The background of the cover is a microscopic image of a blood smear. It shows numerous red blood cells of various sizes and shapes, some appearing normal and others slightly irregular. Two white blood cells with distinct, multi-lobed nuclei are visible on the right side of the cover. The overall color palette is muted, with various shades of brown, tan, and purple against a light greyish-blue background.

Bench Aids
for the
morphological
diagnosis of
anaemia



World Health Organization
Geneva

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Cover photomicrograph: Severe megaloblastic anaemia

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Laboratory investigations

Depending on the morphological features, the tests summarized below may help to elucidate the cause of anaemia. In all cases, the most important laboratory test is the *full blood count*, which consists of: haemoglobin concentration (Hb); packed cell volume (haematocrit) (PCV); red blood cell count (RBC); mean cell volume (MCV); mean cell haemoglobin (MCH); mean cell haemoglobin concentration (MCHC); white blood cell count (WBC). Platelet count is also included where appropriate facilities are available.

In the following lists, individual measurements are specified where relevant. Tests marked with an asterisk (*) will usually need to be sent to a higher-level laboratory.

Infections

Haemoglobin

PCV and MCHC

Total leukocytes (WBC)

Slide test for infectious mononucleosis

Erythrocyte sedimentation rate (ESR)

Ziehl-Neelsen stain on sputum and urine

HIV test if available, when appropriate

* C-reactive protein

Leukocytosis

A differential leukocyte count on the blood film will show a relative increase in neutrophils, lymphocytes, etc. To diagnose an *absolute* increase a total count (WBC) is necessary. However, it is often possible to determine from the film whether there is a high total leukocyte count.

Common causes of neutrophil leukocytosis (see also Plate 6, Infections)

Bacterial infections and inflammations

Tissue necrosis, e.g. cardiac infarct

Burns

Malignant disease

Uraemia

Acute haemorrhage or haemolysis

Normal pregnancy (mild leukocytosis and left-shift)

Eclampsia (marked leukocytosis with left-shift; see **h**, Plate 5, Leukocytes)

Common causes of eosinophilia

Parasitic infections

Allergic conditions

Skin diseases

Hyper eosinophilic syndrome (see **e**, Plate 7, Miscellaneous)

Lymphocytosis (see Plate 6, Infections)

Acute infections:

infectious mononucleosis; viral hepatitis; HIV infection; rubella; pertussis; typhoid; diphtheria

Chronic infections:

tuberculosis; syphilis; toxoplasmosis; brucellosis

Chronic lymphocytic leukaemia

Note: Lymphocytes are the predominant cells of the differential count in infants and young children.

Monocytosis (see Plate 6, Infections)

Chronic bacterial infections:

tuberculosis; brucellosis

Protozoal infections:

malaria; trypanosomiasis; leishmaniasis; toxoplasmosis

Hypochromic anaemias

Haemoglobin

PCV and MCHC

Haemoglobin electrophoresis and Hb-A₂ quantitation, if necessary, to exclude β -thalassaemia trait and haemoglobinopathies

Bone marrow examination, including iron staining

Occult blood tests on stools

* Serum iron

* Serum ferritin

Macrocytic anaemias

Haemoglobin

PCV

RBC and MCV

Reticulocyte count (to exclude haemolytic anaemia)

Bone marrow examination for megaloblasts and giant metamyelocytes

Urinary bilirubin (by test strip) and serum bilirubin to exclude liver disease

* Serum vitamin B₁₂ assay

* Red cell and/or serum folate assay

* Intrinsic factor antibody

* Vitamin B₁₂ absorption (Schilling test)

Haemolytic anaemias

Haemoglobin

Reticulocyte count

Direct antiglobulin (Coombs) test if there are spherocytes

Urinary haemosiderin

Serum bilirubin

Urinary urobilinogen

G6PD screen test, especially when there are irregularly contracted cells

Blood films on patient's family

When there are features of microangiopathic anaemia (see **g**, Plate 3, Haemolytic anaemias):

coagulation screening; prothrombin time; thrombin time; detection of fibrinogen degradation products; renal function tests (blood urea and creatinine)

Haemoglobinopathies

Haemoglobin

PCV and MCHC

Sickle-cell wet preparation

Hb-S solubility test

Reticulocyte count

Brilliant cresyl blue or New methylene blue stain for Hb-H

* Haemoglobin electrophoresis

Thalassaemia

Haemoglobin

PVC and MCHC

Brilliant cresyl blue or New methylene blue stain for Hb-H

RBC by automated counter with calculated MCV and MCH

* Haemoglobin electrophoresis

* Estimation of Hb-A₂ and Hb-F

* Serum iron

* Serum ferritin

Platelets

A platelet count can be obtained from the ratio of platelets to red cells in a well spread film in which the cells are evenly distributed. This requires a reliable red cell count by an automated cell counter. With normal-sized red cells, however, a reasonable estimate of the red cell count can be made from the haemoglobin.

Introduction

In any health clinic or peripheral hospital it should be possible to detect anaemia and to estimate its severity by a simple method (e.g. the Haemoglobin Colour Scale). In larger health clinics a haemoglobinometer may be available, and a laboratory in a district hospital is more likely to measure haemoglobin concentration by means of a spectrophotometer. Determination of packed cell volume can also be used to assess anaemia. Anaemia may be the primary disorder or it may be secondary to an underlying communicable or noncommunicable disease, as indicated below. For correct management of a patient it is therefore essential to identify the cause of the anaemia. In many cases, the type of anaemia can be diagnosed by examining a blood film for abnormal features; this is especially important when other laboratory tests are not immediately available.

This set of bench aids has been produced to help health workers to become familiar with the appearance of normal blood films and to identify, in the red cells, leukocytes and/or platelets, characteristic morphological abnormalities that may occur in a range of relatively common diseases. Features in a stained blood film can be readily compared with the photomicrographs showing these abnormalities. Most of the photomicrographs are high-power magnifications and each is accompanied by a description of the principal morphological features. Appropriate laboratory tests that should help to confirm a diagnosis are listed on the back of this sheet. It is important to appreciate that reliable interpretation of blood cell morphology depends on the quality of preparation and staining of blood films: badly prepared and/or poorly stained films may give rise to misleading artefacts.

In endemic countries, malaria is an important cause of anaemia – particularly in children under 2 years old. However, morphological diagnosis of malaria from blood smears is included in another WHO publication (*Bench aids for the diagnosis of malaria infections*) and is therefore not dealt with here.

Summary of causes of anaemia

Blood loss

Acute or chronic haemorrhage

Blood destruction (haemolysis)

Congenital: hereditary spherocytosis; thalassaemia; sickle-cell disease; other haemoglobinopathies

Acquired: toxic drugs and chemicals; G6PD reaction to non-toxic substances; burns; bacterial infections; *Clostridium welchii* toxin; uraemia; disseminated intravascular coagulation; snake venom; malaria

Immune: transfusion reactions; auto-antibody; haemolytic disease of the newborn

Impaired erythropoiesis

Congenital: thalassaemia

Nutritional: iron deficiency; vitamin B₁₂ deficiency; folate deficiency

Marrow failure: aplastic anaemia; leukaemia: carcinoma; myelofibrosis; hypothyroidism

Chronic inflammatory diseases

Multiple factors

Liver disease; AIDS; tuberculosis

Glossary

Anisocytosis. The size distribution of erythrocytes deviates considerably from the norm.

Anisochromasia. Twin populations of erythrocytes (e.g. in iron deficiency being treated with iron).

Basophilic stippling. Multiple blue-black dots in the erythrocyte cytoplasm, consisting of ribosome remnants.

Döhle bodies. Small light-blue bodies that occur in neutrophils in infection, usually associated with toxic granules.

Echinocyte (Burr cell, crenated cell). Round, spiculated erythrocyte.

Elliptocyte (ovalocyte). Oval or cigar-shaped erythrocyte.

Howell-Jolly body. Dark red-violet dot in erythrocyte cytoplasm, originating from nuclear remnant.

Hypochromia. Pale erythrocytes.

Lymphocytosis and/or monocytosis. Increased proportion of lymphocytes and/or monocytes. In malignant disease, immature cells of the lymphatic system may be present in addition to lymphocytes.

Macrocyte. Erythrocyte with diameter > 10 µm.

Microcyte. Erythrocyte with diameter < 5 µm.

Neutrophil leukocytosis (neutrophilia). The number of neutrophils is increased and their nuclei vary in their segmentation, from band cells (see **h**, Plate 5, Leukocytes) to cells with 4 or 5 lobes. The cytoplasm often shows heavily stained (toxic) granules and vacuoles. This pattern occurs in infections and other conditions listed overleaf. From a film it is possible to diagnose a relative neutrophilia but a total white blood cell count (WBC) may be required to determine whether there is absolute leukocytosis.

Neutrophil leukocytosis with left-shift (leukaemoid reaction). In addition to mature neutrophils with segmented nuclei there are non-segmented myelocytes/metamyelocytes with nuclei that are only slightly dented and a band cell with horseshoe-shaped nucleus with parallel edges. The cytoplasm shows toxic granulation. This reaction occurs in severe infections, especially in children, and in very severe forms of the conditions listed overleaf.

Pelger cells. Mature neutrophils that have failed to lobulate; normally an inherited feature but may occasionally occur in leukaemias.

Poikilocyte. Pear-shaped or tear-shaped erythrocyte.

Polychromasia. Mixture of erythrocytes with normal-staining cytoplasm and usually slightly larger erythrocytes with bluish grey cytoplasm.

Reticulocyte. Erythrocyte with blue dots after vital staining of ribosome remnants.

Rouleaux. Aggregated erythrocytes, arranged in columns like stacks of coins.

Schistocyte. Fragmented erythrocyte.

Sickle cell (drepanocyte). Crescent-shaped erythrocyte.

Spherocyte. Spherical erythrocyte.

Target cell. Erythrocyte with a dark core and periphery, and a pale zone in between.



Preparation of blood films

Introduction

A blood film is made from blood obtained directly by finger-prick or from a sample collected into EDTA anticoagulant; a drop of blood is spread evenly on a slide and stained. It is important to ensure that the film is well made and correctly stained with a Romanowsky stain. There are a number of different Romanowsky stains, which vary in their staining characteristics; it is therefore essential for the laboratory worker to become familiar with one of these stains and, as far as possible, to use only this stain. The blood films shown in these bench aids have been stained by the May-Grünwald-Giemsa (MGG) method. This is a more elaborate staining technique than some, but the results obtained justify the extra time and effort involved.

Making a thin blood film

Preliminary preparation

Spreaders

- Select a glass microscope slide with at least one smooth end.
- Using a glass-cutter, break off both corners of this smooth end, leaving a length of about 18 mm to serve as the spreader (see Fig. 1).
- Each spreader can be used repeatedly provided that the spreading edge remains smooth. The edge must be wiped carefully and dried before the spreader is used to make another blood film; the slide must be discarded if the spreading edge becomes chipped.

Clean slides

It is essential to use clean, dry, dust-free slides: remember that grease and residual detergent are equally likely to spoil a blood film.

- *New slides.* Boxes of clean, grease-free slides may be available. If not, proceed as follows. Leave slides overnight in a detergent solution. Wash the slides thoroughly in running tap water, rinse in distilled water (if available), and leave in methylated spirit (95% ethanol) or methanol until required. Just before use, dry with a clean linen cloth and keep covered until needed to avoid dust settling on the surface of the slides.
- *Used slides.* Place used slides in a detergent solution and heat to about 60°C for 20 minutes. Wash the slides thoroughly in running tap water, rinse in distilled water (if available), then treat as for new slides.

Spreading the blood

Place a small drop of blood on a slide, about 1 cm from the end. Without delay, place a spreader in front of the drop, at an angle of about 30° to the slide; move it backwards to make contact with the drop. The blood should run quickly along the contact line. With a steady movement, spread the drop of blood along the slide (Fig. 1). Do not lift the spreader off until the last trace of blood has been spread out; if the drop of blood was the correct size, the film should be about 3 cm long. It is important that the blood film finishes at least 1 cm from the end of the slide. The thickness of the film can be regulated by varying the size of the drop of blood, the pressure and speed of spreading, and the angle at which the spreader is held. For "thin" anaemic blood with low haemoglobin, the correct thickness is achieved by using a wider angle; for "thick" polycythaemic blood with very high haemoglobin the angle should be narrower.

All blood samples, including films, must be regarded as potentially biohazardous and must be handled with care, especially avoiding contact with the skin if there are cuts, scratches, or abrasions.

After the film has been made, let it dry in the air. Write the patient's identification (and date if appropriate) in pencil on the edge of the film at its origin.

The macroscopic appearance of well and poorly made films is shown in Fig. 2: (a) satisfactory, well made film; (b) too short and too thick – spreader held at wrong angle; (c) film extending to end of slide – blood drop too large; (d) long tail – spreader had chipped edge; (e) patchy spots on surface – dust on slide; (f) film extending to edge of slide – spreader too wide or incorrectly positioned; (g) irregular, streaky surface and round gaps in film – greasy slide; (h) film slightly too thick and too close to edge of slide – a common fault.

Fixing the films

To preserve the morphology of the cells, films must be fixed as soon as possible after they have been dried; it is important to prevent contact with water before fixation is complete. Methanol is the alcohol of choice for the procedure, although ethanol ("absolute alcohol") can be used. Methylated spirit must not be used because it contains water. To prevent the alcohol from being contaminated by absorbed water it must be stored in a tightly stoppered bottle and not left exposed to the atmosphere (especially in humid climates).

Films to be fixed should be placed in alcohol in a covered staining jar or tray for 2-3 minutes. In humid climates fresh methanol is required 2-3 times a day; old portions can be used for storing slides (see above).

Fig.1 Method for spreading a drop of blood on a slide to make a film

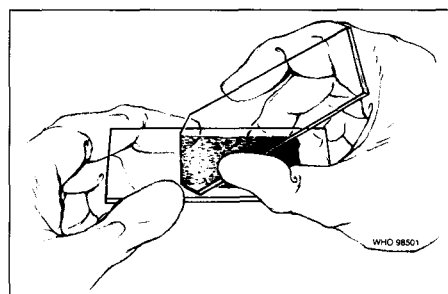
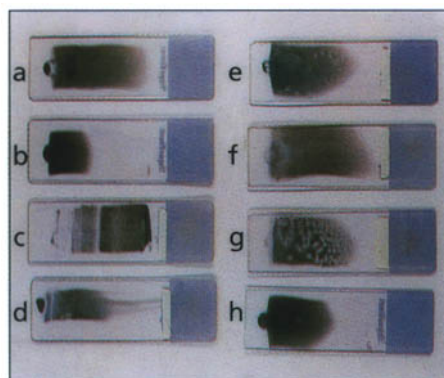
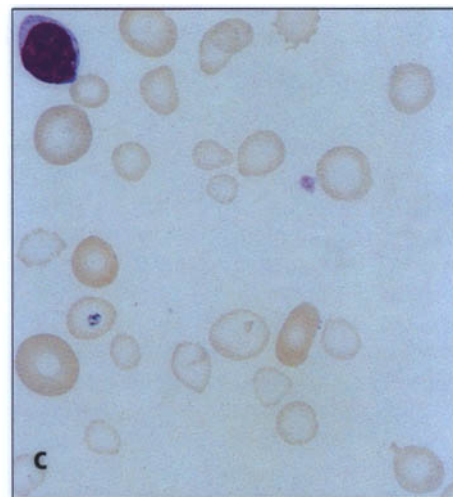
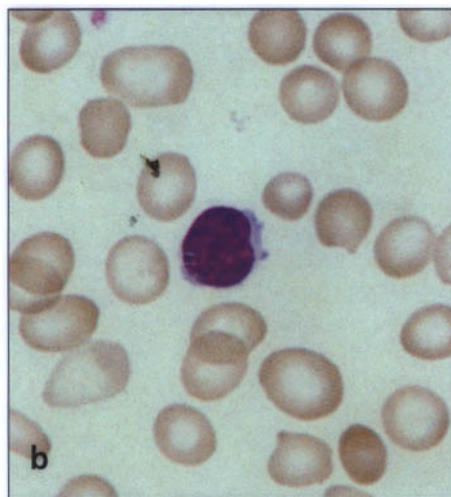
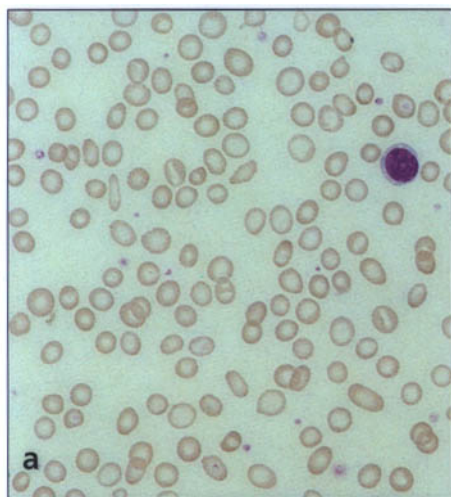


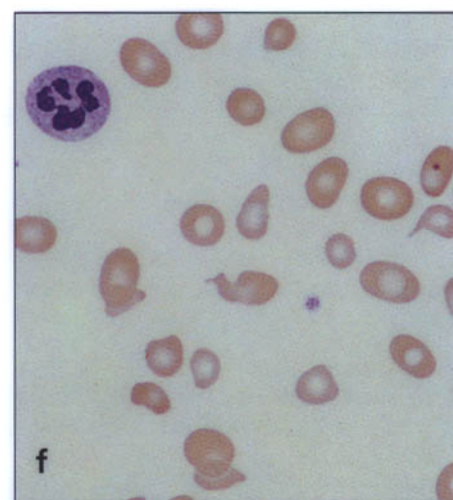
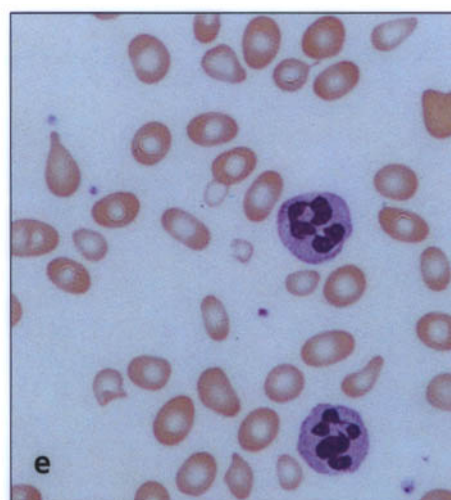
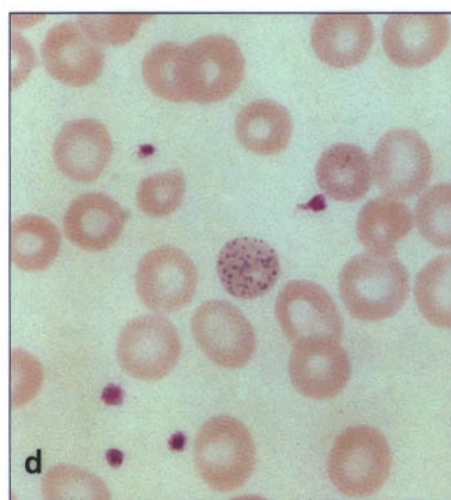
Fig.2 Blood films



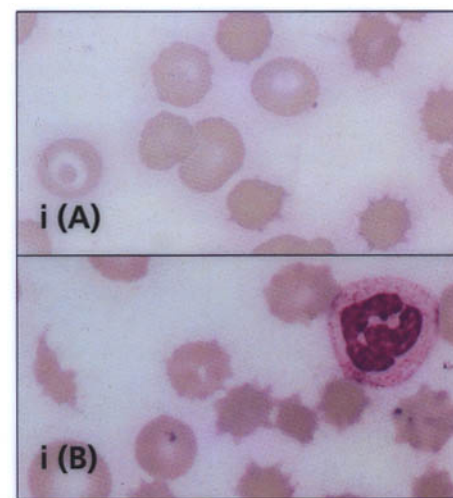
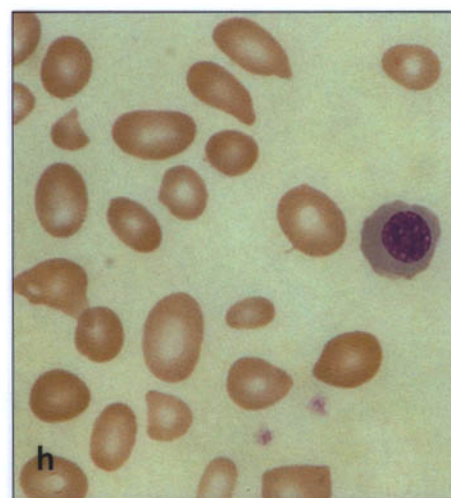
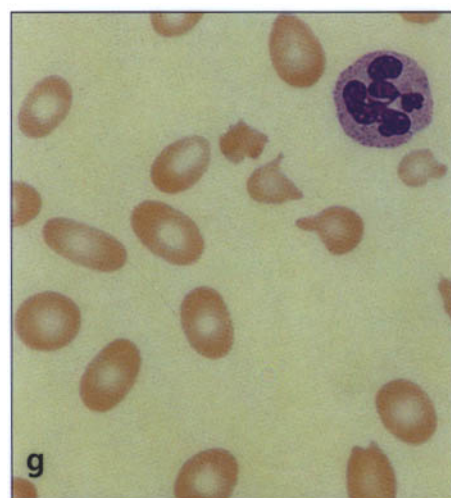
Nutritional anaemias (microcytic and macrocytic) and liver disease



a. Moderately severe iron deficiency anaemia (low power). Marked hypochromia and microcytosis, together with poikilocytosis and marked anisocytosis. **b. Moderately severe iron deficiency anaemia.** Microcytosis, hypochromia, poikilocytosis, and anisocytosis. **c. Very severe iron deficiency anaemia.** Marked microcytosis and anisocytosis, very marked hypochromia, mild poikilocytosis.



d. Lead poisoning. Mild hypochromia. One cell with characteristic marked basophilic stippling. **e. Severe megaloblastic anaemia** (low power). Marked anisocytosis and poikilocytosis (some tear-drop), and some macrocytes that are mainly oval. Note especially hypersegmented neutrophils. **f. Severe megaloblastic anaemia** (low power). Poikilocytosis, macrocytosis, anisocytosis, and one hypersegmented neutrophil. Macrocytes are both round and oval (ovalocytes); one ovalocyte (right margin) contains a Howell-Jolly body.



g. Severe megaloblastic anaemia. One hypersegmented neutrophil, anisocytosis, poikilocytosis, and macrocytosis. **h. Moderately severe megaloblastic anaemia.** Anisocytosis; poikilocytes and macrocytes. There is also one megaloblast (centre right). Megaloblastosis is usually identified in bone marrow preparations but may occasionally be seen (as here) in the circulation. **i. Liver disease** (A) Moderate acanthocytosis and one target cell (left). (B) Macrocytosis and marked acanthocytosis; two cells contain small dark-staining inclusions, which are Pappenheimer bodies.



Staining of blood films

Reagents

- Stock solution of 0.3% May-Grünwald stain in methanol. For a working solution, dilute with an equal volume of buffered water (distilled if available), pH 6.8.
- Stock solution of 1% Giemsa stain in methanol. For a working solution, dilute with 9 volumes of buffered water (distilled if available), pH 6.8.

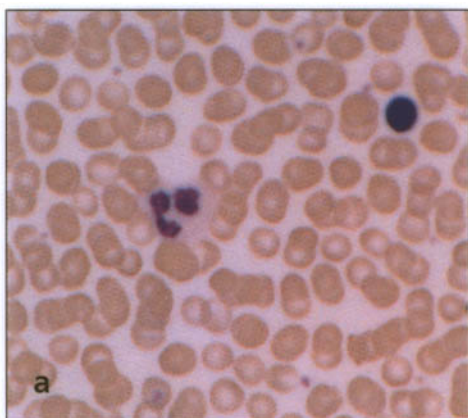
Method

1. Place the fixed slides in a staining jar or tray containing the May-Grünwald working solution. Leave for 15 minutes.
2. Transfer the slides, without washing, to a staining jar or tray containing the Giemsa working solution. Leave for 10 minutes.
3. Wash the slides in running tap water, then leave undisturbed for 3-4 minutes in buffered water, pH 6.8, in the staining jar (but see "Quality control" below).
4. Wipe the back of each slide to remove traces of stain. Allow slides to dry in the air, in a draining rack if available or on end against a flat surface.
5. Check the quality of staining by examining the film by low-power microscopy.

Microscopic examination of blood films

Three levels of magnification are considered here – low power (x10), medium power (x40), and high power with an oil-immersion lens (x100). Every film should be inspected first at low power, before being examined with the x40 lens. The oil-immersion lens should generally be reserved for examining unusual cells and for looking for fine details of cytoplasmic granules, punctate basophilia, etc. It is essential to cover the blood film with a coverglass; this allows it to be examined with the x10 and x40 lenses. Once the film is completely dry, cover it with a rectangular coverglass, either permanently using a neutral mountant, or temporarily using an immersion-lens oil (such as oil of cedarwood) so that the coverglass can be reused. The coverglass must be wider than the blood film so that cells at the edges of the film can be properly examined.

Survey the film at x10 magnification to get a general impression of its quality. Then find an area where the red cells are evenly distributed, just touching but not overlapping, and examine them at x40 magnification. Scan the film for an indication of whether the leukocytes are increased or decreased in relation to the red blood cells, to identify any unusual or abnormal cells, and to estimate the relative proportion of platelets and note the presence of abnormally large platelets. Use the x100 lens for studying fine details of cell morphology.



a. x 40 magnification

Quality control

Staining faults that should be noted on examination by low-power microscopy

Too blue

- smear too thick
- inadequate time in buffer
- buffer pH too high
- diluted stain overused, should be renewed
- stock MGG solution incorrectly made/made with impure dye
- stock MGG solution left exposed to bright daylight

Too pink

- excessive time in buffer
- buffer pH too low
- stock MGG solution incorrectly made/made with impure dye

Stain deposit

- stain solution left in uncovered jar or tray
- solution not filtered

Blue background

- inadequate fixation
- prolonged storage before fixation
- blood anticoagulated with heparin

Irregular leukocyte or platelet distribution (especially in tail)

- poor spreading technique

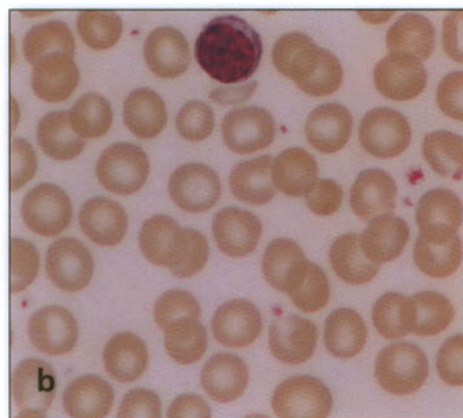
Note: As a quality control check a normal film should also be included from time to time.

Staining characteristics of a correctly stained normal film

<i>Nuclei</i>	purple
<i>Cytoplasm</i>	
erythrocytes	deep pink
reticulocytes	grey-blue
neutrophils	orange-pink
lymphocytes	blue; some small lymphocytes deep blue
monocytes	grey-blue
basophils	blue
<i>Granules</i>	
neutrophils	fine, purple
eosinophils	red-orange
basophils	purple-black
monocytes	fine, reddish (azurophil)
<i>Platelets</i>	purple

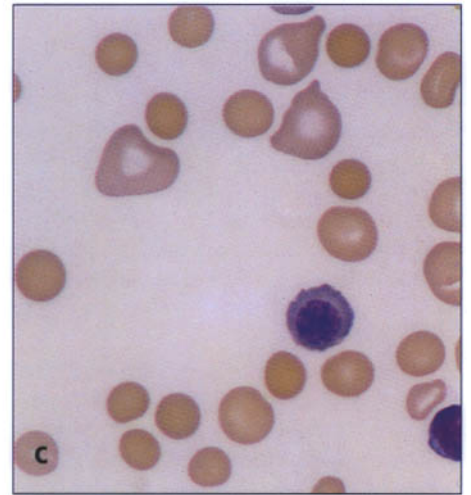
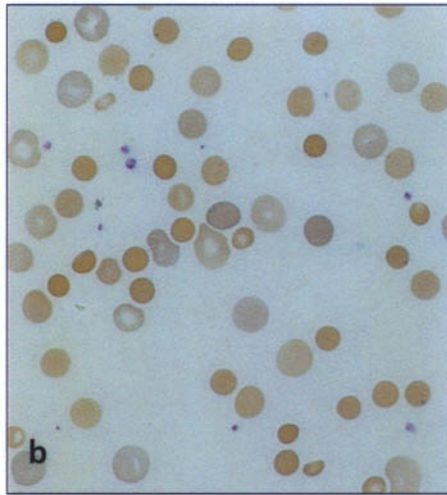
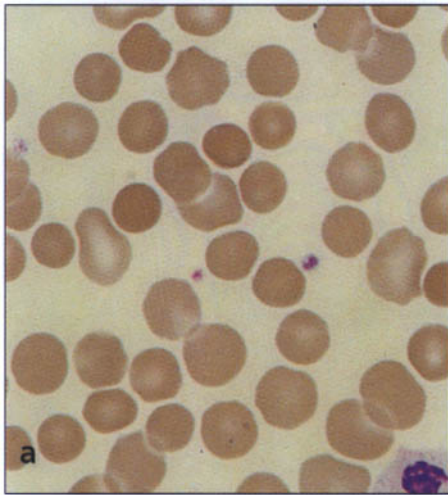
Cellular degenerative changes

Cellular degenerative changes may be caused by prolonged storage of the blood in EDTA, delay in fixing, inadequate fixing time, or use of methanol contaminated with water.

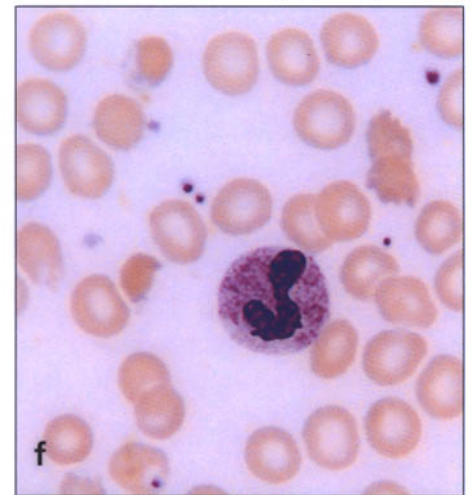
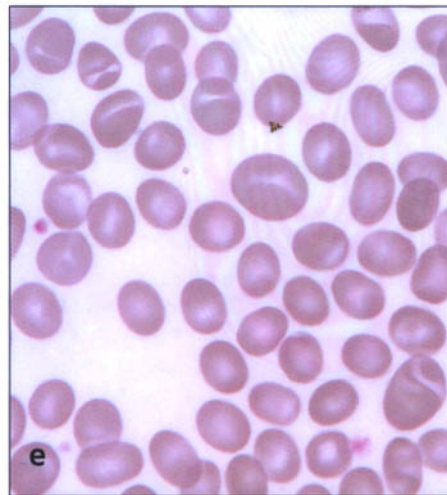
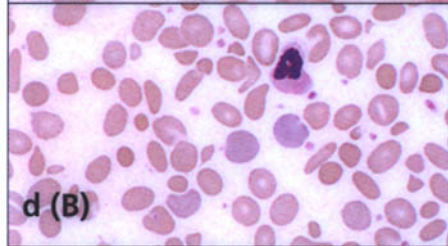
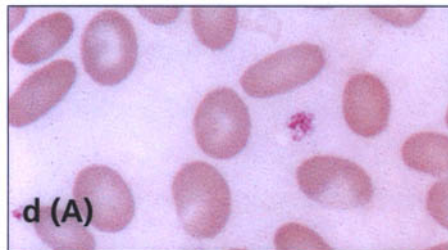


b. x 100 magnification

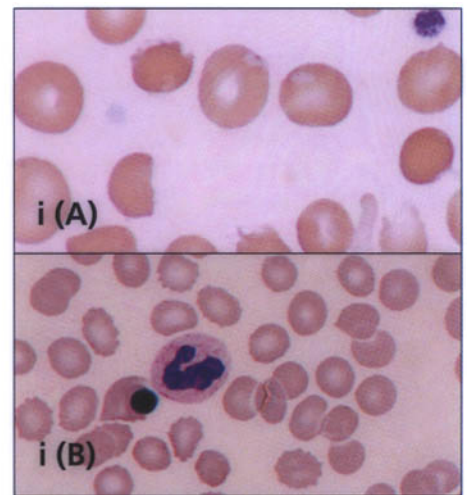
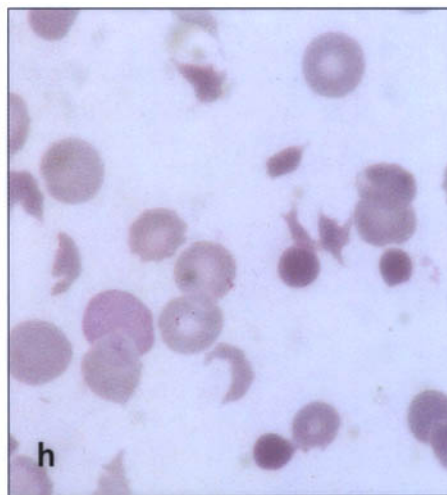
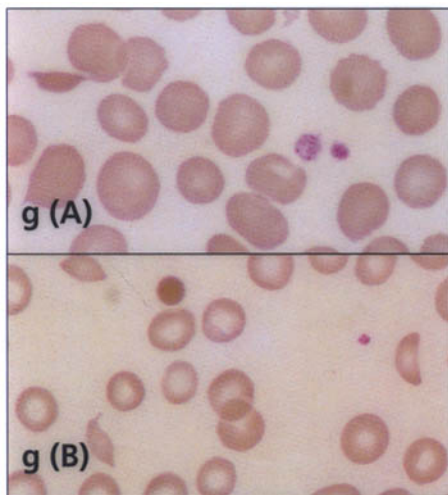
Haemolytic anaemias



a. Hereditary spherocytosis. Numerous spherocytes and several polychromatic macrocytes indicating increased reticulocyte count. This shows well compensated haemolysis, with Hb 14.9 g/dl. **b. Severe autoimmune haemolytic anaemia.** Spherocytes and polychromatic macrocytes, indicating increased reticulocyte count. **c. Haemolytic disease of the newborn.** Severe ABO type, showing spherocytes, polychromatic macrocytes (top), and a nucleated red cell (centre right).



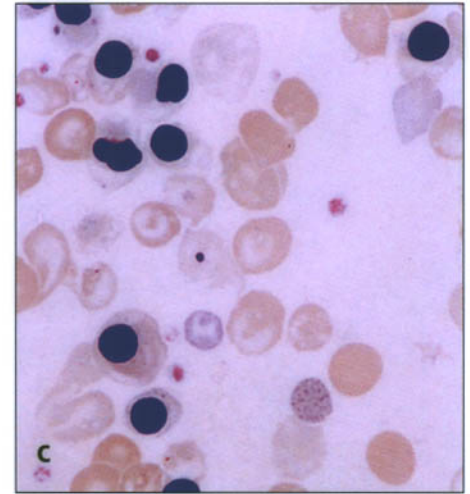
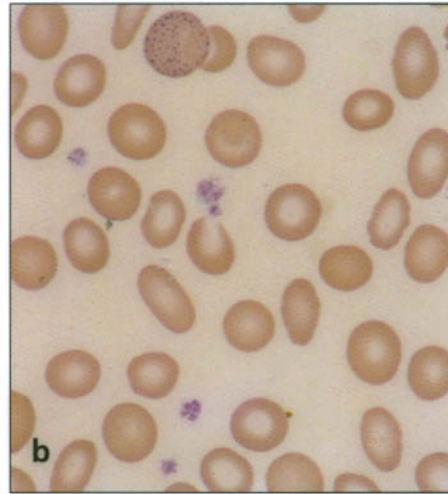
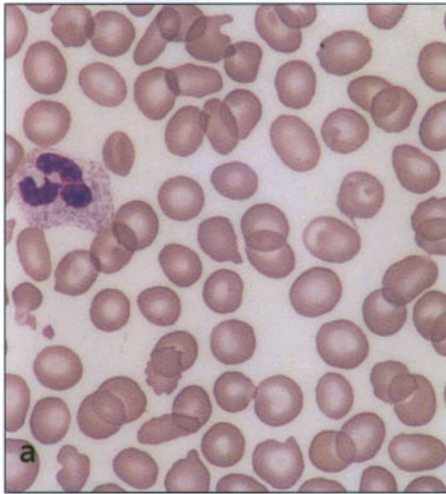
d. (A) Hereditary elliptocytosis. Elliptocytes and ovalocytes. **(B) Hereditary pyropoikilocytosis (low power).** Marked poikilocytosis with some elliptocytes and ovalocytes, microspherocytes, and cell fragments. Several polychromatic macrocytes (reticulocytes). **e. South-east Asian ovalocytosis.** Stomatocytes and ovalocytes. Note two macrocytes (which are also stomatocytes) – the diagnostic feature of this condition. **f. G6PD deficiency.** Film made during a haemolytic episode, showing several irregularly contracted cells which differ from spherocytes (see a and b) in their irregular outline and, in some, retraction of the haemoglobin from the cytoplasmic membrane.



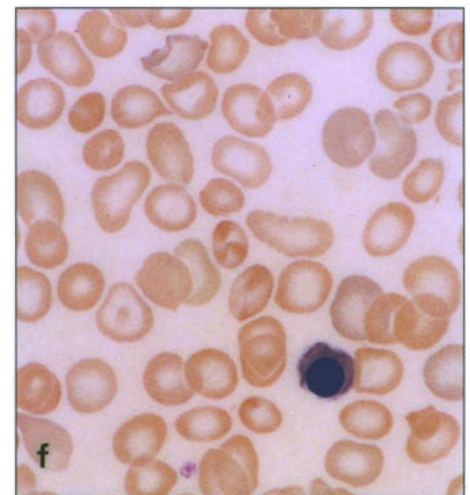
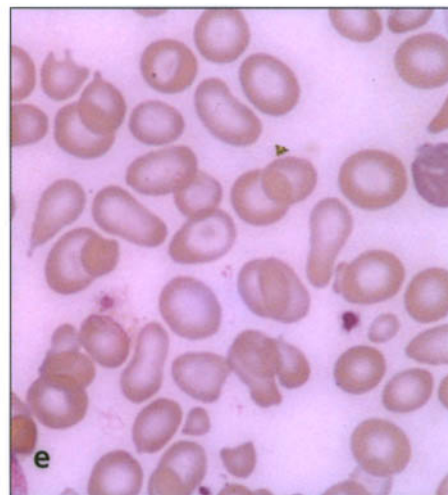
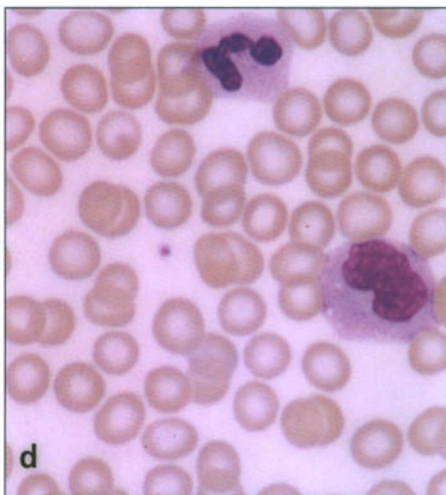
g. (A) Severe microangiopathic haemolytic anaemia. Numerous red cell fragments. **(B) Moderately severe microangiopathic haemolytic anaemia.** Several red cell fragments; one is a microspherocyte. This pattern may occur in pregnancy-associated hypertension, haemolytic-uraemic syndrome, and thrombotic thrombocytopenic purpura. **h. Viper-bite haemolysis.** Numerous microspherocytes and virtual absence of platelets caused by disseminated intravascular coagulation. **i. (A) Dapsone toxicity.** Numerous irregularly contracted cells. Note that one cell (left side) shows apparent retraction of haemoglobin from the cytoplasmic membrane. **(B) Sodium chlorate poisoning.** Marked crenation (echinocytosis) and irregularly contracted cells. There is one nucleated red cell to the left of the neutrophil.



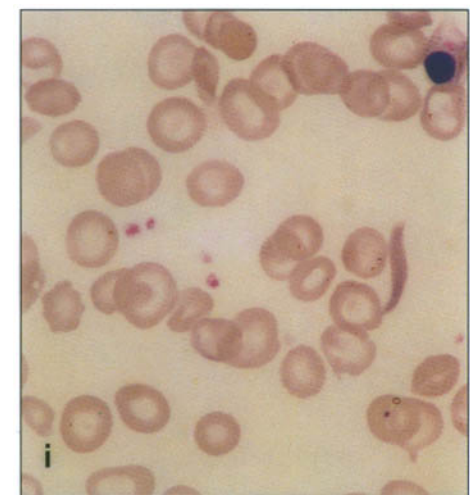
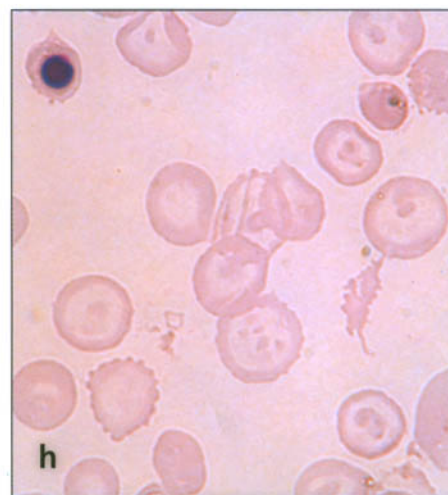
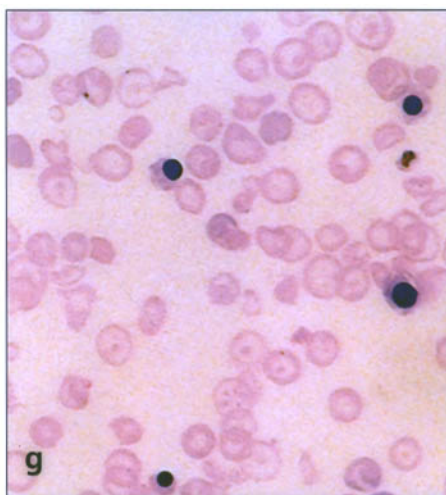
Thalassaemias



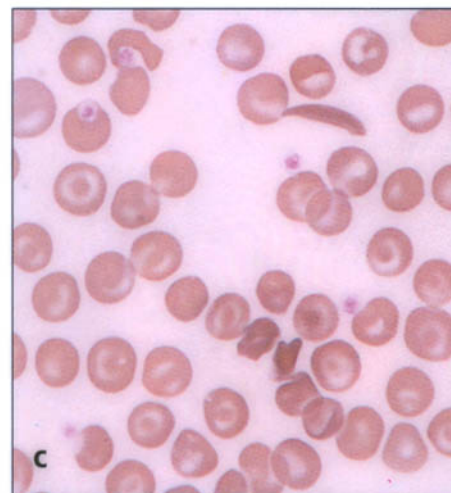
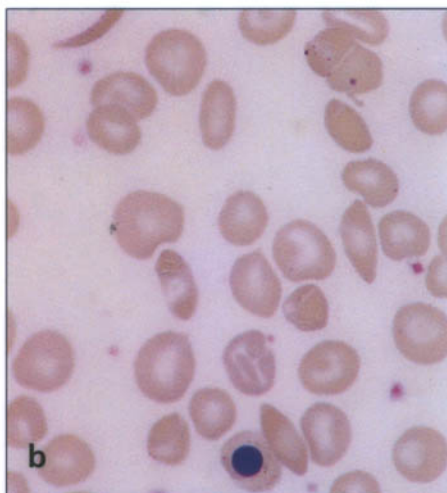
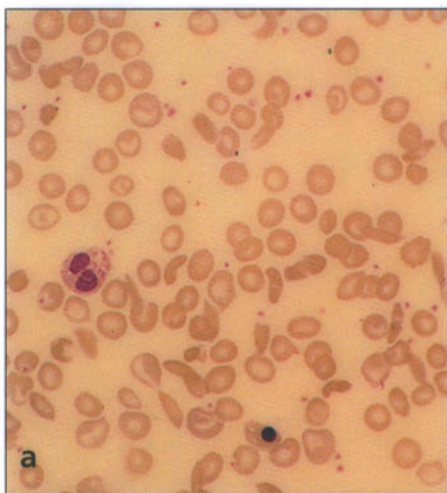
a. β -Thalassaemia trait. Microcytosis, anisocytosis, and poikilocytosis. Family studies showed this case to be heterozygous for β^0 -thalassaemia. **b. β -Thalassaemia trait.** Microcytosis, anisocytosis, and poikilocytosis. Some red cells are hypochromic and one (at top) shows basophilic stippling. Note that some poikilocytes are elliptocytes: these are more common in iron deficiency but are sometimes seen in β -thalassaemia. Target cells often occur but are not seen here. **c. β -Thalassaemia major.** Numerous nucleated red cells (normoblasts). The red cells are a mixture of the patient's own (microcytic and poorly haemoglobinized) and normal transfused cells. There are some target cells and a cell with basophilic stippling (centre right). One cell (centre) shows a Howell-Jolly body.



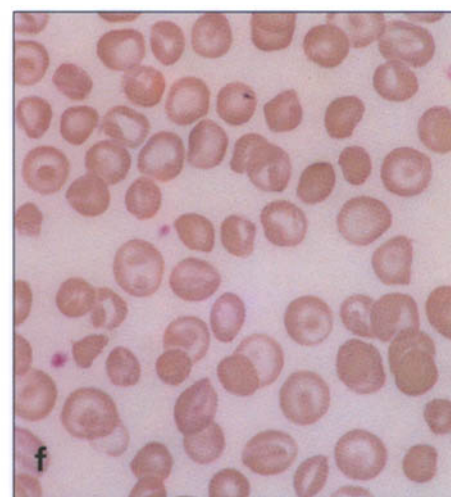
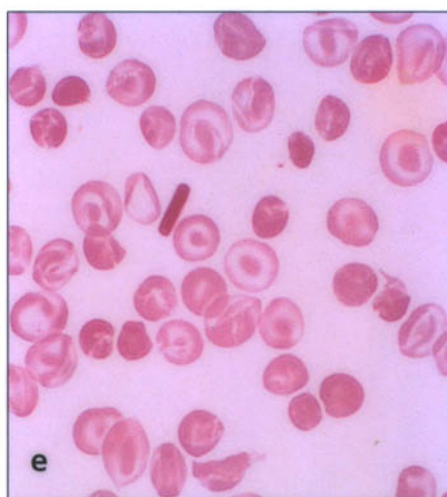
d. α -Thalassaemia trait. Microcytosis and hypochromia. Note the normal neutrophil and the monocyte. **e. Haemoglobin-H disease.** Very marked anisocytosis, poikilocytosis, and moderate hypochromia. The poikilocytes include both ovalocytes and tear-drop poikilocytes. **f. Haemoglobin-H disease.** Anisocytosis, poikilocytosis, one target cell, and one nucleated red cell.



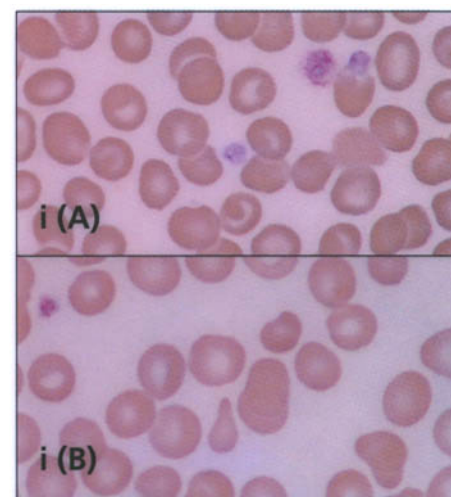
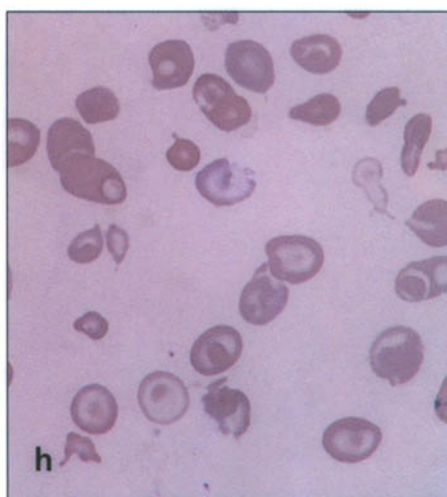
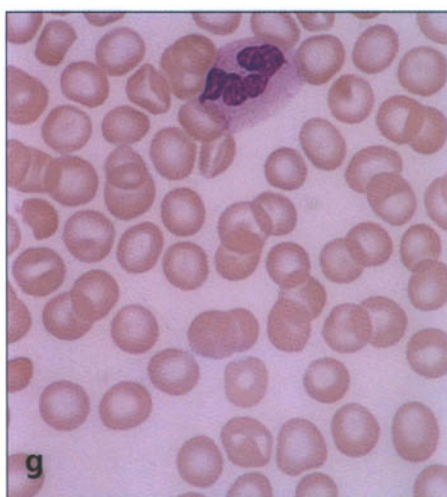
g. Haemoglobin Bart's hydrops foetalis (low power). Numerous nucleated red cells, anisocytosis, poikilocytosis, and hypochromia. **h. Haemoglobin Bart's hydrops foetalis.** Anisocytosis, poikilocytosis, hypochromia, and one nucleated red cell. **i. Sickle-cell/ β^0 -thalassaemia (compound heterozygosity).** Anisocytosis, poikilocytosis, one nucleated red cell, and one sickle cell. Note the greater hypochromia than in sickle-cell anaemia (a and b, front of this plate).

Haemoglobinopathies

a. Sick cell anaemia (low power). Anisocytosis and poikilocytosis. The poikilocytes include typical sickle cells (crescent-shaped) and partially sickled cells. Some red cells are hypochromic; there is one nucleated red cell (bottom). **b. Sick cell anaemia.** One sickle cell (top left), one nucleated red cell (at bottom), a Howell-Jolly body (top right), and a polychromatic macrocyte (centre). **c. Sick cell/haemoglobin-C compound heterozygosity.** One sickle cell (top), several target cells, and an SC poikilocyte (below centre). Compared with sickle-cell anaemia, sickle cells are uncommon in blood films from the Hb-S/Hb-C compound heterozygosity, whereas SC poikilocytes are seen in many patients with this condition.



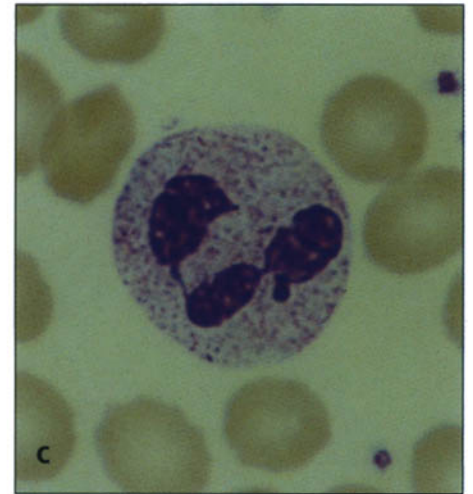
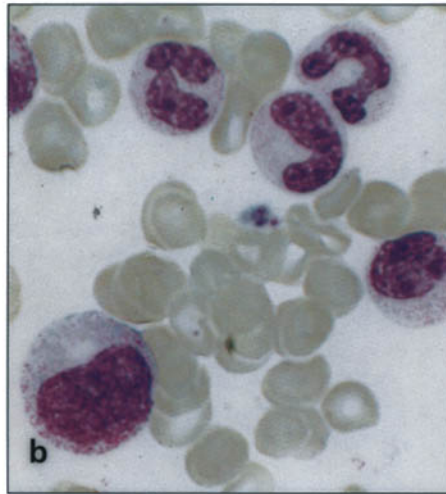
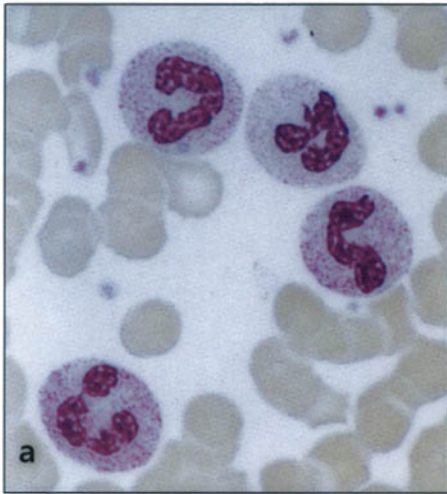
d. Haemoglobin-C disease. Target cells and irregularly contracted cells. At the top there is one "hemighost", i.e. all the haemoglobin is contracted into one half of the cell. **e. Haemoglobin-C disease.** Target cells and irregularly contracted cells. There is one characteristic straight-edged Hb-C crystal; crystals are usually seen inside a red-cell membrane which is otherwise empty of haemoglobin, but in the crystal shown here no cell outline is apparent. Compare the straight edges of the Hb-C crystal with the more complex shape that occurs in the SC poikilocytes, which contain both Hb-S and Hb-C. **f. Haemoglobin-E disease.** Mild anaemia, hypochromia, and an irregularly contracted cell (left). The term "haemoglobin-E heterozygosity" may be preferred, since the condition is usually asymptomatic.



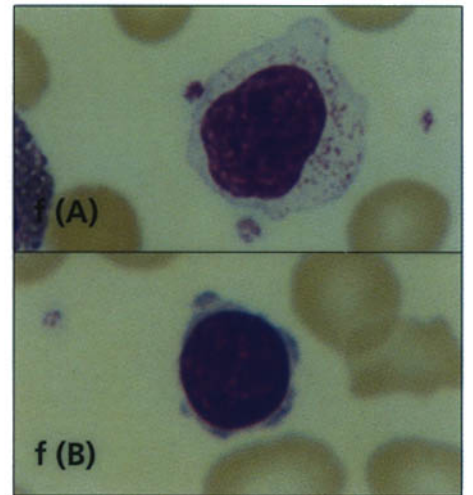
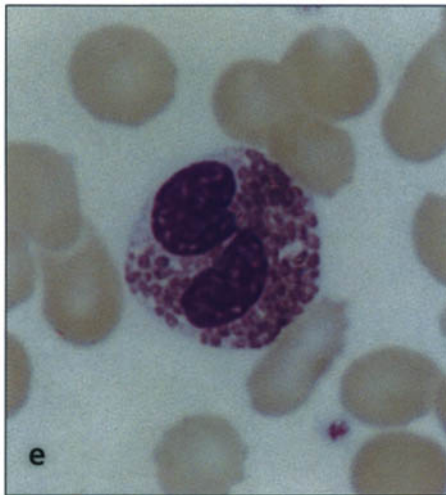
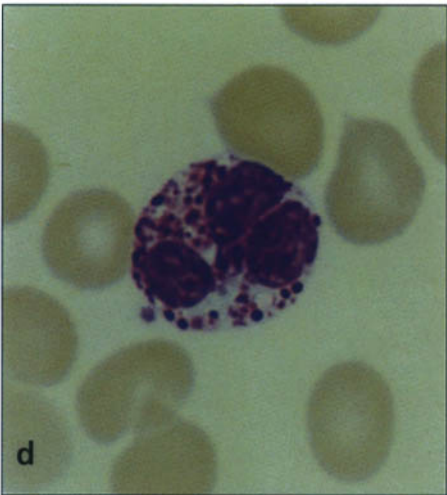
g. Haemoglobin-E trait. Anisocytosis and a few target cells. **h. Haemoglobin-E/ β -thalassaemia compound heterozygosity.** Marked anisocytosis and poikilocytosis. There are numerous target cells, and some red cells are hypochromic. **i. Haemoglobin-E trait** (A) Target cells. The large platelets are due to an incidental inherited platelet abnormality and are not related to the haemoglobinopathy. (B) Two irregularly contracted cells.



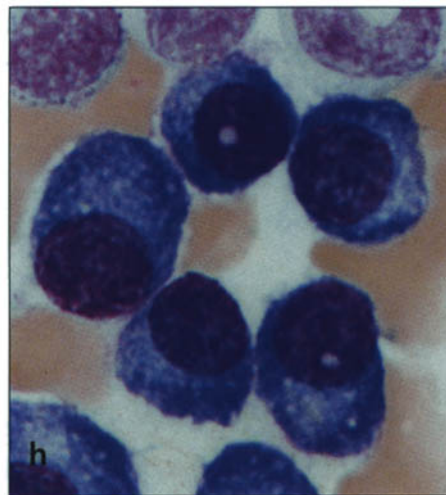
Leukocytes in stained blood films



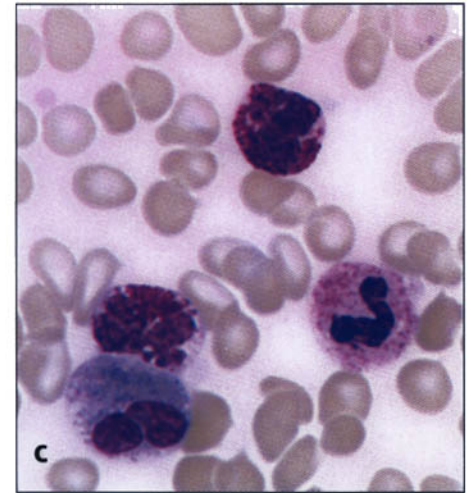
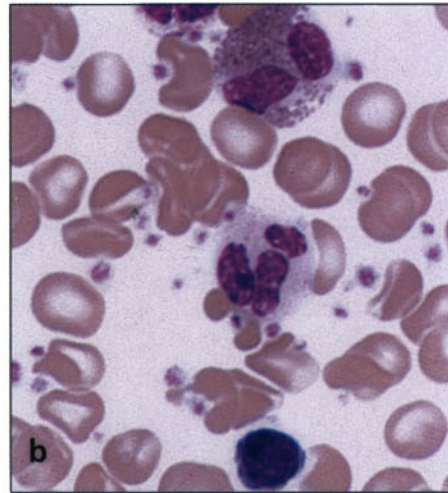
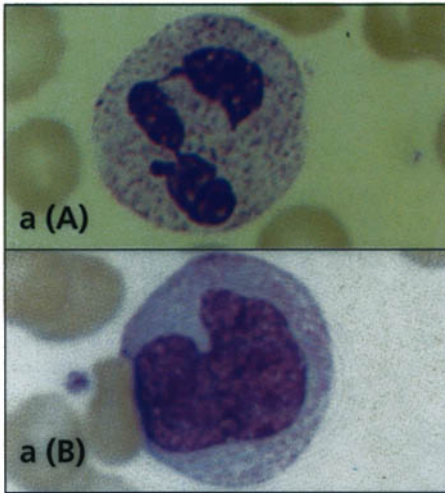
a. Polymorphonuclear neutrophils. Mature cells with nuclei that are segmented into two or three lobes. **b. Myeloid cells.** Young neutrophils with nuclei that are not segmented into lobes ("band" cells), cells with slightly indented nuclei (metamyelocytes), and a cell with a round nucleus (myelocyte). **c. Neutrophil with three lobes.** The right-hand lobe has a small attached body – this occurs only in females.



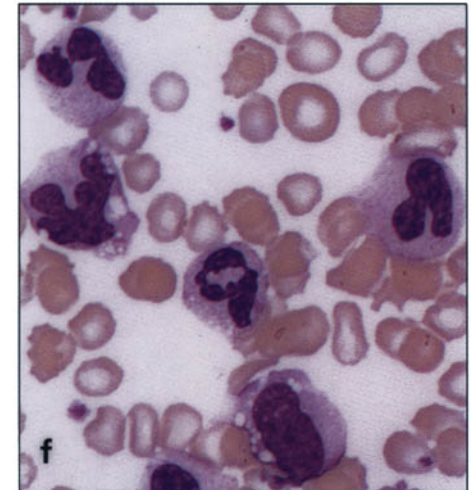
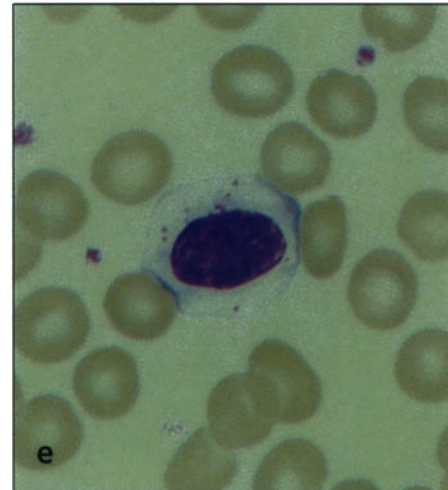
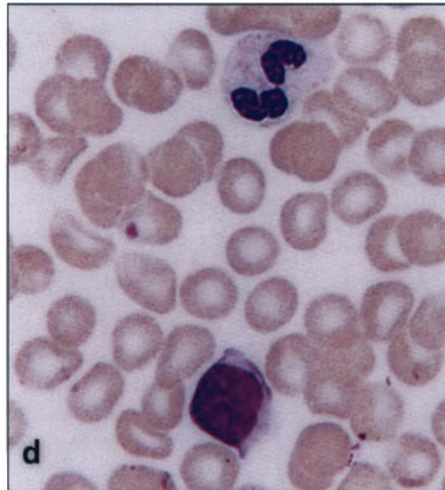
d. Basophil e. Eosinophil f. (A) Large lymphocytes (B) Small lymphocytes



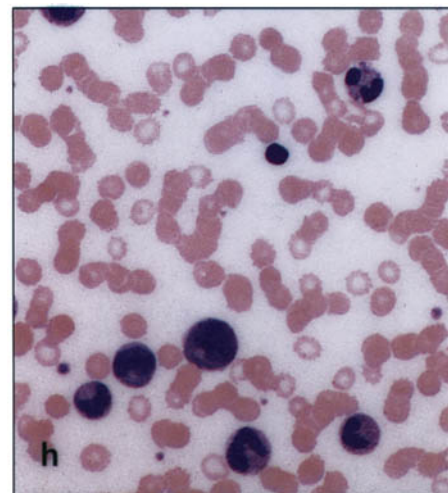
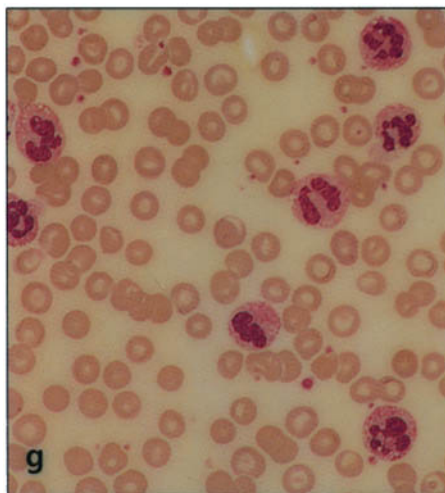
Leukocytes



a. (A) Normal neutrophil. The nucleus is segmented, usually with 2-4 (occasionally 5) lobes, interconnected by a thin strand of nuclear material. The cytoplasm stains pink-orange with a finely granular appearance. **(B) Normal monocyte.** The largest normal blood cell. The large nucleus is curved in a horseshoe shape or folded, but not segmented. The cytoplasm is greyish blue with some fine reddish (azurophil) granules. **b. Normal eosinophil, neutrophils, and a small lymphocyte.** The eosinophil (top) is a little larger than a neutrophil and the nucleus usually has two lobes; the cytoplasm is packed with large refractile orange-red granules. The small lymphocyte (bottom) has a round non-segmented dense nucleus surrounded by a thin rim of cytoplasm. Lymphocyte nuclei are uniformly about 9 μm in diameter, which provides a useful guide for estimating whether the red cells are normocytic (about 7-8 μm diameter), microcytic (smaller than normal), or macrocytic (larger than normal). **c. Basophils.** Two basophils can be seen, together with an eosinophil (right) and a neutrophil (top). Basophils have nuclear segments which may fold up on each other and are often obscured by the distinctive large blue-black cytoplasmic granules.



d. Lymphocyte and neutrophil. By contrast with the small lymphocyte in **b**, this is a large lymphocyte with a less dense nucleus and abundant pale blue cytoplasm. The neutrophil is hypogranular; this may be the result of poor staining or it may indicate an abnormality in granulopoiesis. **e. Large granular lymphocyte.** By contrast with the lymphocytes in **b** and **d**, the large granular lymphocyte has abundant pale blue cytoplasm containing prominent azurophil granules. **f. Neutrophils and monocytes.** Note that the nucleus of the monocyte varies in shape and is not segmented; the cytoplasm is bluish grey and contains vacuoles and fine azurophil granules.



g. Neutrophil leukocytosis (neutrophilia). There is an obvious increase in the proportion of neutrophils, and their nuclei vary in segmentation from band cells (see **h**) to cells with 4 or 5 lobes. The cytoplasm shows heavily stained (toxic) granules and vacuoles. **h. Neutrophil leukocytosis with left-shift (leukaemoid reaction).** In addition to mature neutrophils with segmented nuclei, there are non-segmented myelocytes/metamyelocytes with nuclei that are only slightly dented, and a band cell with a horseshoe-shaped nucleus with parallel edges. The cytoplasm shows toxic granulation.



Care of the microscope

Daily maintenance

- *After use.* Clean optical surfaces and objectives carefully with a small camel-hair brush or lens paper. Remove immersion-oil residues using lens paper soaked with an optical cleaning solution:

petroleum ether or toluene	40%
ethanol	40%
ether	20%

 Remove heavy contamination with mild soap solution.
- *At the end of the day.* Clean optical surfaces with lens paper or fine tissue soaked with an optical cleaning solution. Use a small camel-hair brush or a blower to remove dust particles. Clean the mirror with a fine tissue soaked with alcohol. Clean the support with a 50/50 solution of distilled water/96% ethanol. (Note: this solution is not suitable for cleaning optical parts.)

Storage

- *In hot dry climates.* The main problem is dust. Always keep the microscope in an airtight plastic cover when it is not in use.
- *In hot humid climates.* If the microscope is not properly stored, fungus may develop, particularly on the surfaces of the lenses, in the grooves of the screws, and under the paint. Fungus can corrode lenses, so that the microscope will quickly become useless and have to be replaced. Always keep the microscope in an airtight plastic cover when it is not in use, with a dish of blue silica to desiccate the air inside the cover. (The silica will turn red when it has lost the capacity to absorb moisture from the air, but can easily be regenerated by heating, e.g. in an oven.) Store the microscope in a well ventilated area away from direct light. Clean dust from the microscope daily.

Remember:

- Never dip objectives in xylene, ethanol, or acetone.
- Never use ordinary paper to clean lenses.
- Never touch objectives with your fingers.
- Never clean the supports or stage with xylene or acetone.
- Never clean the inside lenses of the eyepieces with cloth or paper (this might remove the anti-reflecting coating): use a fine paint brush only.
- Never store separate eyepieces and objectives without sealing each in an airtight plastic bag (plus blue silica in humid climates).
- Never leave the microscope without the eyepieces unless the openings are plugged to exclude dust.
- Never keep the microscope in a closed wooden box in hot humid climates.
- Never put the microscope away with immersion oil on the objective.
- Never push the objective through a slide: both slide and objective may break.

Use of the microscope

All microscopes need to have a good light source – either daylight or electric light. Modern microscopes have a built-in lamp; some may have a detachable lamp. A microscope lamp should have a blue “daylight” filter, which converts the yellow light of the electric lamp into white “natural” light. For good microscopy it is essential to be able to control the intensity of light.

When examining a slide, move the microscope objective close to the slide by means of the screws for coarse and fine adjustment. Verify proper illumination of the slide by aligning the substage condenser and light diaphragm into the correct position. On some microscopes the intensity of light provided by the light source can be adjusted.

Difficulties that may be encountered during microscopy

Absence of images:

- The objective has been moved too fast and is too high. Repeat the adjustment.
- The wrong side of the slide is uppermost. Turn the slide over and replace on the microscope stage.

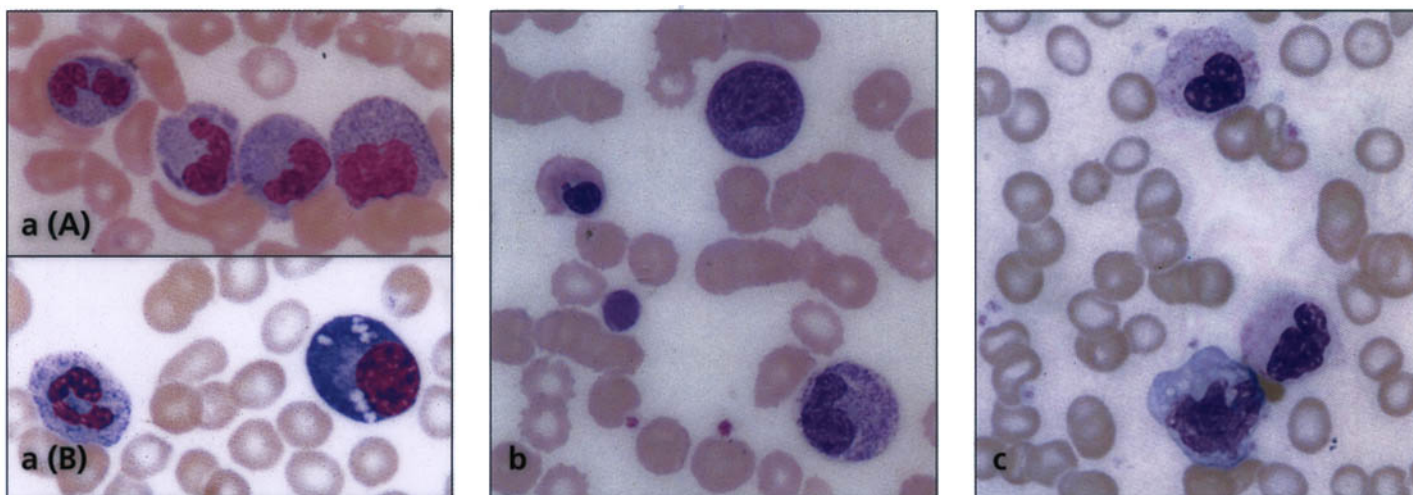
Absence of light:

- The illumination system is not functioning or the mirror is not correctly positioned to reflect daylight through the optical pathway of the microscope. Check the electrical support or correct the position of the mirror. The flat side of the mirror should be used to reflect artificial light; when the light source is daylight, the concave side of the mirror should be used, without the substage condenser.
- The objective has not been turned into the fixed position. Turn the revolving nosepiece slightly to immobilize the objective in the axis of the tube.
- The iris diaphragm is closed. Open it.
- The substage condenser is incorrectly positioned. Switch on the lamp or turn the mirror to reflect light into the light pathway. Raise the condenser to the maximum extent and open the iris diaphragm to two-thirds of its maximum aperture. Remove an eyepiece and look down the tube. If necessary, realign the condenser and lamp or condenser and mirror, so that the brightest light appears in the centre of the condenser. Replace the eyepiece.

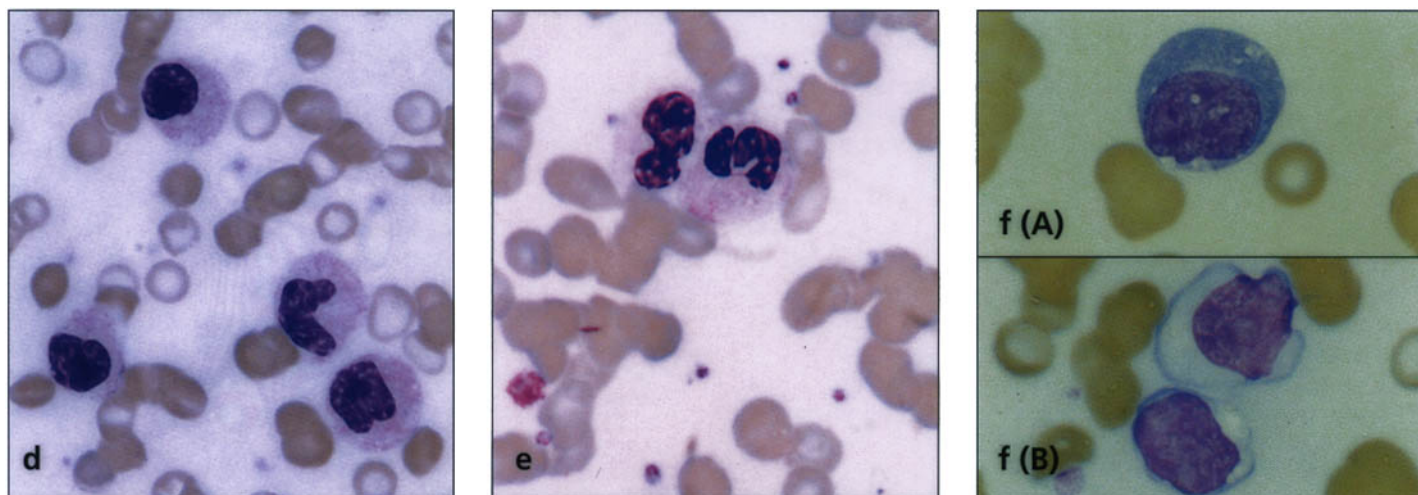
Absence of clear images:

- The lenses are dirty. Verify that the lenses of the ocular eyepieces are clear by turning them in the tube. Traces of grease or dust can be detected by the presence of spots that move with the movement of the eyepiece. Clean the lenses carefully with lens paper and an optical cleaning solution as necessary.
- There is immersion oil on the objectives, either because the oil was not removed after previous microscopy or because excessive oil was used. Remove the oil carefully and repeat the examination in another area of the slide.
- The microscope slide or the coverglass is dirty. Repeat the preparation using a clean slide and coverglass.
- The fine adjustment screw is blocked. Turn the screw, without using force, in the reverse direction and repeat the adjustment.

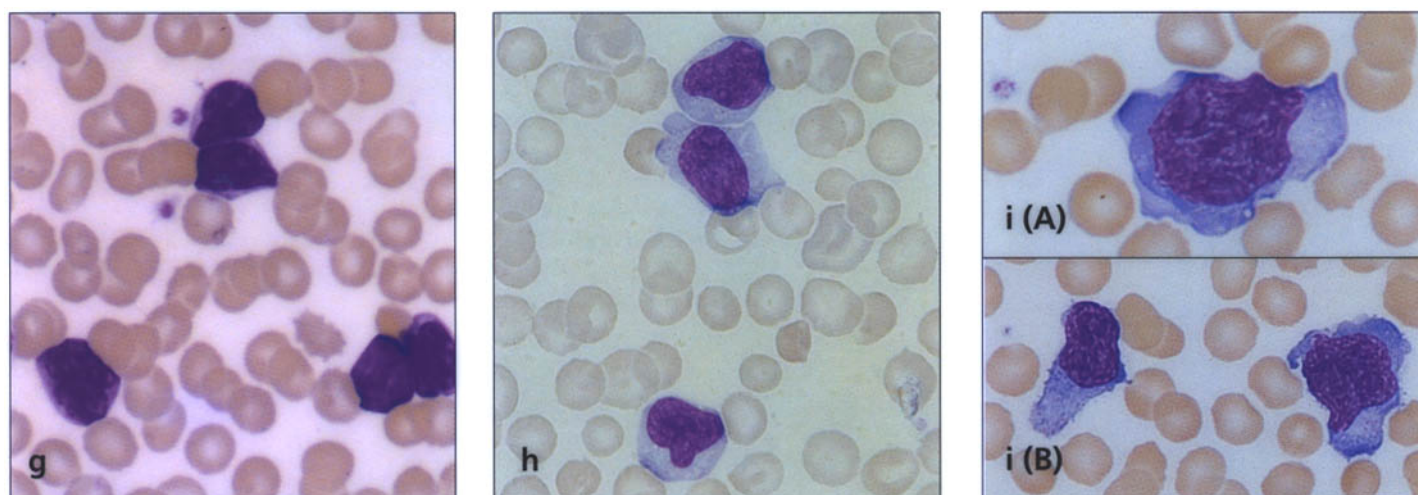
Infections



a. (A) Neutrophilia. Showing toxic granulation and left-shift. Two very distinct Döhle bodies can be seen – in the two neutrophils on the left; these are typical changes in severe bacterial infection. **(B) Reactive plasma cell.** Occurs in infections. The plasma cell has characteristic dense clumped chromatin and an eccentric nucleus. The other leukocyte is a neutrophil with some dark-staining granules in the cytoplasm. Some red cells are slightly hypochromic, suggestive of "anaemia of chronic disease", which occurs in chronic infection or inflammation; the anaemia is initially normocytic and normochromic, then microcytic and hypochromic (see **a-c**, Plate 2, Nutritional anaemias). **b. Leuko-erythroblastic blood film in severe infection.** A promyelocyte (top) and a metamyelocyte with toxic granulation (bottom). Red cells show rouleaux and some are crenated; there is also one nucleated red cell. A leuko-erythroblastic picture can also result from acute blood loss and from bone marrow infiltration. **c. Tuberculosis.** A monocyte and two neutrophils with toxic changes and poor lobulation. The red cells are hypochromic and microcytic (compare with **a(A)**).



d. Tuberculosis. Neutrophilia with toxic changes and a left-shift. There is poor lobulation, with two non-segmented neutrophils resembling Pelger cells. The red cells are hypochromic with marked rouleaux. **e. AIDS.** Dysplastic neutrophils and marked rouleaux in an untreated case of AIDS. Both neutrophils are hypogranular and the left-hand one shows poor lobulation. **f. (A), (B) AIDS.** Plasmacytoid reactive lymphocytes, rouleaux, and increased background staining in a patient with advanced AIDS and *Pneumocystis carinii* pneumonia.



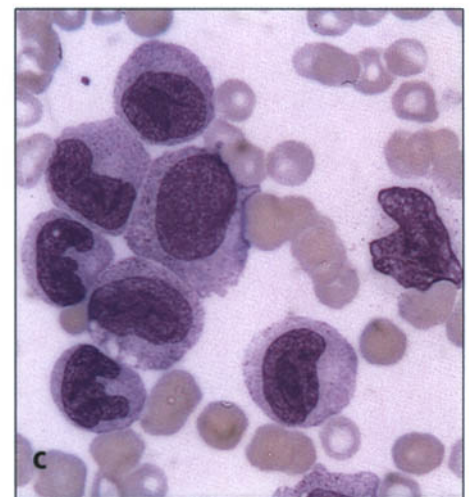
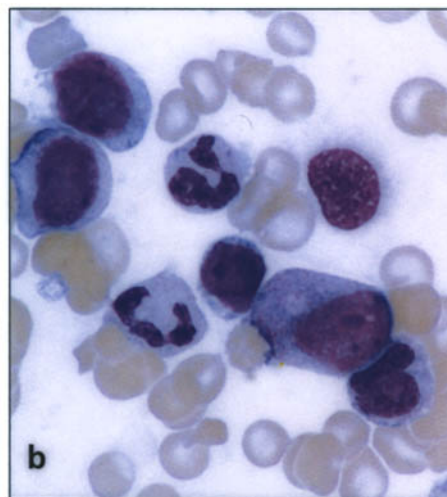
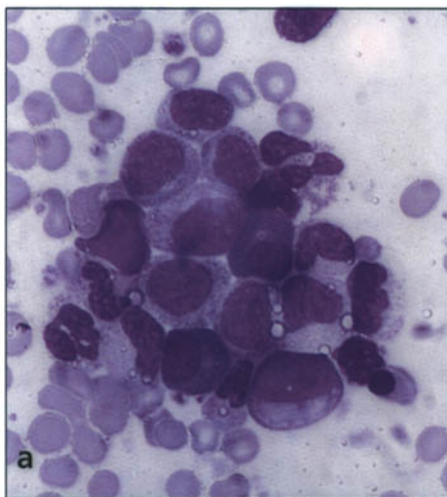
g. Pertussis (whooping cough). Small and mature lymphocytes with no obvious morphological abnormality. **h. Infectious mononucleosis (glandular fever).** There are three reactive lymphocytes, which are larger than normal lymphocytes with more abundant cytoplasm and irregular nuclear shape. Equally atypical cells can also be seen in other infections and in children with bacterial infections. **i. Infectious mononucleosis. (A)** An atypical lymphocyte (or atypical mononuclear cell), which is very large with abundant basophilic cytoplasm showing a scalloped edge around red cells. There are several large nucleoli. This type of cell is very characteristic of the disease. **(B)** Two atypical lymphocytes.



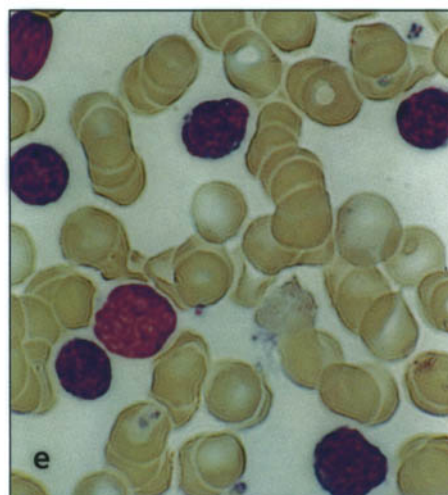
Leukaemias

Leukaemias are uncommon conditions, but it is essential to be aware that they may occasionally be identified as the cause in a patient who presents with anaemia.

When any of the features illustrated below is seen, the blood film should be reviewed by an experienced haematologist. Diagnosis and classification of the type of leukaemia will often require examination of the patient's bone marrow, special cytochemical stains, and immunophenotyping, which are beyond the resources of a health clinic or small hospital laboratory.

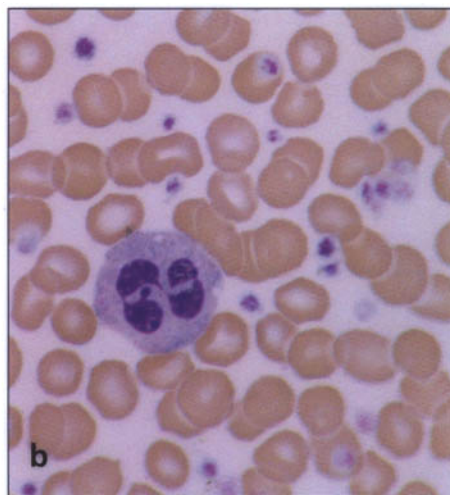
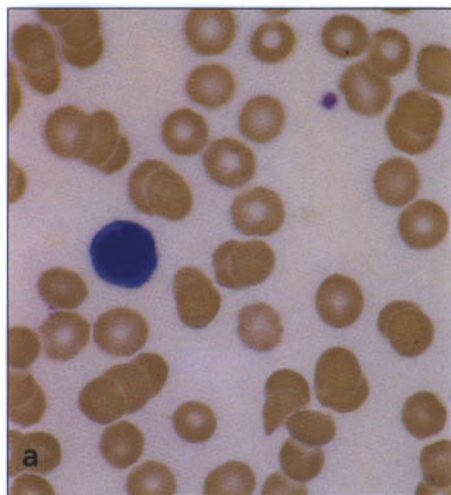


a. Chronic myeloid leukaemia. There is a very high leukocyte count and the blood film shows myeloid cells at all stages of maturation from myeloblasts to neutrophils. There is one normoblast on the right, towards the bottom. The film differs from that in leukaemoid reaction (see **h**, Plate 5, Leukocytes) and in leukoerythroblastic conditions (see **b**, Plate 6, Infections) in that greater numbers of eosinophils and basophils are also seen. **b. Acute myeloid leukaemia.** Most cases are characterized by a preponderance of myeloblasts. In this film there are three myeloblasts, a promyelocyte, and two neutrophils with no cytoplasmic granules. **c. Acute monoblastic leukaemia.** Blast cells and maturing monocytic cells with folded nuclei (compare with normal monocytes in **a** and **f**, Plate 5, Leukocytes).

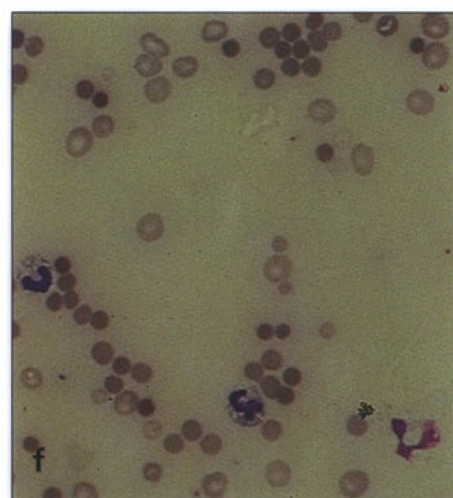
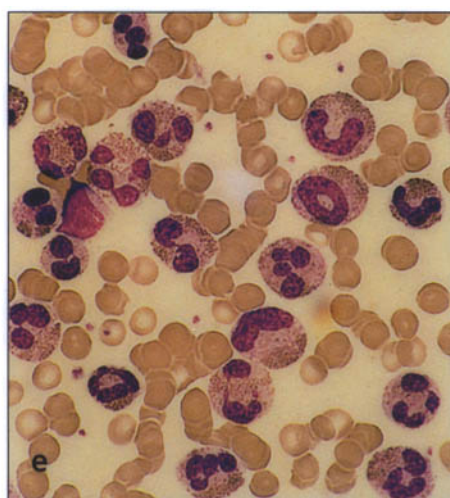
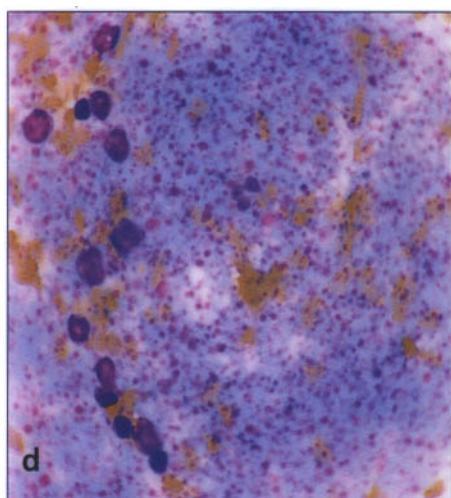


d. Acute lymphoblastic leukaemia. There are four lymphoblasts. It may sometimes be difficult to distinguish between lymphoblasts and myeloblasts (see **b**); it may also be difficult to distinguish lymphoblasts from reactive lymphoid cells (see **f-i**, Plate 6, Infections). **e. Chronic lymphocytic leukaemia.** Marked lymphocytosis with small mature lymphocytes and distorted cells without cytoplasm ("smear cells"), i.e. fragile cells disrupted when the film was made.

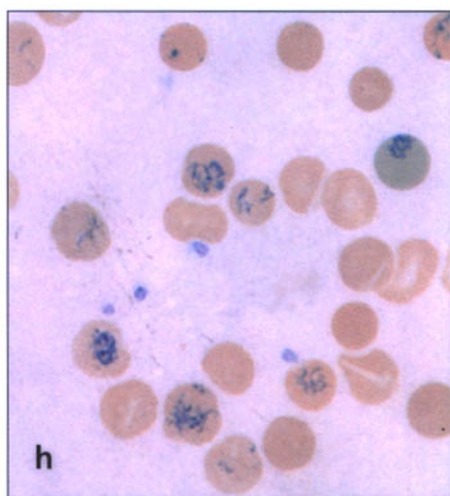
Miscellaneous



a. Thrombocytopenia. Only one platelet is seen: the platelet count was $70 \times 10^9/\text{litre}$. Note the marked rouleaux. **b. Thrombocytosis.** Numbers of platelets relative to the red cells are increased. The platelet count was $1135 \times 10^9/\text{litre}$. Note that the (normal) neutrophil has four lobes, together with a drumstick protuberance indicating that the blood film is from a female. **c. Platelet clumping.** Platelets are aggregated into moderately large clumps (compare with **d**). An accurate platelet count from the ratio of platelets to red cells would be impossible, but there are enough platelets to suggest that the count is normal.



d. Platelet clump. This massive clump of platelets was in the tail of the blood film, with very few platelets in the body of the film, demonstrating that the entire film should be surveyed to avoid a mistaken impression of thrombocytopenia. **e. Severe hypereosinophilia.** The eosinophils have one to four lobes; there are two eosinophil band forms and one with a ring-shaped nucleus. Some are hypogranular. Such striking eosinophilia may be caused by parasitic infection, drug hypersensitivity, reaction to tumours (e.g. Hodgkin disease), and eosinophilic leukaemia (rare); there are some unexplained cases, termed "idiopathic eosinophilia". **f. Clostridium welchii sepsis** (low power). There is severe haemolytic anaemia. Note the numerous spherocytes.



g. Clostridium welchii sepsis (high power). Spherocytes and microspherocytes can be seen. There is one red cell ghost (top left) caused by total leakage of haemoglobin from the cell. **h. Reticulocyte preparation.** This film, from a patient with hereditary spherocytosis, was made from a blood sample incubated with a solution of New methylene blue. The proportion of reticulocytes was very high: more than one-third of the cells are reticulocytes, compared with the normal level of 0.5-2 per 100 red cells.