瑞氏吉姆萨染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
离心机	DRAGONLAB	D3024R
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
二甲苯	国药集团化学试剂有限公司	10023418
瑞氏吉姆萨染液套装	Wanwu	G1009
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

1、涂片制备：取细胞悬液，离心，弃上清液，加适量 PBS 缓冲液，混匀后取适量滴于载玻片前端，另取一块边缘光滑的载玻片做推片，将其一端置于液滴前方，向后移动至接触液滴使液滴均匀分散在推片与载片的接触处。然后呈 30-40°角均匀用力向另一端平稳地推出。涂片放置自然晾干。

2、瑞士吉姆萨染色：

1、血涂片染色：

①取一张血涂片，组化笔沿着载玻片的边缘尽量画一个最大的长方形；若是其它细胞涂片，则不需要画圈。

②滴加适量瑞氏染液(完全覆盖细胞即可)染色 1min，用盛装纯水的洗瓶冲洗组织上的染液，至玻片流水呈无色即可；

③胶头吸管滴加 10 滴吉姆萨染色液染色 30s(洗耳球轻轻吹打至染液完全覆盖细胞)

用胶头吸管直接滴加 20 滴瑞氏吉姆萨 C 溶液于吉姆萨染色液上染色 2min(洗耳球轻轻吹打混匀)。用盛装纯水的洗瓶冲洗组织上的染液，至玻片上流水呈无色即可；

④稍微甩下载玻片上多余的水份，显微镜快速观察：白细胞胞核分叶清晰，部

分白细胞胞浆着淡红色，红细胞呈红色圆饼状，无核，数量较多。立即将染好的涂片置于65℃烤箱烘烤 4h 以上

2、组织石蜡切片染色：

①石蜡切片脱蜡至水，自来水洗 5min；

②取一张切片，组画笔沿着载玻片边缘画大圈圈住组织，胶头吸管滴加 10 滴瑞氏染液染色 2min，直接滴加 0.5ml 吉姆萨：瑞氏吉姆萨 D 溶液=1:19 混匀的混合液于瑞氏染液上染色 30s(洗耳球轻轻吹打混匀)；

③用盛装纯水的洗瓶冲洗组织上的染液，至玻片上流水呈无色即可；稍微甩下载玻片上多余的水份，显微镜快速观察：肥大细胞呈紫红色，细胞核清晰呈蓝色，部分白细胞胞浆着淡红色，红细胞呈红色圆饼状，无核。立即将染好的涂片置于 65℃烤箱烘烤 4h 以上

3、透明封片：干净的二甲苯 5min，中性树胶封片。

4、显微镜镜检，图像采集分析。

三、结果判读：

红细胞数量多，体积小，无核，呈粉色或肉粉色；嗜中性粒细胞体积略大于红细胞，细胞核呈紫蓝色分叶状，细胞质几乎无色；嗜酸性粒细胞体积略大于嗜中性粒细胞，细胞核呈紫色，通常为 2 叶，细胞质充满嗜酸性颗粒呈红色；嗜碱性粒细胞体积略小于嗜酸性粒细胞，细胞质内有大小不等被染成紫色的嗜碱性颗粒，核 1-2 叶，胞质染成淡蓝色。淋巴细胞圆形，体积与红细胞相似，核致密染成深紫色。单核细胞体积最大，细胞质染成灰蓝色，核呈肾型或马蹄型染成紫蓝色。血小板呈红色小颗粒。

四、**注意事项：**

1、制作良好的涂片要求厚薄适宜，分布均匀，边沿整齐。

2、制作的涂片需要充分干燥以防脱片。

3、染色时间可根据染液浓度，室温高低，细胞多少来进行调节。

Wright's Giemsa staining experimental report

1. Lab equipment and reagents

A. Lab equipment

Items	Manufacturer	Model
Centrifuge	DRAGONLAB	D3024R
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
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
Wright's Giemsa staining kit	Wanwu	G1009
Neutral balsam	Sinopharm Chemical Reagent Co., Ltd.	10004160

2. Experimental steps

1.Smear preparation: take the cell suspension, centrifuge, discard the supernatant, add an appropriate amount of PBS buffer, mix and take an appropriate amount then drop it in the front of the slide, and take another slide with a smooth edge as a slide, place one end in front of the droplet, and move it backward until touch the droplet so that the droplet is evenly dispersed at the contact place between pushing piece and the slide. Then push it evenly and forcefully to the other end at an angle of 30-40°. Smears are allowed to dry naturally.

2.Wright's Giemsa staining:

1.blood film staining:

①Take a blood film and draw the largest rectangle along the edge of the slide with the IHC pen; if it is other cell film, it is doesn't need to draw circles.

②Drop appropriate amount of Wright's staining solution (completely cover the cell) to stain for 1min. wash the staining solution on the tissue with pure water until water in the slide is colorless;

③Add 10 drops of Giemsa staining solution to stain for 30s (washing ear bulb gently until the staining solution completely covers the cell) , directly add 20 drops of Wright's Giemsa C solution to the Giemsa staining solution for 2 minutes, wash the staining solution on the tissue with pure water until water in the slide is colorless;

④Slightly shake off the excess water on the slide, and quickly observe under the microscope: the white blood cell nucleus is clearly divided, part of white blood cell cytoplasm is light red, and the red blood cell is in the shape of a red pie without nucleus, large number. Immediately place the stained blood film in the oven at 65℃ for more than 4h.

2.Paraffin section tissue staining:

① Paraffin section deparaffinization and rehydration and wash with tap water for 5 minutes;

②Take 1 pc of slide, draw a large circle along the edge of the glass slide to surround the tissue, add 10 drops of Wright's staining solution to stain for 2 minutes, and directly add 0.5ml of Giemsa: Wright's Giemsa solution D(1:19) the mixed solution on the Wright's dyeing solution for 30s .

③Rinse the staining solution on the tissue with pure water, until the running water on the slide is colorless; slightly shake off the excess water on the slide, and quickly observe under the microscope: the mast cells are purple-red and the nucleus are clear blue , part of the white blood cell cytoplasm is pale red, and the red blood cell is in the shape of a red pie without nucleus. Immediately place the stained blood film in the oven at 65℃ for more than 4h.

3. Dehydration and sealing:clean xylene for 5 minutes and neutral balsam sealing.

4. Microscope examination, image collection and analysis.

3. Results

Large number of red blood cell, small size, without nucleus, pink or flesh pink; neutrophils are slightly larger than red blood cells, nucleus is purple-blue leaf-shaped,, cytoplasm is almost colorless; eosinophils are slightly larger than neutrophils granulocytes, the nucleus is purple, usually 2 leaves, the cytoplasm is filled with eosinophilic granules with red colorvolu;volume of basophils are slightly smaller than eosinophils, and there are basophilic granules of different sizes stained into purple in the cytoplasm, nucleus 1-2 leaves, cytoplasm stained light blue. Lymphocytes are round, volume is similar to red blood cell, and the nucleus are densely stained to dark purple. Mononuclear cell has the largest volume, the cytoplasm stained to grayish blue, and the nucleus is kidney-type or horseshoe-type stained to purple-blue. Platelets are small red particles.

4. Note

1. Well-made smear should be appropriate thickness, uniform distribution, and neat edges.

2. The smear should be fully dried to prevent falling off.

3. Staining time can be adjusted according to the concentration of the staining solution, room temperature and cells number.