



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	Servicebio	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	Servicebio	G1047
分化液	servicebio	G1039
返蓝液	servicebio	G1040
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

1. 抗酸工作液的配制：抗酸染液 A 10ml+抗酸染液 B 90ml，混合均匀。
2. 抗酸分化液的配制：取 10ml 浓盐酸用无水乙醇定容至 1000m。
3. 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

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醇II5min-75%酒精 5min, 水洗。

4. **抗酸染色:** 切片入抗酸工作液中染色 30min, 流水冲洗;
5. **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;
6. **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。
7. **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。
8. **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。
9. 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

1.1 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	Servicebio	G6004
Upright optical microscope	Nikon Japan	Nikon Eclipse E100
Imaging system	Nikon Japan	NIKON DS-U3

1.2 Chemical reagents

Items	Manufacturer	Model
Absolute alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Acid-fast staining kit	Servicebio	G1047
Differentiation solution	.Servicebio	G1039
	Servicebio	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.



- 2.1 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.
- 2.2 Acid-fast staining: put the slides into the Acid-fast staining solution stain for 30 minutes and running water washing;
- 2.3 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;
- 2.4 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.
- 2.5 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.
- 2.6 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.
- 2.7 Microscope examination, images collection and analysis.

3. Results

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

4. Note

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