革兰氏染色实验报告

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
二甲苯	国药集团化学试剂有限公司	10023418
革兰氏染液套装	Wanwu	G1065
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。

2、革兰氏染色：切片滴加革兰氏染液 A 滴染 10s-30s，水洗，甩干；滴加革兰氏染液 B1min，水洗，甩干；巴氏吸管吸取革兰氏染液 C 从载玻片磨砂面一端冲洗组织进行分化，至切片流下的革兰氏染液 C 呈无色即可，水洗，甩干；将切片浸革兰氏染液 D 溶液 1s 水洗，镜检，烤箱烤干。

3、脱水：将烤干的切片快速入三缸无水乙醇快速脱水，依次 1s、3s、5s。

4、透明封片：切片放入干净的二甲苯5min透明，中性树胶封片。

5、显微镜镜检，图像采集分析。

三、结果判读：

革兰氏阳性菌呈紫色，革兰氏阴性菌呈红色。

四、注意事项：

1、染色过程中的革兰氏染液 C 分化是关键步骤，需注意分化程度。

2、尽量不要重复染色，分化液在脱去片子颜色的同时也改变了细胞壁的通透性，影响了最后结果的准确性。

3、冬季气温较低时，染色时间可适当延长。

Gram Staining Experiment Result

I. Experimental equipment and reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
Dehydrator	DIAPATH	Donatello
Embedding center	Wuhan Junjie Electronics Co., Ltd.	JB-P5
Pathological microtome	Shanghai Leica Instrument Co., Ltd.	RM2016
Cooling plate	Wuhan Junjie Electronics Co., Ltd.	JB-L5
Tissue spreading water bath	Zhejiang Jinhua Kedi Instrumental Equipment Co., Ltd.	KD-P
Oven	Tianjin Leibo Terry Equipment Co., Ltd.	GFL-230
Microscope slide	Wanwu	
Upright electron microscope	JAPAN NIKON	NIKON ECLIPSE E100
Imaging system	JAPAN NIKON	NIKON DS-U3

2. Main experiment reagents

Reagent name	Manufacturer	Item No.
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Gram staining solution kit	Wanwu	G1065
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

II. Experimental procedure

1. Deparaffinize the paraffin sections to water: Place the sections in sequence in Xylene I for 20min - Xylene II for 20min - Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - 75% Ethyl alcohol for 5min , wash with tap water.

2. Gram staining: Drop Gram staining solution A on the sections to stain for 10s-30s, wash and shake to make dry. Then add Gram staining solution B for 1min, wash and shake to make dry. And then take the Gram staining solution C with Pasteur pipette and rinse the tissue from the frosted surface side of the slide for differentiation, until the Gram staining solution C flow down from the section is colorless, wash it and shake to get it dry. Dip the sections in Gram

staining solution D for 1s and wash, microscopic examination, bake by oven to dry.

3. Dehydration: Quickly put the dried sections into three Jars of Anhydrous ethanol to dehydrate for 1s, 3s and 5s in sequence.

4. Transparency and sealing: Put the sections into clean Xylene for 5min for transparency, and then seal with neutral balsam.

5. Microscope inspection, image acquisition and analysis.

III. Interpretation of results

Gram-positive bacteria are purple, and Gram-negative bacteria are red.

IV. Precautions

1. The differentiation of Gram staining solution C in the staining process is a key step, and attention should be paid to the degree of differentiation.

2. Try not to repeat the staining. The differentiation solution removes the color of sections, and meanwhile also changes the permeability of the cell wall, which affects the accuracy of the final result.

3. When the temperature is low in winter, the staining time can be extended properly.