

## APPENDIX 5

### Giemsa staining and haematology

#### *Giemsa staining*

#### **Reagents**

Giemsa stain solution (e.g. BDH/Merck Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, R66; Product 35086). The Giemsa stain may have variable batch quality. Each new batch should be checked against a known organism before routine use.

Phosphate-buffered distilled water, pH 7.2.

$\text{KH}_2\text{PO}_4$	0.7 gm
$\text{Na}_2\text{HPO}_4$	1.0 gm
Distilled water	1.0 litre

Absolute methanol (Analar). This must be stored in a tightly stoppered bottle to prevent absorption of water.

#### **Method**

1. Prepare thin film as for routine haematology. Ensure that the film has a good 'tail' and does not reach the edges of the slide laterally.
2. Allow the film to dry in air and fix with methanol for one minute.
3. Tip off excess methanol and place face down on a slide staining tray.
4. Using a 20 ml syringe and a blunt needle, dilute the stock Giemsa 1:10 with buffered distilled water. Mix well and expel air.
5. Infiltrate the stain, using the syringe and needle, under the slide, taking care not to trap large air bubbles. Stain for 25 - 30 minutes.
6. At end of staining time, rinse slides briefly with tap water and allow to drain dry in a vertical position. Possible parasites should be examined in more detail using the oil immersion lens. A 50x or 63x oil immersion objective is invaluable for preliminary examination: at least 1000 fields should be examined by a trained microscopist.

*Notes:*

The syringe method for dilution of Giemsa (point 4 above) is strongly recommended, as once the stain is diluted with water precipitation of the stain begins which is hastened by exposure to air. This dilution must, therefore, be prepared immediately prior to staining; stock diluted stain should not be made. Staining face-downwards in a slide tray also reduces precipitation, and any that does develop falls away from the smear. Cleanly stained smears are very important when searching for small intracellular parasites.

The buffered water at pH 7.2 *must* be used for the dilution of stain for blood parasites. It is only at this alkaline pH that proper differentiation of parasite nuclear and cytoplasmic material takes place.

*Packed cell volume (PCV) determination (haematocrit)*

The packed cell volume (PCV) is the percentage of blood volume taken up by red cells. A low PCV is indicative of anaemia. To determine the PCV a heparinised microhaematocrit capillary tube is filled directly with capillary blood (e.g. from lancet puncture of the finger or ear) or filled with venous blood collected with anticoagulant. Tubes are sealed at one end with Plasticine or by heat. Use capillary action to fill the tubes leaving 10 - 15 mm unfilled at the end of the tube to be sealed. The tube is centrifuged in an haematocrit centrifuge (at 12,000 g) with the sealed end of the tube against the rubber outer edge of the centrifuge plate. Centrifuge for 5 minutes and read the PCV in a microhaematocrit reader by putting the base of the red cell layer on the zero and the top of the plasma layer on the 100 lines. The silver line position is adjusted to touch the red cell/white cell/platelet interface and the PCV volume is read from the scale.

The PCV (haematocrit) is the ratio of packed red cells to the volume of the blood.

*White cell count*

## White cell diluting fluid:

Glacial acetic acid	2 ml
1 % methylene blue	1 - 2 drops
Distilled water	to 98 ml

The red cells are lysed and a stain is used for the white cells. To lyse the cells 20  $\mu$ l of blood is added to 0.38 ml of white cell diluting fluid (1 in 20): mix thoroughly. A

haemocytometer counting chamber is filled and left to stand in a humid chamber for two minutes. All the white cells in an area of 4 mm<sup>2</sup> are counted.

The white cell count

$$= \frac{\text{no. of cells counted}}{\text{volume counted (0.4)}} \times \text{dilution} \times 10^6 = \text{the no. counted} \times 50 \times 10^6/l$$

Normal white cell counts (WBC) in different age groups are:

Age group	Normal WBC
Infants at 1 year	6-18 x 10 <sup>9</sup> /l
Children 1 to 10 years	5-14 x 10 <sup>9</sup> /l
Adults	4-11 x 10 <sup>9</sup> /l

*Determination of haemoglobin (cyanmethaemoglobin method)*

Solution for dilution of blood (Drabkin's solution, pH 7.0 - 7.4)

Potassium ferricyanide	200 mg
Potassium cyanide	50 mg
Potassium dihydrogen orthophosphate	140 mg
Nonidet P40	1 ml
Distilled water	to 1 l

Store at room temperature in the dark (do not freeze). *This solution is highly toxic.*

This solution can be prepared by dissolving standard tablets of components or by diluting an ampoule of commercially available concentrate.

Add 20 µl of blood collected with anticoagulant to 4 ml of reagent, mix well and leave at room temperature for three minutes. The absorbance is read on a colorimeter at 540 nm against a blank of the reagent solution alone. The absorbance of a reference standard of cyanmethaemoglobin is also measured separately against a reagent blank.

Haemoglobin concentration (gm/l) is calculated by:

Absorbance of test sample x Concentration of standard x dilution factor

Absorbance of standard 100

Normal ranges are:

Age group	Normal ranges
Children 1 to 6 years	110 - 140 gm/l
Adult males	130 - 180 gm/l
Adult females	115 - 165 gm/l

*Total serum protein*

Biuret reagent:

Copper sulphate (5 H <sub>2</sub> O)	3 gm
Potassium sodium tartrate	9 gm
Potassium iodide	5 gm
NaOH	24 gm
Distilled water	up to 1 l

Alkaline tartrate reagent:

Potassium sodium tartrate	9 gm
Potassium iodide	5 gm
NaOH	24 gm
Distilled water	up to 1 l

Add 0.1 ml of serum to 5 ml of biuret reagent.

Add 0.1 ml of serum to 5 ml of alkaline tartrate solution.

Allow both tubes to stand for 30 min at room temperature.

Blank a spectrophotometer with a 540 nm wavelength filter, using 5 ml of biuret reagent mixed with 0.1 ml of distilled water.

Read the absorbance of the patient's serum in biuret reagent and subtract the absorbance in alkaline tartrate solution.

The total protein in the patient's sample can be read from a calibration curve prepared from 5 dilutions of a protein standard solution or a control serum containing a known quantity of protein. Lyophilised standards containing known quantities of total protein are available commercially.