肠道黏液层制片及 PAS 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
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	G6012-1
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
卡诺氏固定液	Wanwu	G1120
甲醇	国药集团化学试剂有限公司	100141580
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
PAS 染液套装	Wanwu	G1008
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

1、肠组织取材固定：动物处死以后迅速切取目的区域肠段（要求大于 5 mm）。取材时保留肠道内容物，不能冲洗和挤压肠道内容物否则会破坏黏液层和影响组织结构完整。取材后立即投入卡诺氏固定液中固定，固定时间最少 4h，不超过 7 天，可较好保存肠道粘液层。

2、组织脱水包埋切片：组织固定好以后甲醇 2 次每次 40-60min，无水乙醇两次，每次 40-60min，二甲苯透明 20-40min，石蜡包埋，制备 4um 石蜡切片，65°烤片一小时备用。

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-无水乙醇II5min-75%酒精 5min，自来水洗。

4、切片入 PAS 染色液 B 中染色 10-15min，自来水洗，蒸馏水洗两遍；

5、切片入 PAS 染色液 A 浸染 25-30min，避光，流水冲洗 5min；

6、切片入 PAS 染色液 C 染 30S，自来水洗，盐酸水溶液分化，自来水洗，氨水返蓝，流水冲洗。

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 -二甲苯 5min-二甲苯II5min 透明，中性树胶封片。

8、显微镜镜检，图像采集分析。

三、结果判读：

紫红色的黏液层位于肠道内容物及肠粘膜上皮之间，要么包裹于肠道内容物，要么附着于粘膜上皮。

四、注意事项：

1、由于肠道黏液是由粘液细胞分泌到肠腔，附着于粘膜上皮极易脱落，很难保留非常完整的黏液层。并且黏液属于糖类物质必须用不含水的卡诺氏固定液固定，否则黏液层会被溶解。

2、PAS染色液B要经常更换，避免影响染色效果；

3、PAS染色液A染色时要避光。

Mucus Layer Preparation and PAS Staining

Experimental Report

I. Experimental equipment and reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
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	G6012-1
Upright electron microscope	JAPAN NIKON	NIKON ECLIPSE E100
Imaging system	JAPAN NIKON	NIKON DS-U3

2. Main experiment reagents

Reagent name	Manufacturer	Item No.
Carnoy's Fluid	Wanwu	G1120
Methyl alcohol	Sinopharm Chemical Reagent Co., Ltd.	100141580
Anhydrous ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
PAS Staining solution suit	Wanwu	G1008
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

II. Experiment steps

1. **Draw materials and Fixation of intestinal tissue:** After the animal is killed, quickly cut out intestinal segment of target area (greater than 5 mm required). Keep the intestinal internal contents when taking the intestinal segment samples. Do not rinse and squeeze the intestinal contents. Otherwise it will damage the mucus layer and affect the integrity of the tissue structure.

After taking the sample, immediately put it into Carnoy's Fluid for fixation, for a minimum of 4 hours and no more than 7 days, thus to better preserve the intestinal mucus layer.

2. Tissue dehydration, embedding, sectioning: After the tissue is fixed, put into Methyl alcohol for 2 times, 40-60min each time, Anhydrous ethanol twice, 40-60min each time, Xylene transparency for 20-40min, Paraffin embedding, cut 4um paraffin sections, and bake the sections at 65 ° for an hour.

3. Deparaffinize the paraffin sections to water: Put the sections in order in Xylene I for 20min - Xylene II for 20min - Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - 75% Alcohol for 5min, wash with tap water.

4. Put the sections into PAS staining solution B to stain for 10-15 minutes, wash with tap water and wash with distilled water twice.

5. Dip the sections into PAS staining solution A to stain for 25-30min and protect from light, rinse with running water for 5min.

6. Put the sections into PAS staining solution C for 30S, wash with tap water, differentiate with hydrochloric acid aqueous solution, wash with tap water, put in ammonia to return blue, and rinse with running water.

7. Dehydration and sections sealing : Put the sections in sequence into Anhydrous ethanol I for 5min - Anhydrous ethanol II for 5min - Anhydrous ethanol III for 5min - Xylene for 5min - Xylene II for 5min for transparency, seal the sections with neutral balsam.

8. Microscope inspection, image acquisition and analysis.

III. Interpretation of results:

The purple-red mucus layer is located between the intestinal contents and the intestinal mucosal epithelium, either is wrapped in the intestinal internal contents or is attached to the mucosal epithelium.

IV. Precautions:

1. Because the intestinal mucus is secreted by mucus cells into the intestinal cavity and attached to the mucosal epithelium, it is easy to fall off. And it is difficult to retain a very complete mucus layer. And the mucus belongs to carbohydrates, it must be fixed with water-free Carnoy's Fluid, otherwise the mucus layer will be dissolved.
2. PAS staining solution B should be replaced frequently, to avoid affecting the staining effect.
3. Avoid light when staining with PAS staining solution A.