessive, the blue of collagen will be too light. If the differentiation is insufficient, it is easy to overlap with red and become purple blue.

5. Technical support

The technical support of Masson staining experiment is provided by Hubei BIOSSCI Biotech Co., Ltd (Wuhan Changyan Pathology technology Co., Ltd).

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