抗酸染色实验报告

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	G6004
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
抗酸染液套装	Wanwu	G1047
分化液	Wanwu	G1039
返蓝液	Wanwu	G1040
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

抗酸工作液的配制：抗酸染液 A 10ml+抗酸染液 B 90ml，混合均匀。

抗酸分化液的配制：取 10ml 浓盐酸用无水乙醇定容至 1000ml

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，水洗。

2、抗酸染色：切片入抗酸工作液中染色 30min，流水冲洗；

3、背景分化：抗酸分化液快速分化2s，水洗终止分化，可反复操作，至镜检时抗酸菌为紫

红色，背景基本无色即可；

4、硫酸分化：若切片背景较深，盐酸分化不掉时，切片入抗酸染液C快速分化1s，快速自来水洗（反复冲洗直至切片上无硫酸残留），重复操作至阳性菌呈紫红色，背景无色。

5、抗酸染液D染色：切片入抗酸染液D染30s，自来水洗，分化液分化2s，自来水洗，返蓝液返蓝3-5s，流水冲洗。

6、**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-二甲苯I5min-二甲苯II5min透明，中性树胶封片。

7、显微镜镜检，图像采集分析。

三、结果判读：

抗酸菌呈紫红色丝状，细胞核呈浅蓝色。

四、注意事项：

1、两次分化都应注意分化程度，分化过度，将抗酸菌的紫红色分掉，分化不足，背景红色。

2、苏木素复染过程中要浅染，不能影响抗酸菌的观察。

Acid-fast 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	G6004
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
Acid-fast staining kit	Wanwu	G1047
Differentiation solution	.Wanwu	G1039
	Wanwu	G1040
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. Experimental steps

Preparation of Acid-fast staining solution: Acid-fast staining solution A 10ml + Acid-fast staining solution B 90ml, well mixed.

Preparation of acid-fast differentiation solution: take out 10ml of concentrated hydrochloric acid and dilute to 1000ml with absolute ethanol.

(1)Paraffin section deparaffinize and rehydrate: 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, then tap water washing.

(2)Acid-fast staining: put the slides into the Acid-fast staining solution stain for 30 minutes and running water washing;

(3)Background differentiation: acid-fast differentiation solution rapidly differentiates for 2s. Terminate differentiation by water washing, which can be repeated. By the time of microscope examination, the acid-fast bacteria are purple-red, and the background is basically colorless;
(4)Sulphuric acid differentiation: If the background is deep and the hydrochloric acid differentiation does not fall, put the slides into acid-red staining C for rapid differentiation for 1s, tap water washing (repeatedly rinse until no sulfuric acid remains on the slide), and repeat operations until the positive bacteria are purple-red , the background is colorless.
(5)Acid-fast solution D staining: put the slides into acid-fast solution D stain for 30s, tap water washing, differentiate for 2s, tap water washing, return blue solution to blue for 3-5s, rinse with running water.
(6)Dehydration and sealing: put the slides into absolute ethanol I 5min-anhydrous ethanol II 5min-anhydrous ethanol III 5min-xylene I 5min-xylene II 5min -transparent, neutral balsam sealing.
(7)Microscope examination, images collection and analysis.

3. Results

The acid-fast bacteria are purple-red filaments, and the nucleus is light blue.

4. Note

1. Pay attention to the degree of differentiation, over-differentiate, and differentiate the purple color of acid-fast bacteria., insufficient differentiation, the background is red.
2.Light staining during hematoxylin counterstaining process, should not affect the observation of acid-fast bacteria.