Practical Session 1- Making and Staining Peripheral Blood Smears

Learning Outcomes

- **To understand the principles used in staining white blood cells, red blood cells and platelets using a Romanowsky stain.**
- **To be able to prepare a blood smear suitable for analysis using the correct technique.**
- **To be able to identify white blood cells, red blood cells and platelets.**
- **To perform a manual white cell differential.**

Reagents and materials	**Notes**
Microscope	x 40 / x50 / x100 objectives
Control levels 1 , 2 and 3	Representing different sample types
Methanol	Flammable
Wright-Giemsa stain	Wear appropriate PPE
Glass slides	Dispose used slides in sharps bin
Capillary tube	For dispensing blood drop
Coverslips	(if required)
Buffered water	pH 6.8
Immersion Oil	(if required)

<u>Exercise 1: Blood film morphology - Preparing and staining</u> (1.hr)

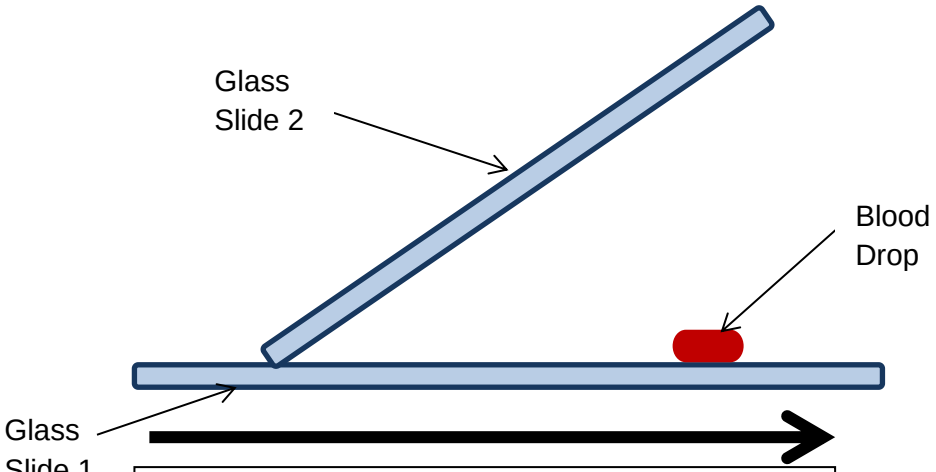
Task

To prepare blood films for control levels 1, 2 and 3 and stain them using a modified Romanowsky Stain, Wrights- Giemsa. Romanowsky stains are a family of which have the ability to make subtle distinctions in shades of staining and differentially stain granules. Romanowsky stains are neutral stains composed of a mixture of oxidized methylene blue (azure) dyes and Eosin Y. The azures are basic dyes that bind acid nuclei and result in a blue to purple colour. The acid dye, eosin, is attracted to the alkaline cytoplasm, producing red coloration.

Method

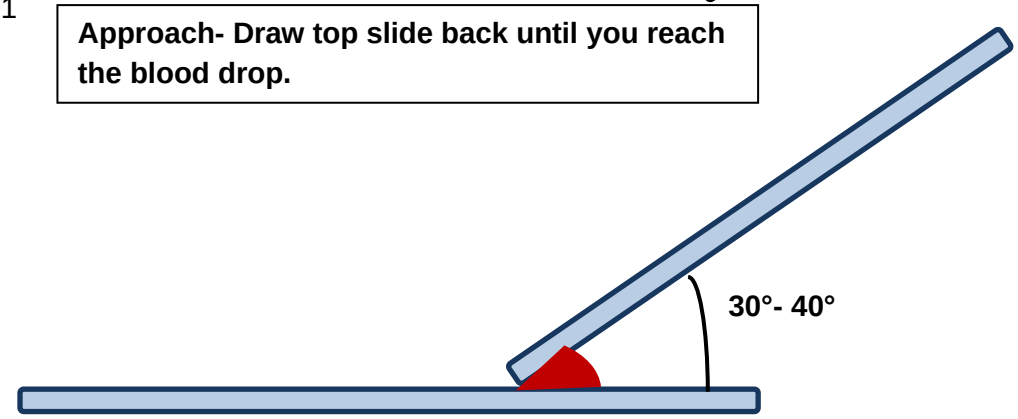
1. Ensure the three samples provided are adequately mixed
2. Label a glass slide and ensure free from dust.
3. Using a capillary tube dispense one drop of mixed whole blood to the end of the slide.
4. Use another glass slide to spread the blood drop to produce a blood film as show below:

A



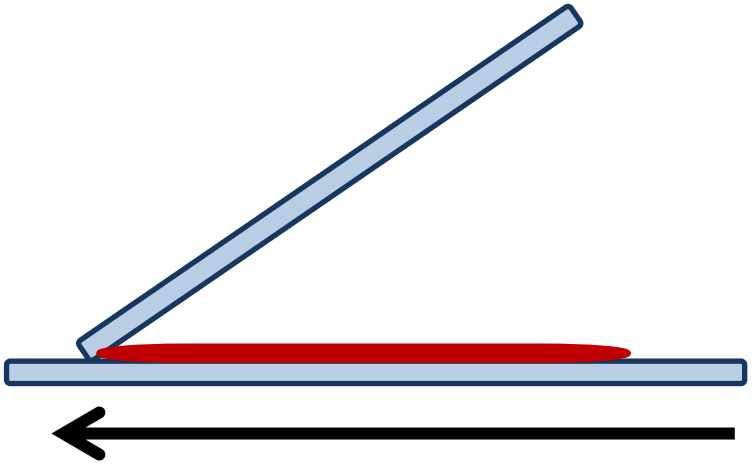
Approach- Draw top slide back until you reach the blood drop.

B



Adhesion- Hold the top slide at a 30°-40° angle.

C



Advancement- To spread to blood drop, move the slide forward smoothly.

5. Leave for 5 minutes to allow blood film to air dry thoroughly.
6. Bath 1: Fix film in Methanol for 1 minute.
7. Bath 2: Stain in Wright-Giemsa for 2 minutes.
8. Bath 3: Rinse in buffered water for 3 minutes.
9. Allow films to air dry.

(Whilst the films are drying please move to exercise 2. Complete exercise 1 once films have sufficiently dried.)

Results for Exercise 1

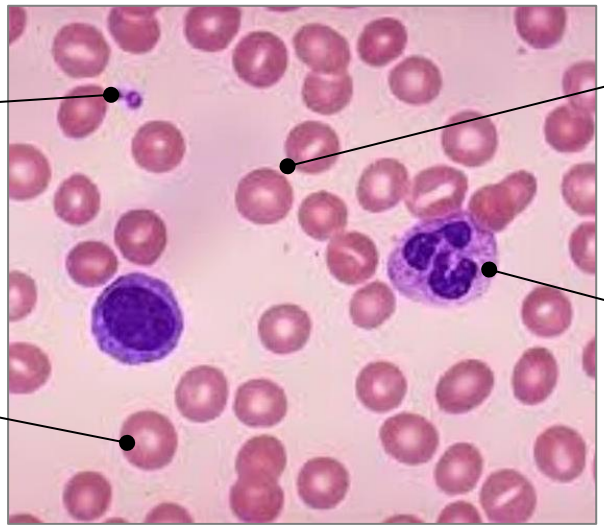
Each group to present their best stained blood film for each level:

Film level	Appearance / score (1 – 5)			
	Drop	Tail	Body	Inclusions
Low Control				
Med Control				
High Control				

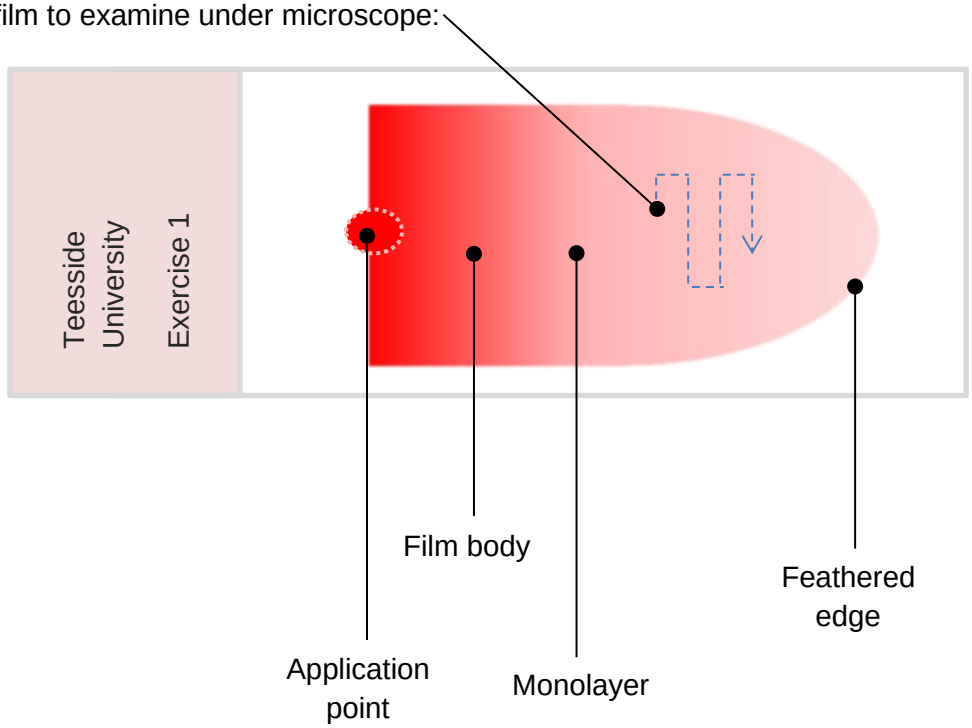
What do you think are the most important factors for making a good blood film?

Additional information for this exercise

What a stained blood film should look like:



Areas of film to examine under microscope:

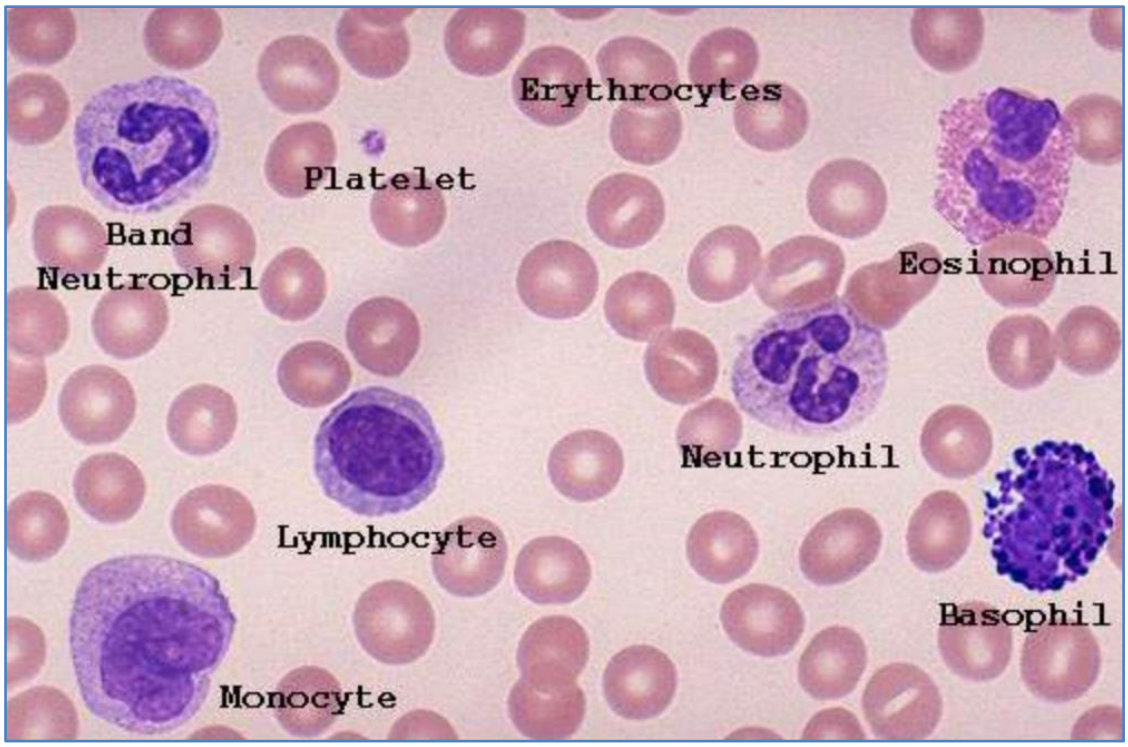


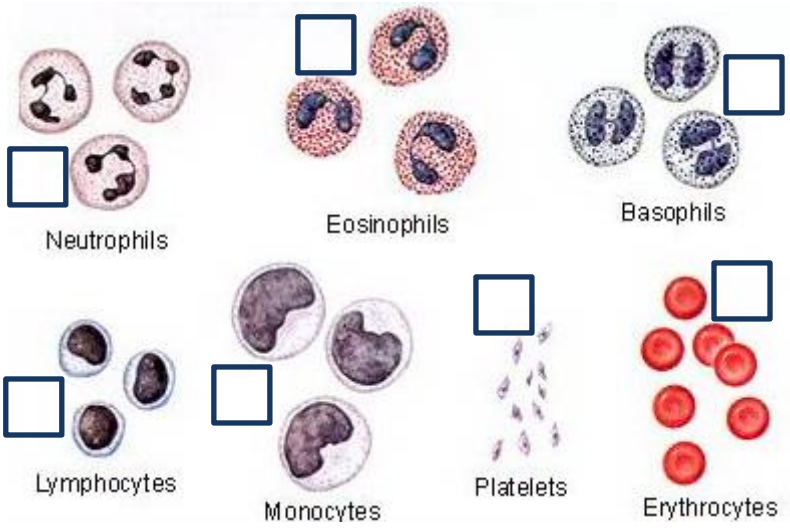
<u>Exercise 2: Blood Film Morphology – Reading and Interpretation</u> (1 hour)

Task

Examine the pre-prepared blood film provided to identify and describe common WBC and RBC morphology types.

1. using the key below identify the following cell types and tick when you have seen them in the normal film.





Exercise 3: Blood film morphology –Manual WBC Differential (30 minutes)

Task

To perform a manual WBC differential to determine what illness the patient is suffering from.

Method

Count 100 white blood cells (under x ☐ magnification) and record the numbers in the boxes below

Neutrophil		%
Lymphocyte		%
Monocyte		%
Eosinophil		%
Basophil		%

Laboratory comments on diagnosis:

Film Ref: