Gordon-Sweets 网状纤维染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
病理切片机	上海徕卡仪器有限公司	RM2016
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230
载玻片	Wanwu	
正置光学显微镜	日本尼康	NIKON ECLIPSE E100
成像系统	日本尼康	NIKON DS-U3

2、 主要实验试剂

试剂名称	厂家	货号
无水乙醇	国药集团化学试剂有限公司	100092683
二甲苯	国药集团化学试剂有限公司	10023418
中性树胶	国药集团化学试剂有限公司	10004160
氨水	国药集团化学试剂有限公司	10002118
硝酸银	国药集团化学试剂有限公司	10018461
KOH	国药集团化学试剂有限公司	10017018
高锰酸钾	国药集团化学试剂有限公司	10017318
硫酸	国药集团化学试剂有限公司	10021618
草酸	国药集团化学试剂有限公司	10014818
硫酸铁铵	国药集团化学试剂有限公司	10001818
甲醛	国药集团化学试剂有限公司	100100620

二、 试剂配制

1、10%的硝酸银：10g 硝酸银+100ml 蒸馏水

2、3%的 NaOH： 3g 氢氧化钠+100ml 蒸馏水

3、0.5%的高锰酸钾：0.5g 高锰酸钾+100ml 蒸馏水

4、0.5%的硫酸：500ul 浓硫酸+100ml 蒸馏水

5、2%的草酸：草酸 2g+100ml 蒸馏水

6、2%的硫酸铁铵：硫酸铁铵 2g+100ml 蒸馏水

7、10%的中性甲醛：浓甲醛 10ml+蒸馏水 90ml

三、**实验步骤**

网状纤维孵育液：10%的硝酸银 2ml，逐滴加入浓氨水，边加边摇匀，出现沉淀后继续滴加浓氨水，至沉淀恰好溶解（若沉淀在滴加浓氨水时的一瞬间澄清，说明氨水过量，需要重新配制），加入 3%的 NaOH 2ml，再次形成沉淀，逐滴加入浓氨水，至沉淀刚好溶解，加入超纯水定容至 40ml。（用棕色瓶盛装，4℃保存 1-2 个月，用前复温）

1、石蜡切片脱蜡至水：依次将切片放入二甲苯I20min-二甲苯II20min-无水乙醇I5min-无水乙醇II5min-75%酒精 5min，自来水洗。

2、组织酸化：组画笔画圈圈住组织，滴加酸化液（0.5%的高锰酸钾与 0.5%的硫酸 1:1 混匀）于组织上氧化 5min，切片入两缸超纯水浸洗共 10s，稍甩干切片多余水分。

3、漂白：滴加 2%的草酸于组织上漂白 2min，切片入 3 缸超纯水浸洗，各 5s，稍甩干切片多余水分。

4、媒染剂染色：滴加 网状纤维染液F于组织上媒染15min（避光），切片入3缸超纯水浸洗，各5s，稍甩干切片多余水分。

5、网状纤维孵育液染色：滴加网状纤维孵育液于组织上处理5min（避光），切片入3缸超纯水浸洗，各5s，稍甩干切片多余水分。

6、还原：滴加 10%的中性甲醛液于组织上还原 3min（避光，3min 后肉眼观察组织未出现黄棕色时，可延长还原时间至 5min，显色效果可能会有改善），切片入 3 缸超纯水浸洗，各 5s，稍甩干切片多余水分。

7、脱水封片：切片依次放入无水乙醇 I 5min -无水乙醇 II 5min-无水乙醇III5min -二甲I5min-二甲苯II5min 透明，中性树胶封片。

四、结果判读：

网状纤维呈黑色，背景棕黄色。

五、注意事项：

1、网状纤维孵育液避光 4℃条件下可保存 7 天左右（当短时间内染液中出现黑色颗粒，或者染液浑浊也可重新配制），使用前复温；NaOH、硫酸和草酸可以不避光常温保存，其余染色全部避光常温保存。

2、染色过程中，使用避光的染色盒进行染色。

3、在配制银氨液过程中，沉淀恰好溶解要严格把控。

Copper red acid staining experimental report

1. Lab equipment and reagents

A. Lab equipment

Items	Manufacturer	Model
Pathology microtome	Shanghai Leica Instruments Co., Ltd.	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd.	JB-L5
Water Bath-Slide Drier	Zhejiang Jinhua Kedi Instrumental Equipment CO.,LTD	KD-P
Laboratory oven	Tianjin Labotery Instrument Equipment Co., Ltd.	GFL-230
Microscope slide	Wanwu	
Upright optical microscope	Nikon Japan	Nikon Eclipse E100
Imaging system	Nikon Japan	NIKON DS-U3

B. Chemical Reagents

Items	Manufacturer	Model
Absolute ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160
Aqueous ammonia	Sinopharm Chemical Reagent Co., Ltd.	10002118
Silver nitrate	Sinopharm Chemical Reagent Co., Ltd.	10018461
KOH	Sinopharm Chemical Reagent Co., Ltd.	10017018
Potassium permanganate	Sinopharm Chemical Reagent Co., Ltd.	10017318
sulfuric acid	Sinopharm Chemical Reagent Co., Ltd.	10021618
Oxalic acid	Sinopharm Chemical Reagent Co., Ltd.	10014818
Ammonium iron(III) sulfate	Sinopharm Chemical Reagent Co., Ltd.	10001818
Formaldehyde	Sinopharm Chemical Reagent Co., Ltd.	100100620

2. Reagent preparation

1. 10% silver nitrate: 10g silver nitrate + 100ml distilled water
2. 3% NaOH: 3g sodium hydroxide + 100ml distilled water
3. 0.5% potassium permanganate: 0.5g potassium permanganate + 100ml distilled water
4. 0.5% sulfuric acid: 500ul concentrated sulfuric acid + 100ml distilled water
5. 2% oxalic acid: oxalic acid 2g+100ml distilled water
6. 2% ammonium iron(III) sulfate: ammonium iron(III) sulfate 2g+100ml distilled water
7. 10% neutral formaldehyde: concentrated formaldehyde 10ml + distilled water 90ml

3. Experimental steps

Reticular fibers incubation solution: 10% silver nitrate 2ml, add concentrated ammonia drop by drop, shake container while adding, and then continue to add concentrated aqueous ammonia drop after precipitation occurs, until the precipitation is just dissolved (if the precipitation is clarified at the moment when adding the concentrated ammonia drop by drop, it means that the ammonia is excessive and should be reconstituted), add 3% NaOH 2ml until precipitate form again, add concentrated aqueous ammonia drop by drop, until the precipitation is just dissolved, add add ultra-pure water to make up to 40ml. (Packed in a brown bottle, stored at 4℃ for 1-2 months, rewarm before usage)

(1) Paraffin section deparaffinization and rehydration: put slides into xylene I 20minutes-xylene II 20 minutes-absolute ethanol I 5 min-absolute ethanol II 5 min-75% alcohol for 5 min, tap water washing.
(2) Tissue acidification: draw a circle to enclose the tissue, then add acidification solution (0.5% potassium permanganate and 0.5% sulfuric acid 1:1 mixed) drop by drop to oxidize the tissue for 5 minutes, put the slides into two cylinders of ultra-pure water washing for 10s, and and dry the slides to remove excess water.
(3) Tissue bleaching: dorp 2% oxalic acid on the tissues bleaching for 2min, put the slides in ultra-pure water for 3 times, 5s each, and dry the slides to remove excess water.
(4) Mordant staining: drop the Gordon-Sweets staining solution F on the tissue for 15min (light-proof), put the slides in ultra-pure water washing for 3 times, 5s each, and dry the slides to remove excess water.
(5) Gordon-Sweets incubation solution staining: drop Gordon-Sweets incubation solution on the tissues for 5 min (light-proof), put the slides in ultra-pure water washing for 3 times, 5s each, and dry the slides to remove excess water.
(6) Reduction: drop 10% neutral formaldehyde solution on the tissue for 3min (lightproof, if the does not appear yellow-brown after 3min, the reduction time can be extended to 5min, the color rendering effect may be improved), put the slides in ultra-pure water washing for 3 times, 5s each, and dry the slides to remove excess water.
(7) Dehydration and sealing: put the slides into absolute ethanol I 5min-absolute ethanol II 5min-absolute ethanol III 5min-dimethyl I 5min-xylene II 5min transparent, neutral balsam sealing.
(8) Microscope examination, image collection and analysis.

4. Results

The reticular fibers are black and the background is brownish yellow.

5. Note

1. The reticular fibers incubation solution can be stored for about 7 days at 4℃(lightproof) (when black particles appear in the staining solution for a short period of time, it can be reconstituted if the staining solution is muddy), rewarm before usage; NaOH, sulfuric acid and oxalic acid can be stored at room temperature without lightproof, and the rest of staining solution should be stored at room temperature with lightproof.

2. During the staining process, use a light-proof staining jack for staining.

3. In the process of preparing silver ammonia solution, precipitation dissolution should be strictly controlled.