六胺银染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
包埋机	武汉俊杰电子有限公司	JB-P5
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
六胺银染液套装	Wanwu	G1059
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

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，蒸馏水洗 1 遍。

3、媒染剂染色：切片入六胺银（PASM）染液 A 浸泡过夜，水稍洗。

4、工作液配制：六胺银（PASM）染液 D：六胺银（PASM）染液 C 20:1 混合成六胺银储备液(现配现用)取六胺银储备液 20ml，加入蒸馏水 15ml，再加入六胺银（PASM）染液 E2ml，混合均匀。

5、组织酸化：切片入六胺银（PASM）染液 B 处理 15 至 20min，蒸馏水洗 3-5 次；

6、孵育染色：工作液提前置于 60℃烤箱预热，滴加预热好的工作液于组织切片上，加盖孵育(56℃-59℃)；

7、显色：孵育 40min 后将切片拿出在镜下观察（观察时工作液不用洗去），肾脏则以肾小球毛细血管基膜呈黑色为准，如果着色不够深，则继续放入烤箱孵育；肺则以真菌变呈黑色为准（如果没有真菌则以支气管变黑为标准）。以后每隔 10min 取出切片在镜下观察，直至染色结果满意为止，蒸馏水洗 3 遍。

8、六胺银（PASM）染液 F 处理：六胺银（PASM）染液 F 稍处理，蒸馏水洗 1 遍。

9、复染六胺银（PASM）染液 F（根据需要选做）：85%、95%的酒精梯度脱水，切片入六胺银（PASM）染液 F 染液 20s。

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

11、显微镜镜检，图像采集分析。

三、结果判读：

肾小球囊基膜、肾毛细血管球基膜和肾小管上皮基膜呈黑色，真菌、胶原纤维也呈灰黑色或棕黑色，背景红色。

四、注意事项：

1、在孵育之前切片必须用蒸馏水清洗干净，配制六胺银储备液和工作液的容器必须洗干净，否则易出现杂质。

2、六铵银储备液和工作液必须现配现用；

PASM staining experimental report

1. Lab equipment and reagents

A. Lab equipment

Items	Manufacturer	Model
Dehydrator	DIAPATH	Donatello
embedding machine	Wuhan Junjie Electronics Co., Ltd.	JB-P5
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 alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
PASM staining kit	Wanwu	G1059
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. Experimental steps

(1) Paraffin section deparaffinization and rehydration: put the 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 and then distilled water washing.
(2) Periodic acid-silver metheramine staining: slides into periodic acid-silver metheramine (PASM) staining solution A, soak overnight, tap water washing.
(3) Working solution preparation: PASM staining solution D: PASM staining solution C 20:1

mixed into PASM stock solution, take PASM stock solution 20ml, adding 15ml of distilled water, And then add PASM staining solution E 2ml and well mixed.

(4) Tissue acidification: put the slides into PAS staining solution B for 15 to 20 minutes, and distilled water washing for 3-5 times;

(5) Incubation and staining: the working solution placed in a 60°C oven for preheating in advance, and add the preheated working solution in the slides drop by drop, incubation with cover (56℃-59℃);

(6) Color rendering: after 40 minutes of incubation, take out the slides and observe under a microscope (the working solution doesn't need to be washed away during observation), and the kidney is subject to the glomerular capillary basement membrane being black. If the coloring is not deep enough, continue to incubate in the oven; Lungs are subject to fungus turning black (if there is no fungus, the bronchial darkening is the standard). After that, take out the slides and observe under a microscope every 10 minutes until staining result is okay, distilled water washing for three times.

(7) PASM staining solution F treatment: after treatment of PASM staining solution F, wash once with distilled water.

(8) Counterstaining PASM staining solution F (optional): 85%, 95% alcohol for gradient dehydration, put the slides into PASM staining solution F for 20 seconds.

(9) Dehydration and sealing: put the slides into absolute ethanol I 5min- absolute ethanol II 5min-absolute ethanol III 5min- xylene I 5min-xylene II 5min transparent, neutral balsam sealing.

(10) Microscope examination, image collection and analysis.

3. Results

The glomerular basement membrane, the renal capillary basement membrane and the renal tubular epithelial basement membrane are black, and the fungi and collagen fibers are also grayish black or brownish black with red background.

4. Note

1. The slides should be cleaned with distilled water before incubation, and the container for preparing the PASM stock solution and working solution must be cleaned, otherwise, impurities will easily appear.

2. PASM stock solution and working solution should be ready for use;