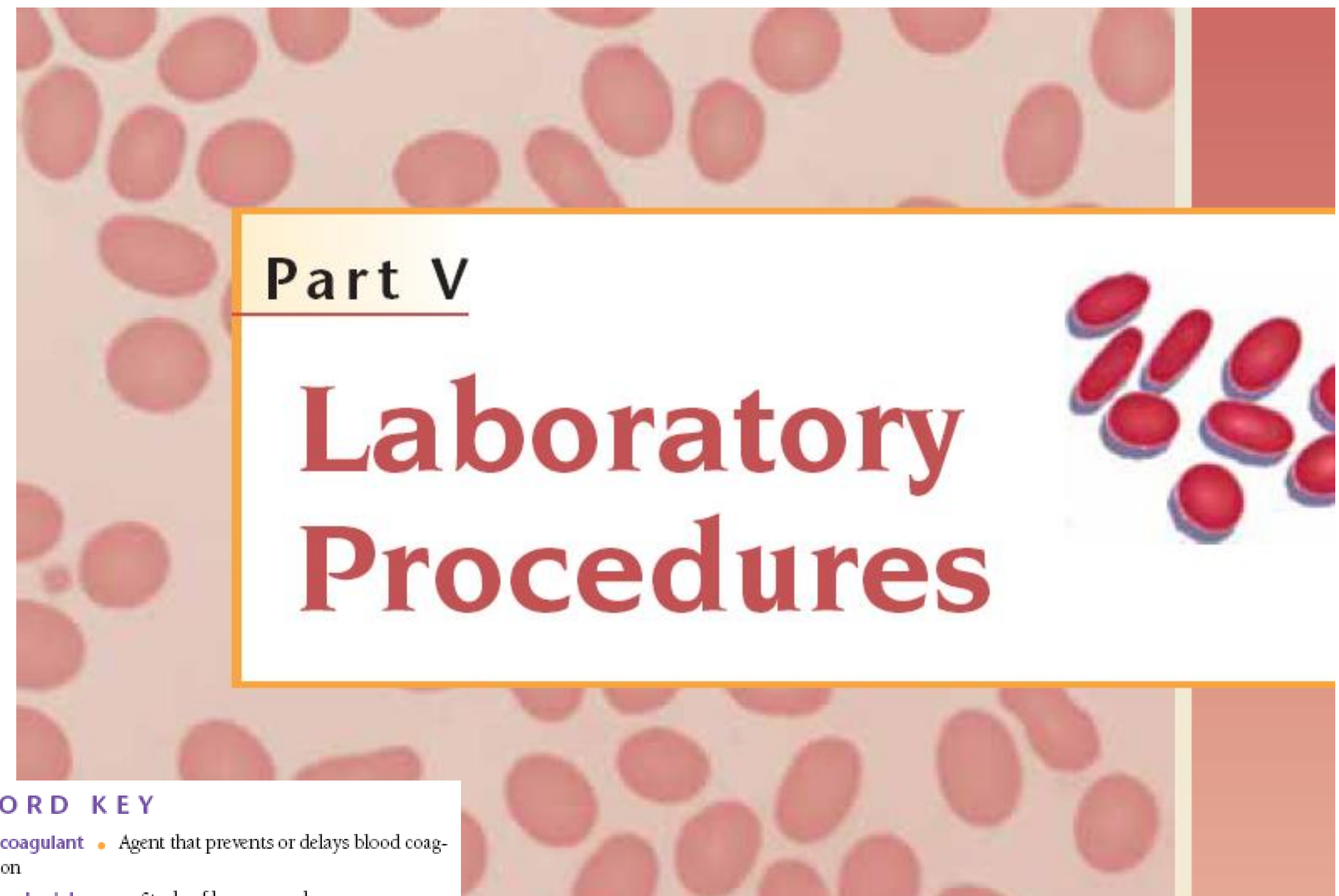


WORD KEY

Anticoagulant • Agent that prevents or delays blood coagulation

Pathophysiology • Study of how normal processes are altered by disease

Protocols • Formal ideas, plan, or scheme concerning patient care, bench work, administration, or research

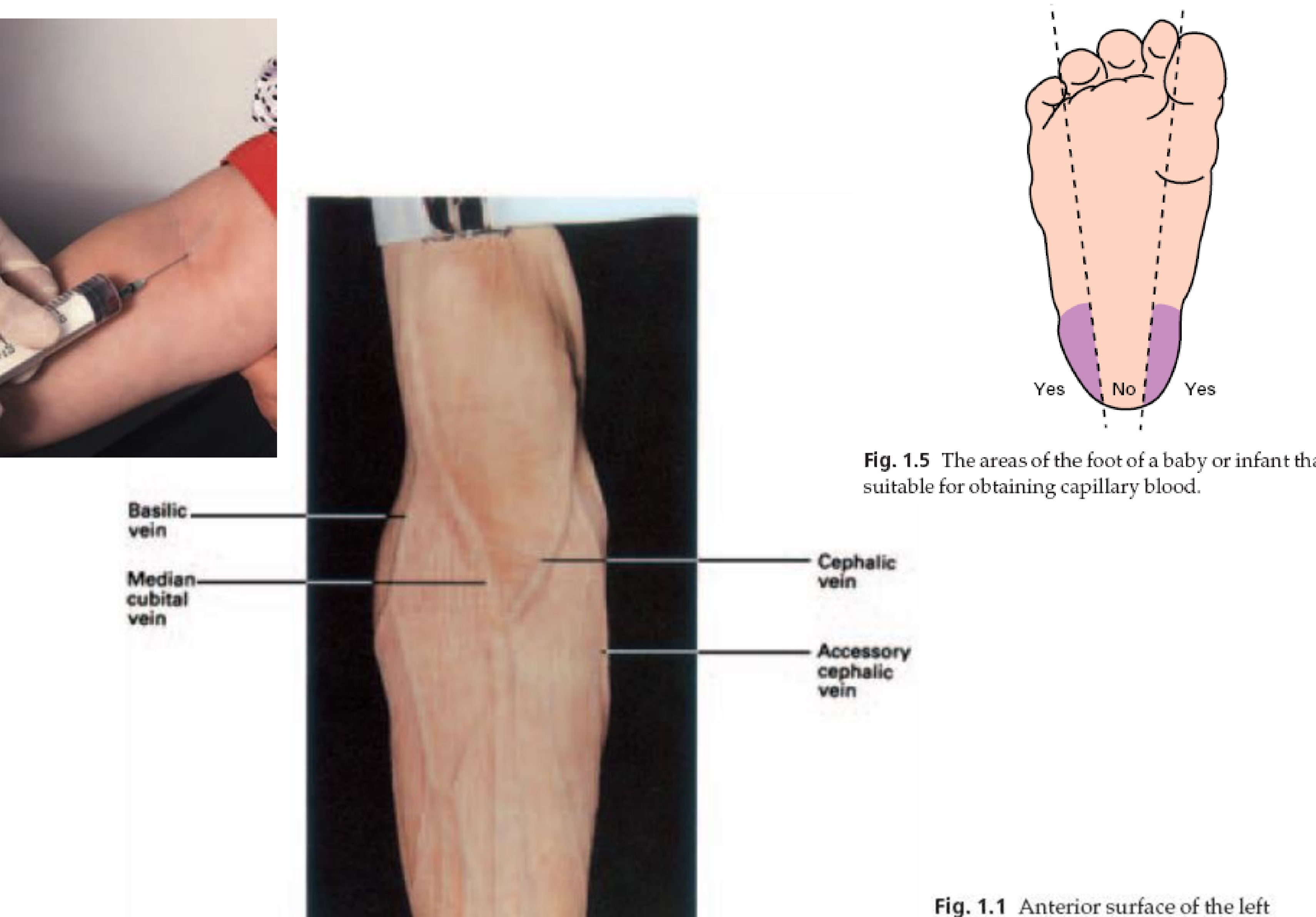


Fig. 1.5 The areas of the foot of a baby or infant that are suitable for obtaining capillary blood.

Fig. 1.1 Anterior surface of the left arm showing veins most suitable for venepuncture.

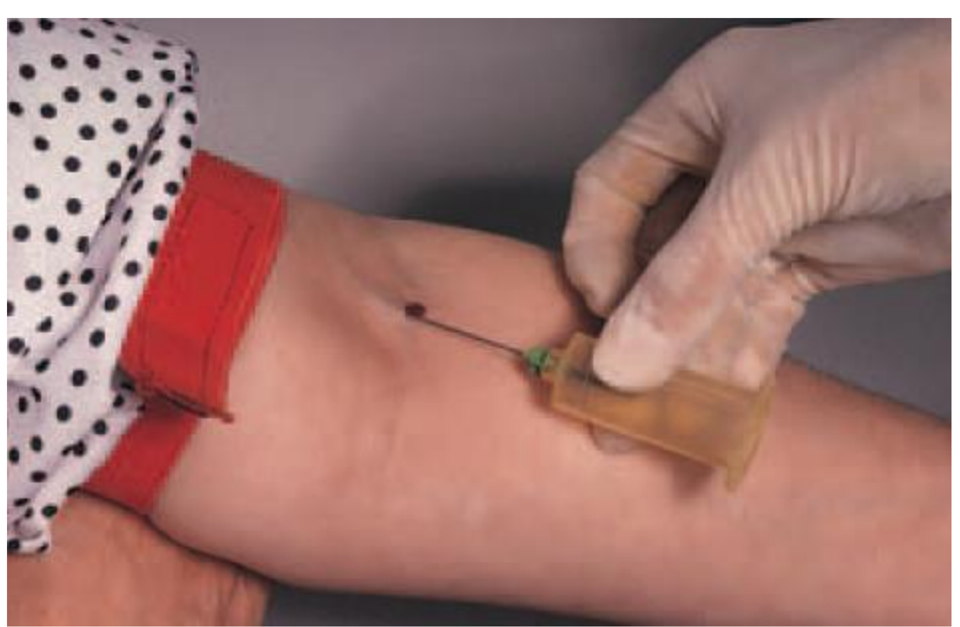
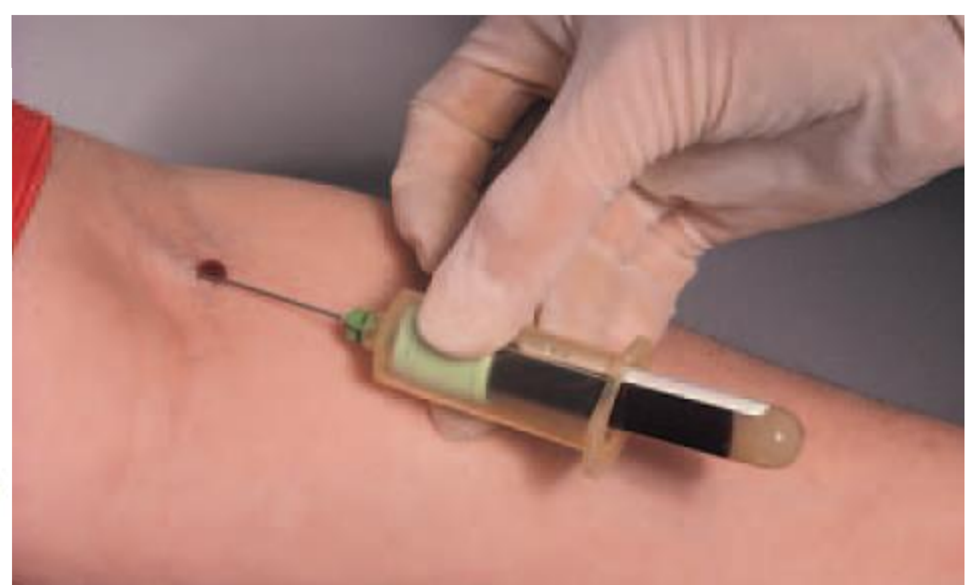


Figure 1.2 Gloves.



ضد انعقادها ی مورد استفاده در هماتولوژی

Anticoagulants in Hematology

EDTA (Ethylen Diamine Tetra Acetic Acid)

- با یون کلسیم ترکیب شده و مانع انعقاد خون می شود.
- ضد انعقادی مناسب برای استفاده در:

✓ شمارش سلولی

✓ هموگلوبین

✓ هماتوکریت

✓ شمارش پلاکت (از تجمع آن جلوگیری می کند.)

❑ میزان مصرف آن بر اساس توصیه ICSH، 1.25-2mg/ml خون است.

نكات كاربردي درمورد EDTA

- مقدار مصرفی متناسب با خون باشد در غیر این صورت گلبول آب را جذب کرده و سبب چروکیدگی می شود.
- افزایش EDTA بیش از 2mg/ml سبب کاهش قابل توجه هماتوکریت و در نتیجه افزایش MCHC خواهد شد.
- این ضد انعقاد بر روی فاکتورهای ۵ و ۸ اثر تخریبی دارد پس در آزمایشات انعقادی کاربرد دندارد.
- پدیده اقماری شدن (Satellite) که پلاکتها دور نوتروفیل و منوسیت به علت حضور یک آنتی بادی فعال شده در مجاورت EDTA حلقه می زنند و باعث کاهش کاذب پلاسما می گردند. در بیماری خاصی گزارش نشده است.
- نوع توصیه شده توسط NCCLS و ICSH نوع پودر K2 می باشد.
- نوعK3 مایع بوده و باعث چروکیدگی سلول (۲٪) شده و باعث کاهش WBC,MCV,RBC, به میزان ۲-۱٪ می شود.

سیترات سدیم

- باعث غیر فعال شدن کلسیم می ششود.
- در آزمایشات انعقادی و سدیمانتاسیون کاربرد دارد.
- کارآیی پلاکتها و فاکتورهای انعقادی حفظ می شود.
- ضد انعقاد انتخابی و سفارش شده برای بررسی عملمرد پلاکت می باشد.
- در دو نوع ۳،۲٪ و ۳،۸٪ وجود دارد.
- برای آزمایشات انعقادی از رقت ۱۰/۱ استفاده می شود.
- برای آزمایش سدیمانتاسیون از رقت ۵/۱ استفاده می شود.

هپارین

- خاصیت آنتی ترومبینی و آنتی ترومبوپلاستینی دارد.
- بر روی اندازه سلول و هماتوکریت تاثیری ندارد.
- ضد انعقاد توصیه شده برای بررسی هماتوکریت و تست شکنندگی اسمزی می باشد.
- برای بررسی گلبولهای سفید و پلاکتها مناسب نیست جرا که باعث تجمع آنها و ایجاد زمینه آبی رنگ می شود.
- میزان مصرف: 0.1-0.2 mg/ml

اگزالات آمونیوم یا پتاسیم

- باعث به وجود آمدن کلسیم اگزالات غیر فعال شده و کلسیم را از خون بر می دارد.
- جهت سدیمانتاسیون کاربرد دارد و لی برای CBC استفاده ندارد چون بر روی لام رسوب کرده و ساختمان سلول را ازبین می برد.
- میزان مصرف: <u>1-2 mg/ml</u>

- **روش ساخت:**
- اگزالات آمونیوم 1.2 gr
- اگزالات پتاسیم 0.8 gr
- فرمالین ۳۸٪ 1 ml
- آب مقطر 100 cc

فلورید سدیم

- با اتصال به کلسیم اثر ضد انعقادی دارد.
- از مصرف گلوکز توسط سلولهای خونی ممانعت می کند.
- در نمونه خون یا مایع مغزی نخاعی که در نیم ساعت نتوان گلوکز آن را اندازه گیری نمود برای جلوگیری از کاهش کاذب آن استفاده می کنند.
- میزان مصرف: 1 mg/ml

ضد انعقادهای مورد استفاده در بانک خون

- ***Heparin***
- ***ACD***
- ***CPD***
- ***CPD-A1***
- ***Additive solutions***
- ***Rejuvenating solutions***

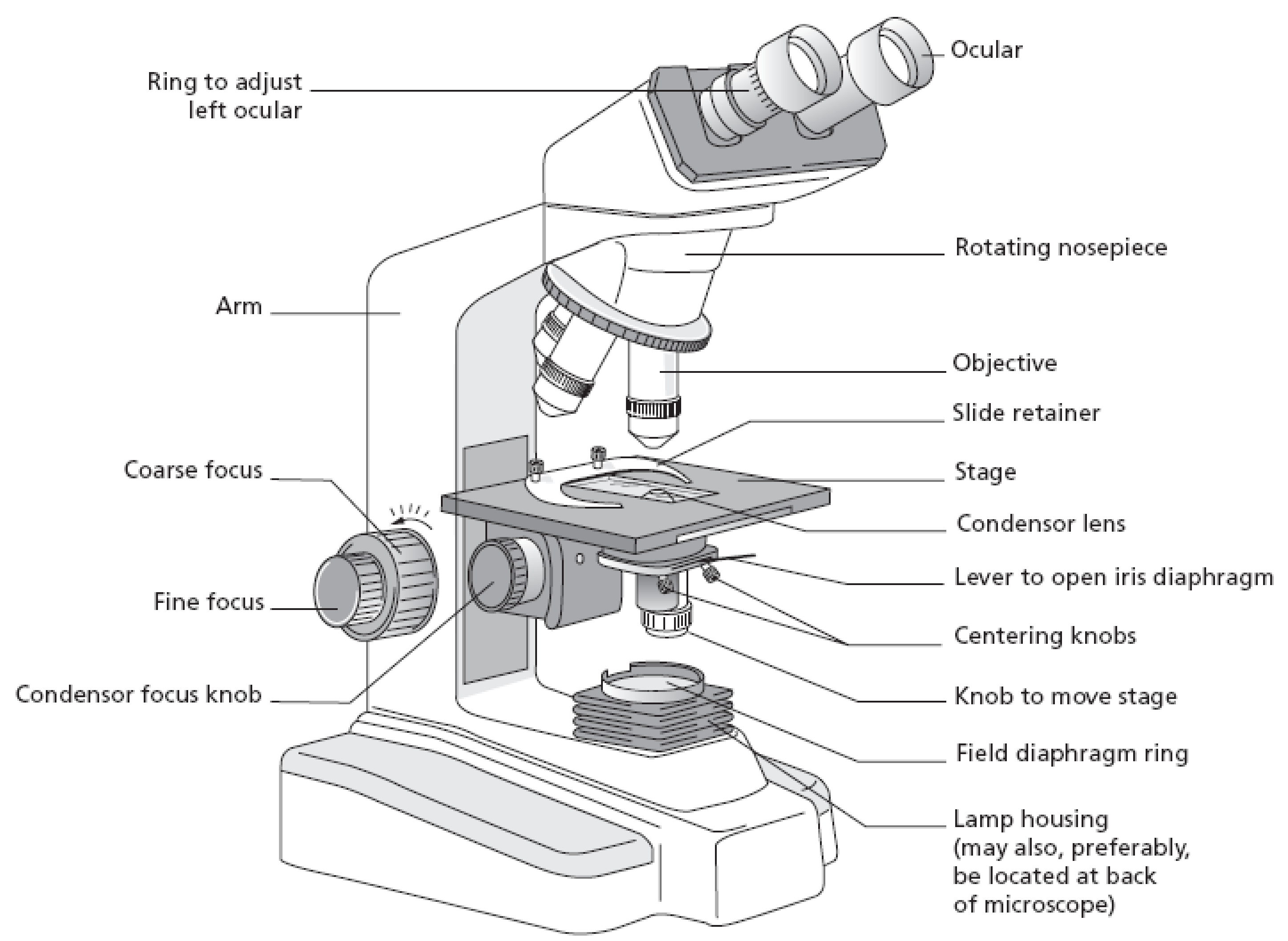

Ring to adjust
left ocular
Ocular
Rotating nosepiece
Arm
Objective
Slide retainer
Coarse focus
Stage
Condensor lens
Lever to open iris diaphragm
Fine focus
Centering knobs
Condensor focus knob
Knob to move stage
Field diaphragm ring
Lamp housing
(may also, preferably,
be located at back
of microscope)

Table 2.5 Units, abbreviations and symbols used for describing haematological variables*.

Variable	Abbreviation	Unit	Symbol
White blood cell count	WBC	number $\times 10^9$/l	
Red blood cell count	RBC	number $\times 10^{12}$/l	
Haemoglobin concentration	Hb	grams/litre OR	g/l
		grams/decilitre OR	g/dl
		millimoles per litre	mmol/l
Haematocrit	Hct	litre/litre	l/l
Packed cell volume	PCV	litre/litre	l/l
Mean cell volume	MCV	femtolitre	fl
Mean cell haemoglobin	MCH	picograms OR	pg
		femtomoles	fm
Mean cell haemoglobin concentration	MCHC	grams/litre OR	g/l
		grams/decilitre OR	g/dl
		millimoles per litre	mmol/l
Platelet count	Plt	number $\times 10^9$/l	
Mean platelet volume	MPV	femtolitre	fl
Plateletcrit	Pct	litre/litre	l/l
Reticulocyte count	Retic	number $\times 10^9$/l	
Erythrocyte sedimentation rate (Westergren, 1 hour)	ESR	millimitres	mm

* In addition, it should be noted that the approved abbreviation for 'international units' is iu.

Table 2.5 Units, abbreviations and symbols used for describing haematological variables*.

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		millimoles per litre	mmol/l
Haematocrit	Hct	litre/litre	l/l
Packed cell volume	PCV	litre/litre	l/l
Mean cell volume	MCV	femtolitre	fl
Mean cell haemoglobin	MCH	picograms OR	pg
		femtomoles	fm
Mean cell haemoglobin concentration	MCHC	grams/litre OR	g/l
		grams/decilitre OR	g/dl
		millimoles per litre	mmol/l
Platelet count	Plt	number × 10^9/l	
Mean platelet volume	MPV	femtolitre	fl
Plateletcrit	Pct	litre/litre	l/l
Reticulocyte count	Retic	number × 10^9/l	
Erythrocyte sedimentation rate (Westergren, 1 hour)	ESR	millimitres	mm

* In addition, it should be noted that the approved abbreviation for 'international units' is iu.

TABLE 1-3 **AUTOMATED BLOOD ANALYZER READOUT (CBC)**

Variable	*Readout*	*Interpretation*
Red cell number	Millions/μL	The number of red cells per volume of blood.
MCV	Femtoliters	The mean volume of a single red cell.
Hb	g/dL	Quantity of hemoglobin in a volume of blood.
MCH	Picograms	The mean hemoglobin content of a single red cell.
MCHC	Percentage	Hemoglobin concentration within individual red cells. Calculated as (Hb/HCT)$\times$ 100
RDW	Percentage	Coefficient of size variation of red cells in a sample = (Standard deviation of red cell volume mean cell volume) $\times$ 100. A larger value indicates greater size variation.
HCT	Percentage	Mathematical derivation of the red cell fraction of the total blood volume.
WBC	Thousands/μL	Number of white cells per unit of blood.[a]
Plts	Hundreds of thousands per μL	Number of platelets per unit of blood.

The Complete Blood Count (CBC)

Component	Reported Units
RBC count	10^6 cells/μL
Hemoglobin	g/dL
Hematocrit	Volumes %
Mean corpuscular volume (MCV)	fL
Mean corpuscular hemoglobin (MCH)	pg
Mean corpuscular hemoglobin concentration (MCHC)	g/dL
Red cell distribution width (RDW)	%
WBC count	10^3 cells/μL
Differential	% and 10^3 cells μL
Neutrophils (segs + bands)	
Lymphocytes	
Monocytes	
Eosinophils	
Basophils	
Platelet count	10^3 cells/μL
Mean platelet volume (MPV)	fL

The most important values of the CBC are listed in bold type.

Table 1–4

Reporting CBCs: Common versus International Units

Component	Common Units	International Units
RBC count	10^6 cells/μL	10^{12} cells/L
Hemoglobin	g/dL	g/L
WBC count	10^3 cells/μL	10^9 cells/L
Platelet count	10^3 cells/μL	10^9 cells/L

$1 \times 10^6/\mu L = 1 \times 10^{12}/L$; $1 \times 10^3/\mu L = 1 \times 10^9/L$; 1 g/dL = 10 g/L.

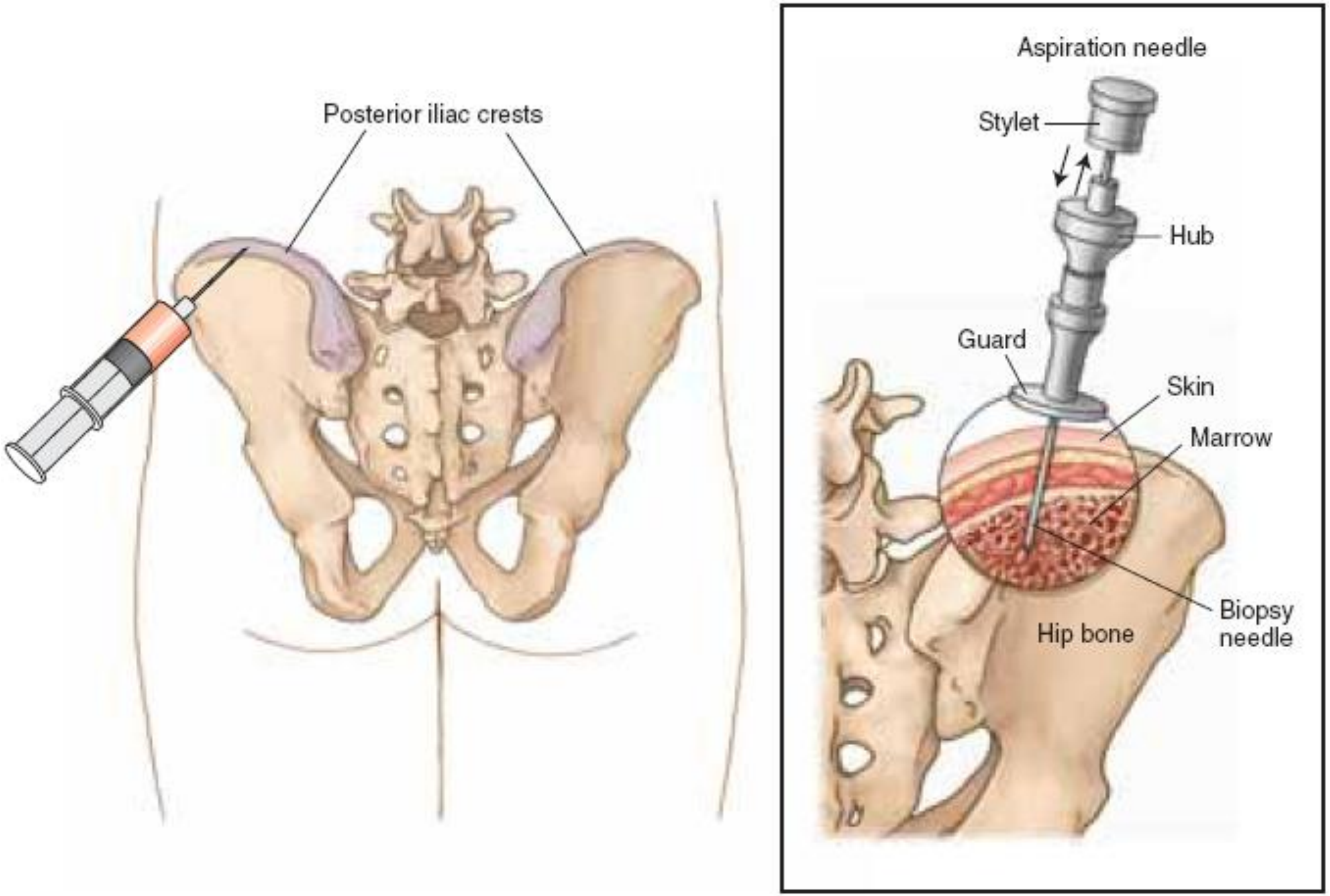


Figure 2.4 Bone marrow aspiration.

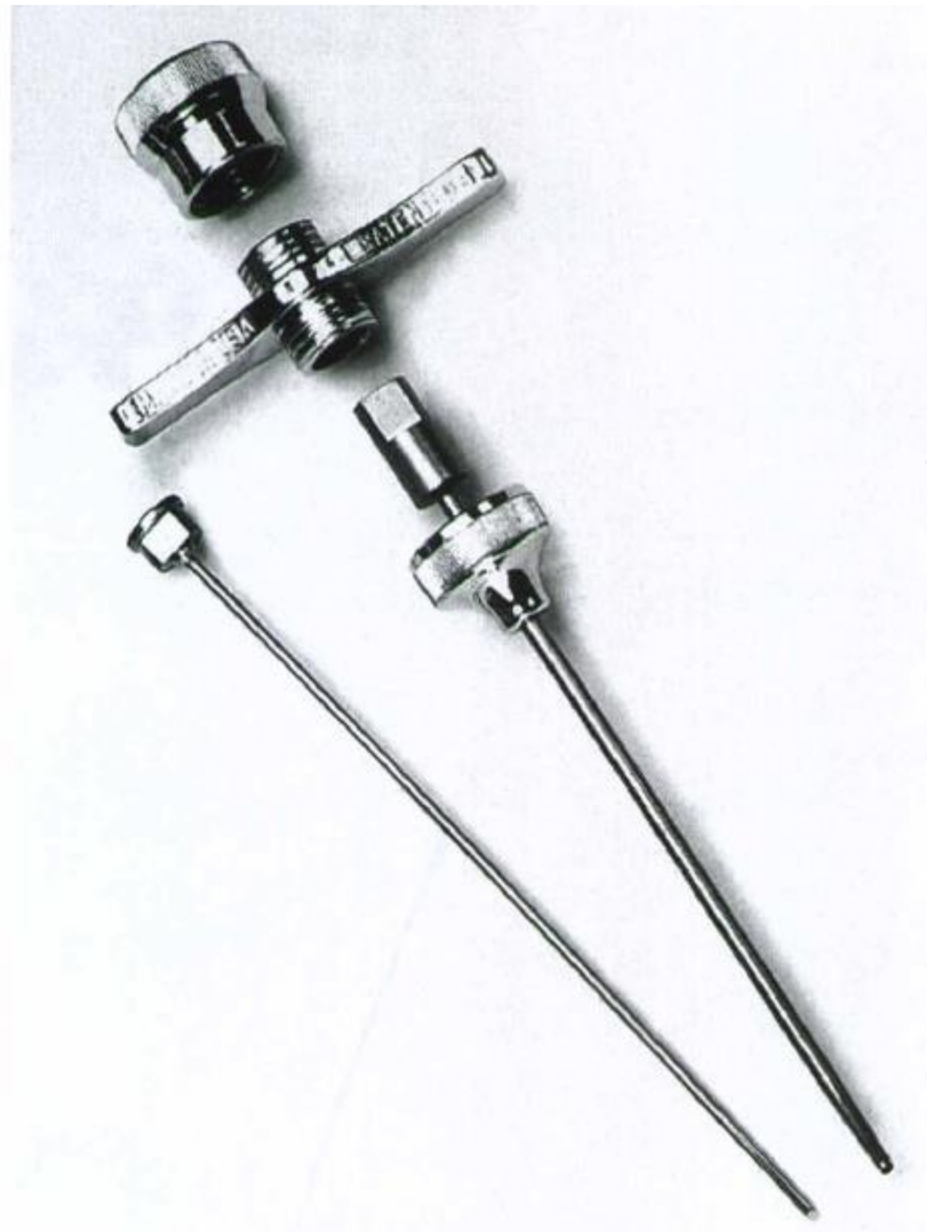


Figure 6.7 Jamshidi trephine for bone marrow biopsy.

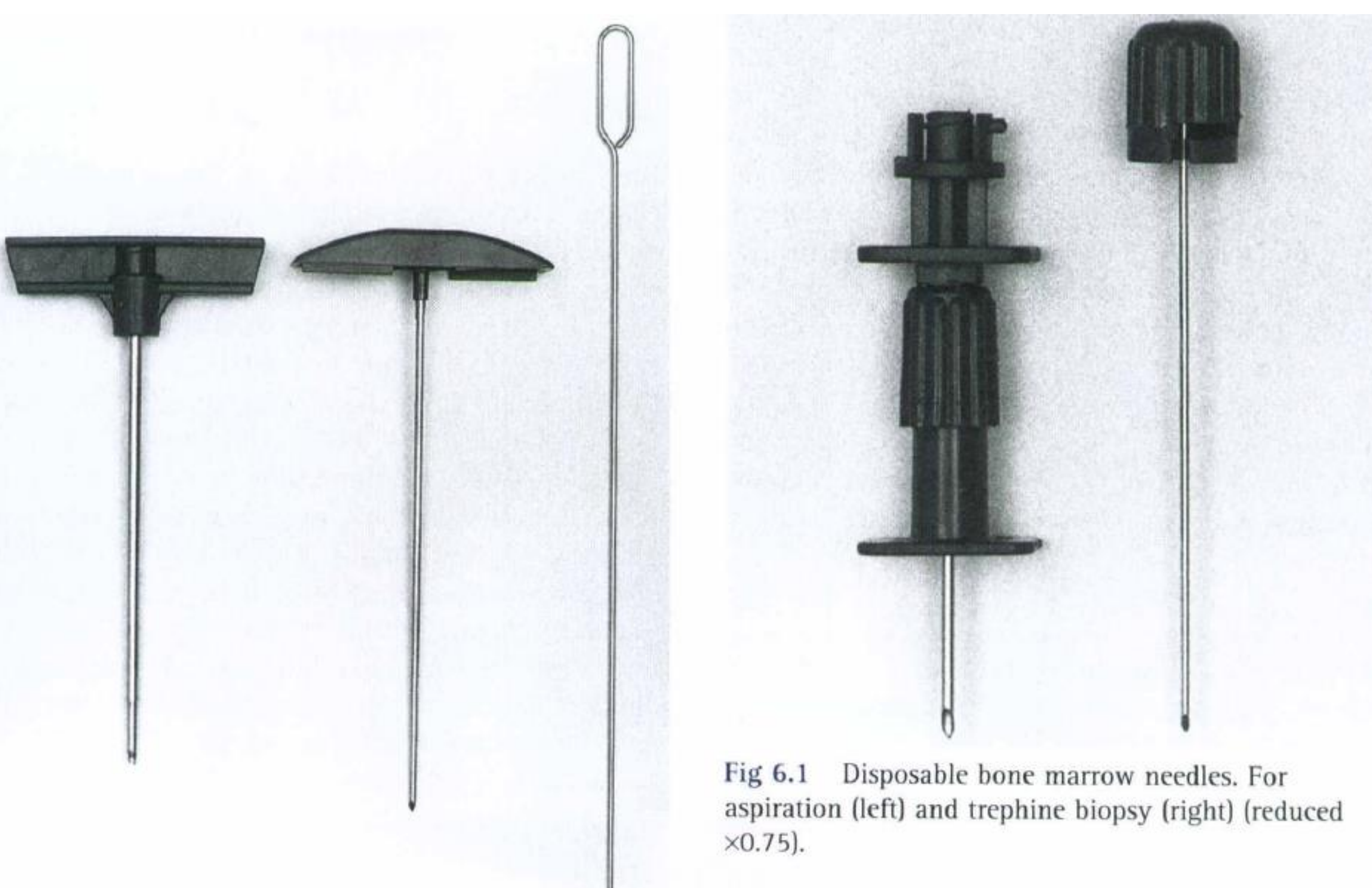


Fig 6.1 Disposable bone marrow needles. For aspiration (left) and trephine biopsy (right) (reduced ×0.75).

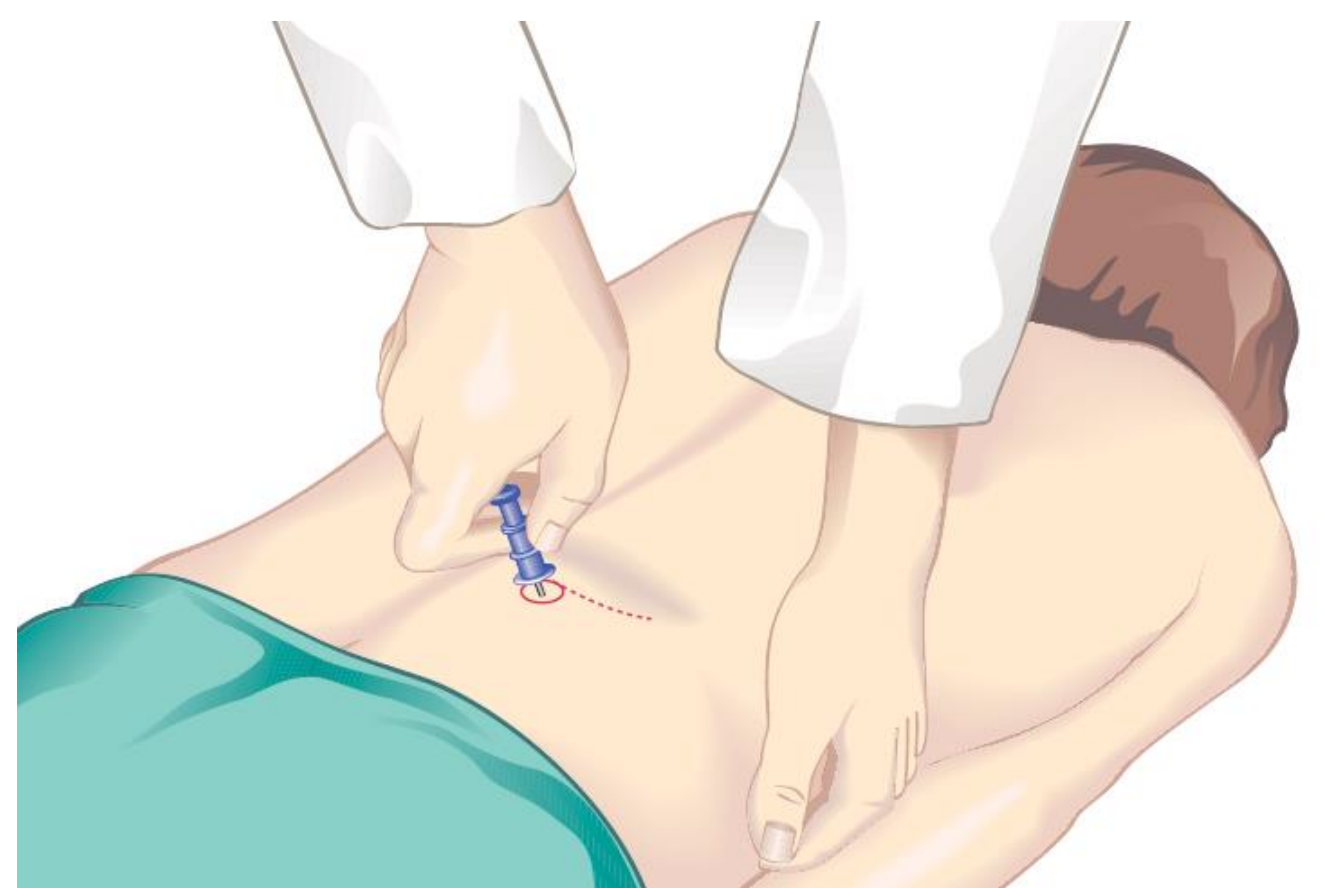


Table 44.2 Special tests on bone marrow cells.

Chromosomes:
- Conventional cytogenetics, e.g. diagnosis and classification of leukaemia, myelodysplasia
- Fluorescent in situ hybridization (FISH) for translocations, location of oncogenes

Molecular tests:
- DNA/RNA analysis/polymerase chain reaction (PCR) eg. diagnosis and classification of leukaemia
- Detection of minimal residual disease

Immune phenotype analysis:
- Diagnosis and classification of leukaemia, lymphoproliferative diseases
- Detection of residual disease

Microbiological cultures, e.g. tuberculosis

Cytochemistry:
- Diagnosis of acute leukaemias

Table 44.1 Indications for bone marrow aspiration (and trephine).*

Unexplained cytopenia:*
- Anaemia, leucopenia, thrombocytopenia

Suspected marrow infiltrate:*
- Leukaemia, myelodysplasia, lymphoma, myeloproliferative disease, myeloma, carcinoma, storage disorders

Suspected infection:
- Leishmaniasis, tuberculosis

*Bone marrow trephine is required for pancytopenia or suspected marrow infiltration.

Surname: ______ First Name: ______ Sex: ______ Date of birth: ______

Consultant: ______

Date taken: ______ Hospital No: ______ Lab No: ______

Clinical details:

WBC Hb MCV Platelet count

Blood film:

Performed by: Aspiration Site: Ease of aspiration:

Particles:

Cellularity: M:E Ratio

Erythropoiesis:

Granulopoiesis:

Megakaryocytes:

Lymphocytes:

Plasma Cells:

Macrophages:

Other Cells:

Blast cells	Promyelocytes	Myelocytes and metamyelocytes	Neutrophils	Eosinophils
Basophils	Monocytes	Lymphocytes	Plasma cells	Erythroid

Storage iron: Siderotic granules:

Other tests to follow:

Conclusion:

Authorized by: Date:

Figure 6.6 Example of report form for bone marrow films.

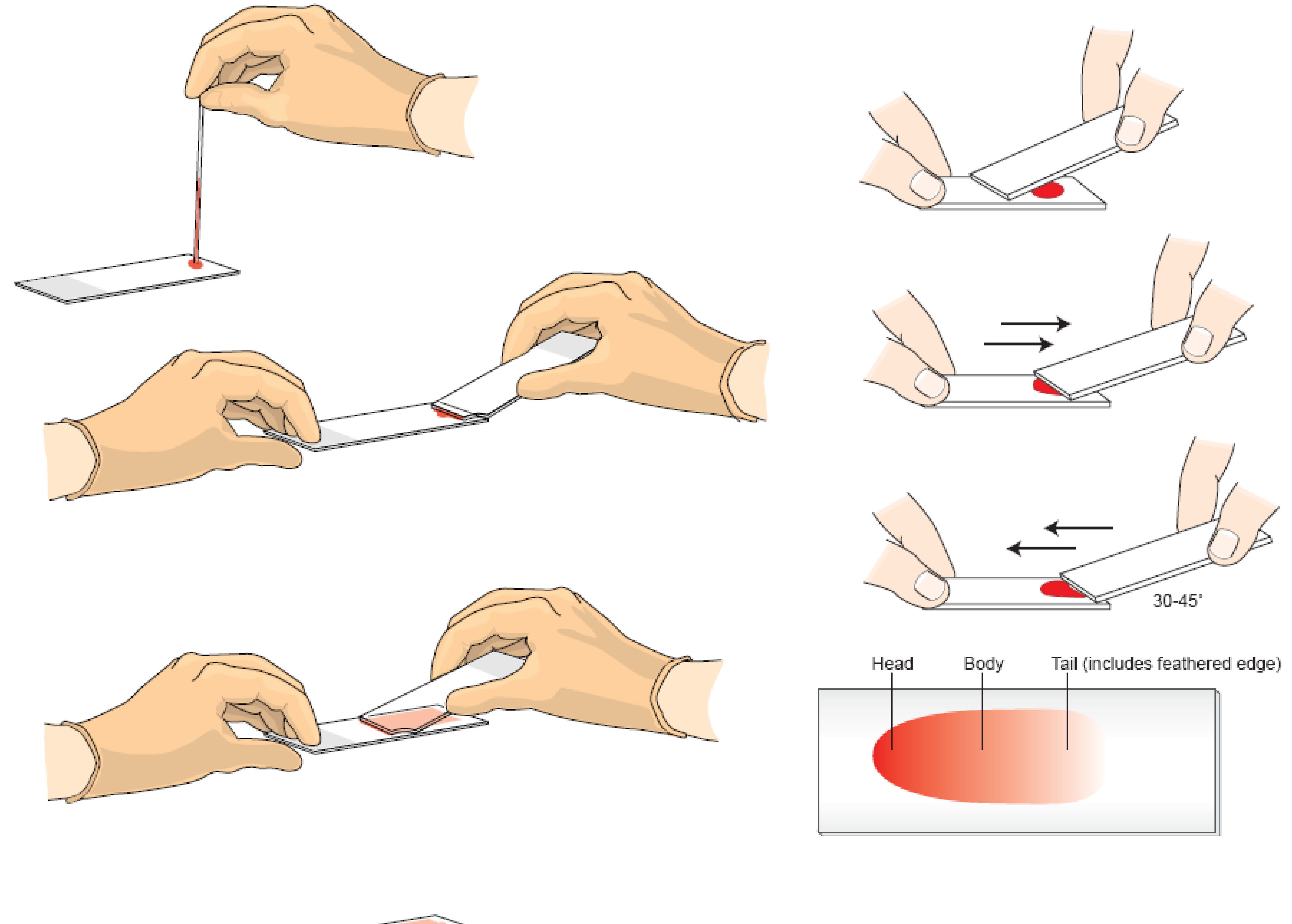


Fig. 1.9 The method of spreading a blood film.

Table 4.1 Colour responses of blood cells to Romanowsky staining

Cellular component	Colour
Nuclei	
Chromatin	Purple
Nucleoli	Light blue
Cytoplasm	
Erythroblast	Dark blue
Erythrocyte	Dark pink
Reticulocyte	Grey–blue
Lymphocyte	Blue
Metamyelocyte	Pink
Monocyte	Grey–blue
Myelocyte	Pink
Neutrophil	Pink/orange
Promyelocyte	Blue
Basophil	Blue
Granules	
Promyelocyte (primary granules)	Red or purple
Basophil	Purple black
Eosinophil	Red–orange
Neutrophil	Purple
Toxic granules	Dark blue
Platelet	Purple
Other inclusions	
Auer body	Purple
Cabot ring	Purple
Howell-Jolly body	Purple
Döhle body	Light blue

Cell component staining	Colour
Chromatin (including Howell–Jolly bodies)	Purple
Promyelocyte granules and Auer rods	Purplish-red
Cytoplasm of lymphocytes	Blue
Cytoplasm of monocytes	Blue-grey
Cytoplasm rich in RNA (i.e. 'basophilic cytoplasm')	Deep blue
Döhle bodies	Blue-grey
Specific granules of neutrophils, granules of lymphocytes, granulomere of platelets	Light purple or pink
Specific granules of basophils	Deep purple
Specific granules of eosinophils	Orange
Red cells	Pink

Table 1.2 Characteristic staining of different cell components with a Romanowsky stain.

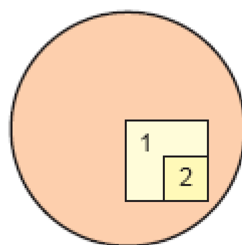


Figure 20.5 Miller Eye Disc. Count RBCs only in the small square; reticulocytes are counted in both squares.

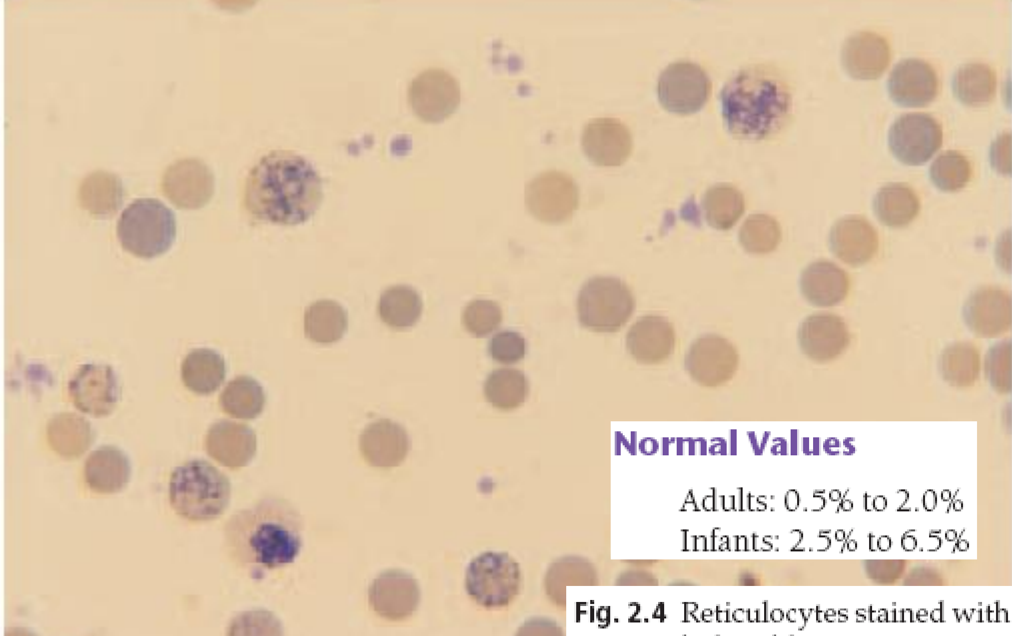
Conditions Associated With...

Decreased Reticulocyte Count

1. Aplastic anemia
2. Exposure to radiation or radiation therapy
3. Chronic infection
4. Medications such as azathioprine, chloramphenicol, dactinomycin, methotrexate, and other chemotherapy medications
5. Untreated pernicious anemia/megaloblastic anemia

Increased Reticulocyte Count

1. Rapid blood loss
2. High elevation
3. Hemolytic anemias
4. Medications such as levodopa, malarial medications, corticotrophin, and fever-reducing medications
5. Pregnancy



Normal Values

Adults: 0.5% to 2.0%
Infants: 2.5% to 6.5%

Fig. 2.4 Reticulocytes stained with new methylene blue.

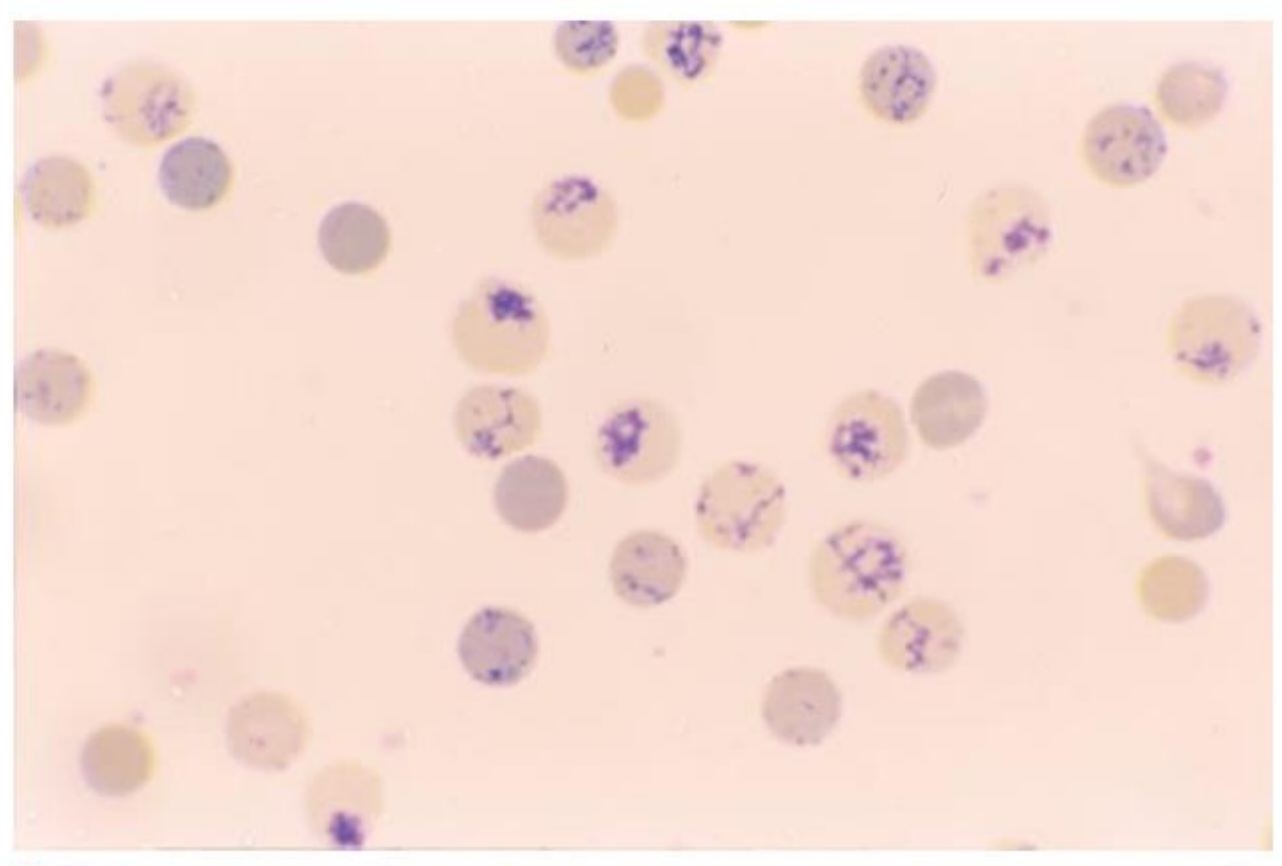


Figure 7–3 Reticulocyte stain. Note the granular aggregates of RNA in the reticulocytes. The reticulocyte count is markedly increased.

Table 2.3 The characteristic appearance of various red cell inclusions on a new methylene blue reticulocyte preparation.

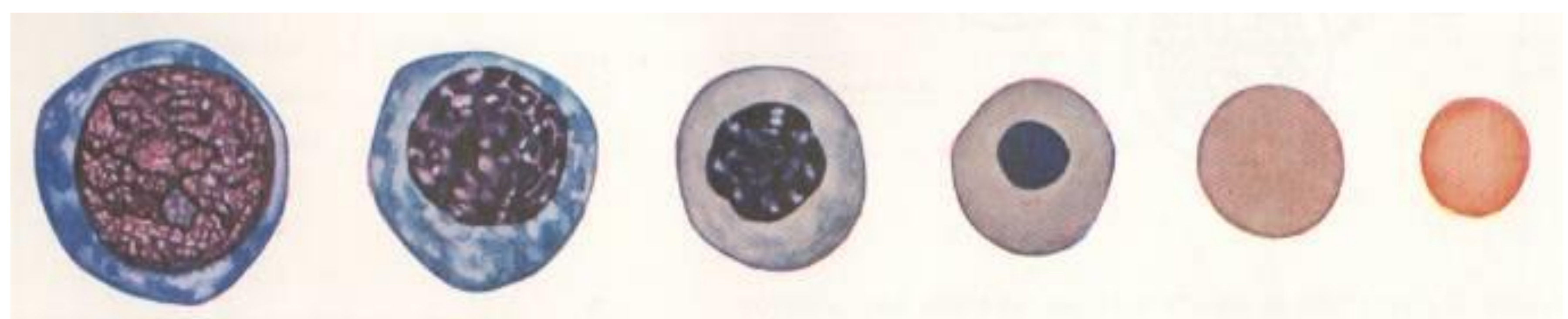
Name	Nature	Appearance
Reticulum	Ribosomal RNA	Reticulofilamentous material or scanty small granules
Pappenheimer bodies	Iron-containing inclusions	One or more granules towards the periphery of the cell, may stain a deeper blue than reticulum
Heinz bodies	Denatured haemoglobin	Larger than Pappenheimer bodies, irregular in shape, usually attached to the cell membrane and may protrude through it, pale blue
Howell–Jolly bodies	DNA	Larger than Pappenheimer bodies, regular in shape, distant from the cell membrane, pale blue
Haemoglobin H inclusions	Denatured haemoglobin H	Usually do not form with short incubation periods; if present they are multiple and spherical giving a 'golf-ball' appearance, pale greenish-blue

Table 4.2 Factors giving rise to faulty staining

Appearances	Causes
Blue to black	Incorrect preparation of stock Stock stain exposed to bright daylight Batch of stain solution overused Impure dyes Staining time too short Staining solution too acid Smear too thick Inadequate time in buffer solution
Too pink	Incorrect proportion of azure B-eosin Y Impure dyes Buffer pH too low Excessive washing in buffer solution
Pale staining	Old staining solution Overused staining solution Incorrect preparation of stock Impure dyes, especially azure A and/or C High ambient temperature
Neutrophil granules not stained	Insufficient azure B
Neutrophil granules Dark blue/black (pseudo-toxic)	Excess azure B
Other stain anomalies	Various contaminating dyes and metal salts
Stain deposit on film	Stain solution left in uncovered jar Stain solution not filtered
Blue background	Inadequate fixation or prolonged storage before fixation Blood collected into heparin as anticoagulant

Steps in Erythropoisis

Early → Intermediate → Late



Proerythroblast (Pronormoblast) | **Polychromatophilic Normoblast** | **Reticulocyte** |

Basophilic Normoblast | **Orthochromatophilic Normoblast** | **Erythrocyte**

Table 2–1
Erythropoiesis

Cell	Appearance
Proerythroblast	14–19 μm diameter; small amount of deeply basophilic cytoplasm; large round nucleus with fine chromatin, nucleolus
Basophilic erythroblast	12–17 μm diameter; deeply basophilic cytoplasm; nuclear chromatin begins to condense
Polychromatophilic erythroblast	12–15 μm diameter; grayish cytoplasm; nucleus is smaller with increased chromatin condensation
Orthochromatophilic erythoblast	8–12 μm diameter; cytoplasm red to pale gray; small totally opaque nucleus
Reticulocyte	7–10 μm diameter; nucleus extruded; ribonucleic acid visible on reticulocyte stain
Erythrocyte	7–8 μm diameter; reddish cytoplasm; anucleate

Table 3.1 Key Features of Red Cell Development

- Nuclei are always "baseball" round.
- Basophila of cytoplasm is an indicator of immaturity.
- Cell size reduces with maturity.
- As hemoglobin develops, the cytoplasm becomes more magenta.
- The N:C ratio decreases as the cell matures.
- The cytoplasm of the red cell does not contain specific granulation.
- Nuclear chromatin becomes more condensed with age.
- Nucleated red cells (orthochromic normoblasts) are not a physiological component of the normal peripheral smear.

Granolopoeisis

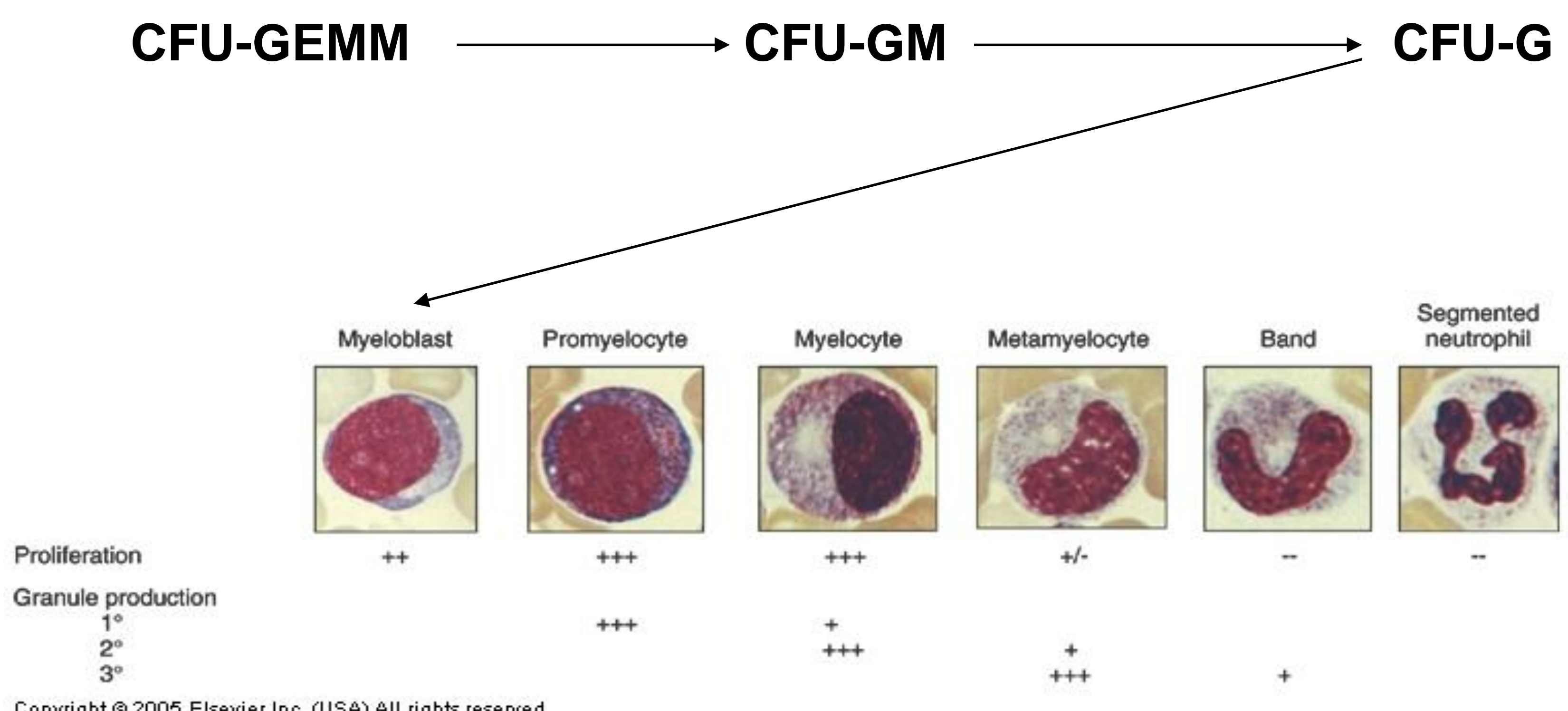


Table 2–2

Granulocytopoiesis

Cell	Appearance
Myeloblast	10–20 μm diameter; large round nucleus with fine chromatin, nucleolus; high nucleus to cytoplasm ratio; small amounts of light gray to pale blue cytoplasm without granules
Promyelocyte	Distinguished by presence of large, reddish-purple primary granules; immature nucleus with nucleolus; grayish to dark blue cytoplasm
Myelocyte	Distinguished by presence of secondary granules; cytoplasm begins to turn light yellow-orange; round nucleus with early chromatin condensation; ± nucleolus
Metamyelocyte	Resembles myelocyte, but with indented (kidney bean–shaped) nucleus with increased chromatin condensation
Band neutrophil	Deeply indented (horseshoe-shaped) but not segmented nucleus; mature cytoplasm
Segmented neutrophil	Nucleus segmented into distinct lobes

Neutrophil, eosinophil, and basophil maturation follows parallel pathways; they can be differentiated at the myelocyte stage, when secondary (specific) granules appear.

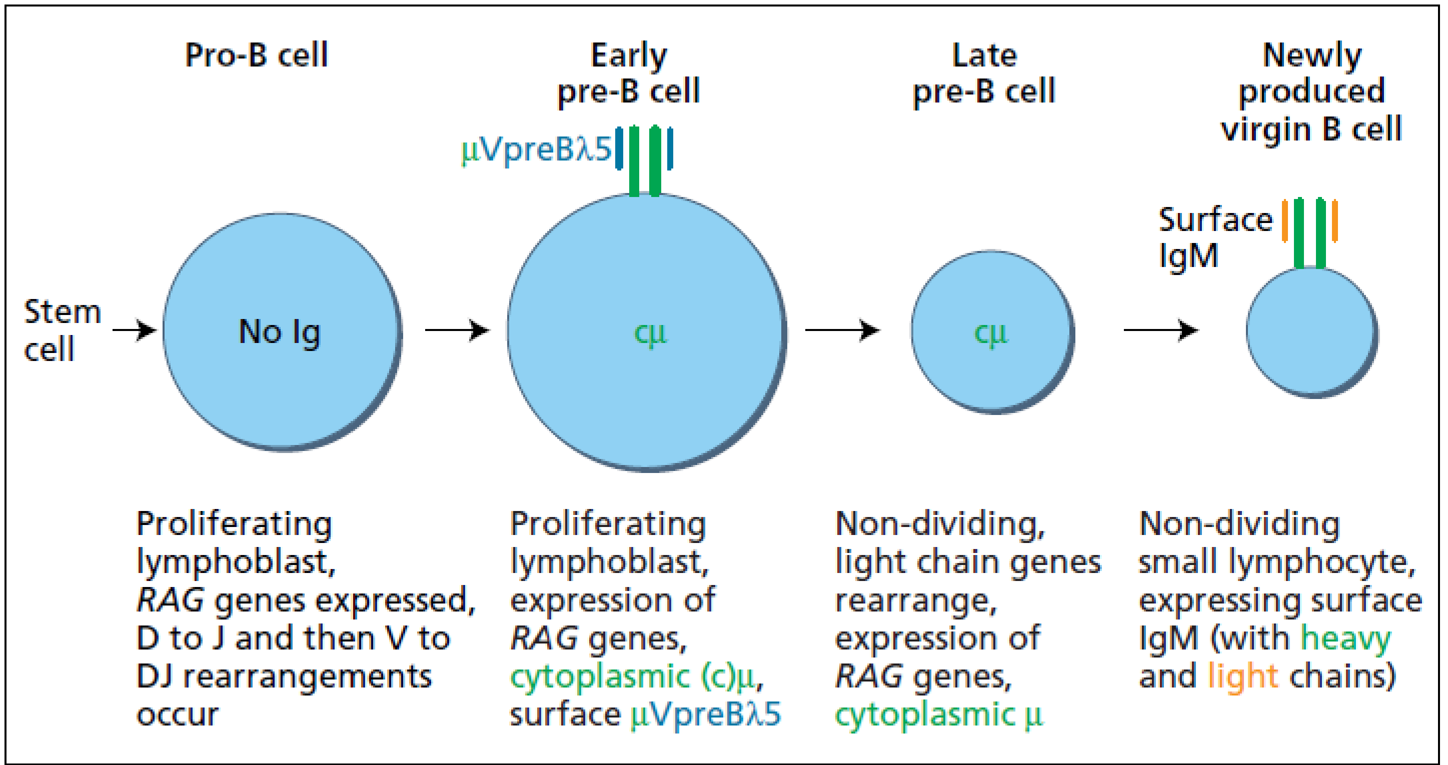


Figure 20.7 Outline of the main stages of B lymphopoiesis. The central process in B-cell formation is the rearrangement of Ig variable-region genes. For rearrangement of V-region genes to occur in either T or B cells, recombinase-activating genes (RAG) 1 and 2 have to be expressed. Absence of these genes totally blocks further differentiation towards B or T cells. After a successful heavy-chain VDJ has been made, a B cell must express the heavy chain with the surrogate light chain composed of V–pre-B and λ5 if further differentiation is to occur. Cells that fail to make either a productive heavy- or light-chain rearrangement destroy themselves by apoptosis.

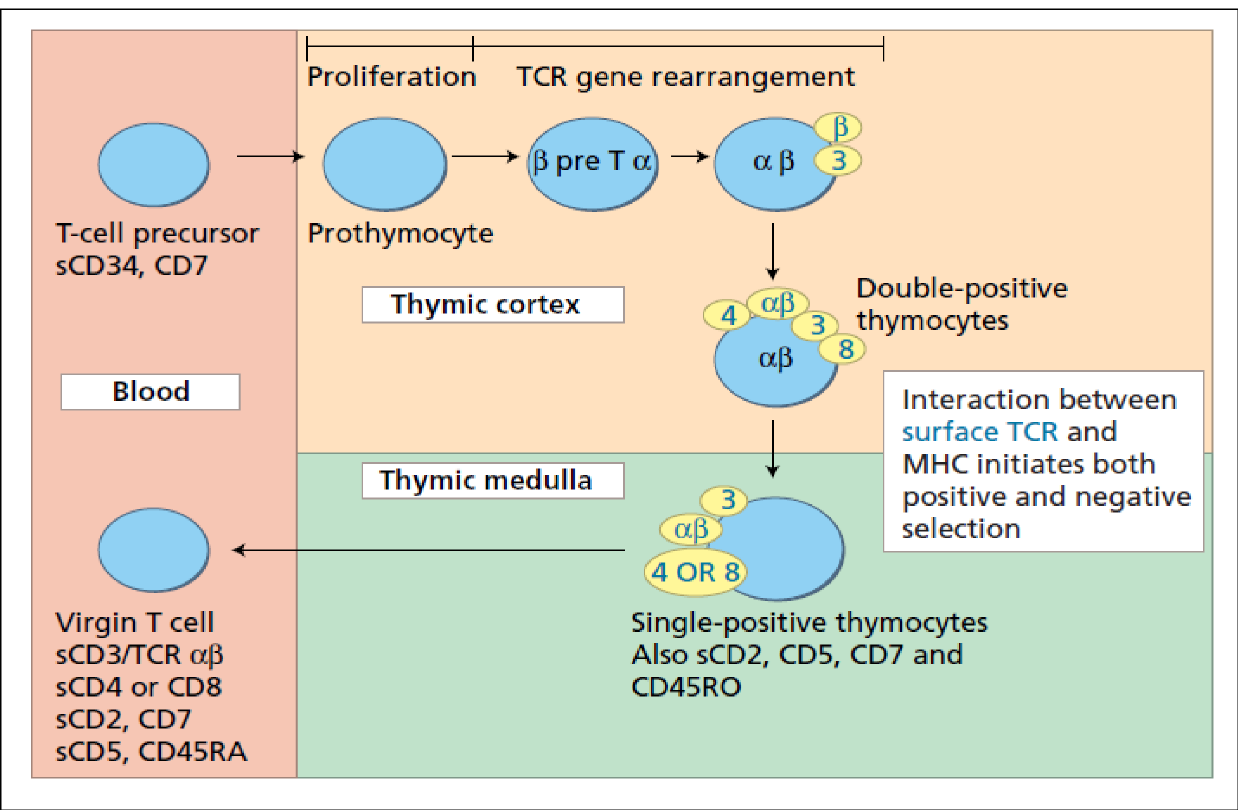


Figure 20.8 T-cell maturation in the thymus. Molecules within cells (red) or on a cell's surface (blue) are depicted without the prefix CD. T-cell progenitors enter the thymus from the marrow or other primary lymphoid site to become a prothymocyte. At this stage their TCR genes are in germline configuration. The proliferative potential and lack of commitment of prothymocytes is reflected in the ability of a single prothymocyte to populate an entire thymic lobe and generate a full T-cell repertoire. Prothymocytes proliferate in the outer cortex. Towards the end of the proliferative phase, TCR rearrangement starts: first β-chain genes are rearranged, then these are expressed with a surrogate α chain – pre-T α–. After this, α-chain genes rearrange. As with B cells, these rearrangements require *RAG1* and *RAG2* genes to be expressed, and junctional diversity is increased by the addition of N sequences using tdt. Selection occurs at the double-positive stage when the full TCR complex is expressed with CD3 and both CD4 and CD8. These cells are selected on their ability to recognize self peptide presented by a self MHC molecule at low avidity. Those cells recognizing peptide with class I go on to become single-positive CD8 expressors; those recognizing peptide with class II continue to express CD4 without CD8. Cells recognizing self peptide at high avidity and cells failing to recognize a self MHC molecule are deleted.

NEUTROPHIL

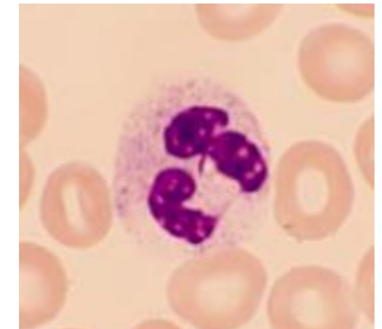
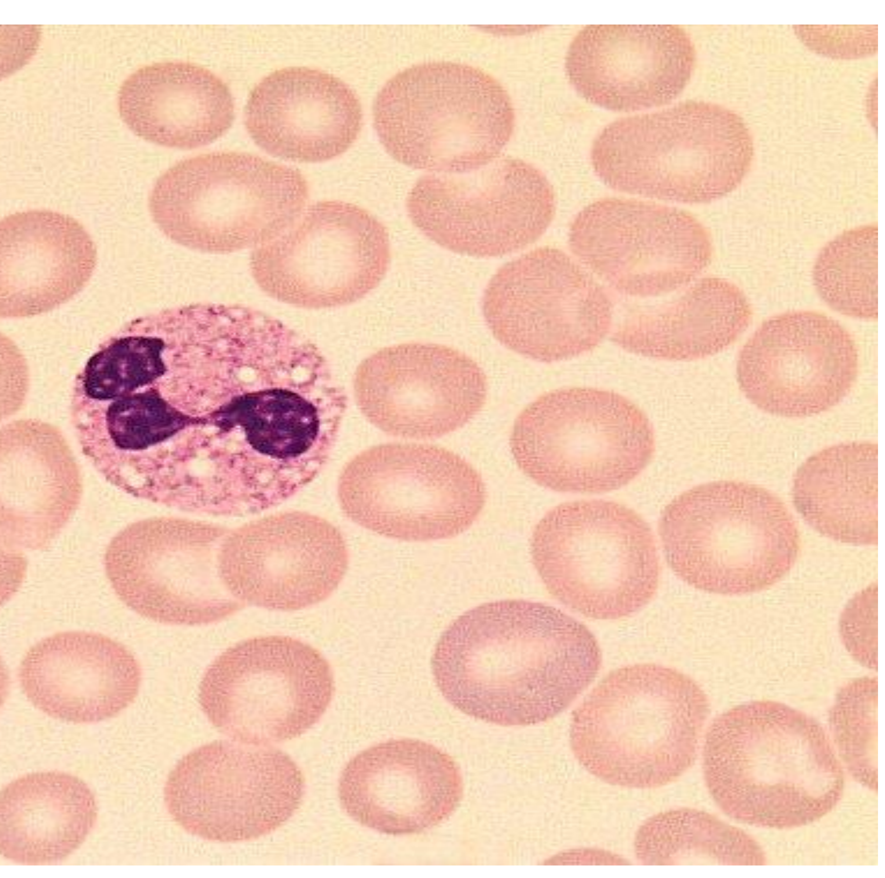


Table 30-3 Neutrophil Constituents and Functions

Neutrophil Constituents and Functions
Azurophilic granules (formed in promyelocyte stage)
Lysosomal enzymes: acid hydrolases, acid phosphatase, beta-glucuronidase
Myeloperoxidase
Elastase
Arylsulfatase
Cationic antibacterial proteins
Specific granules (formed in myelocyte stage)
Lysozyme
Lactoferrin
Collagenase
Plasminogen activator
Aminopeptidase
Tertiary granules
Gelatinase
Cytoplasmic organelles
Alkaline phosphatase
Neutrophil functions
Phagocytosis
Bactericidal activity



EOSINOPHIL

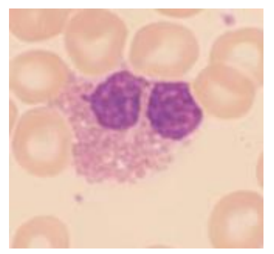
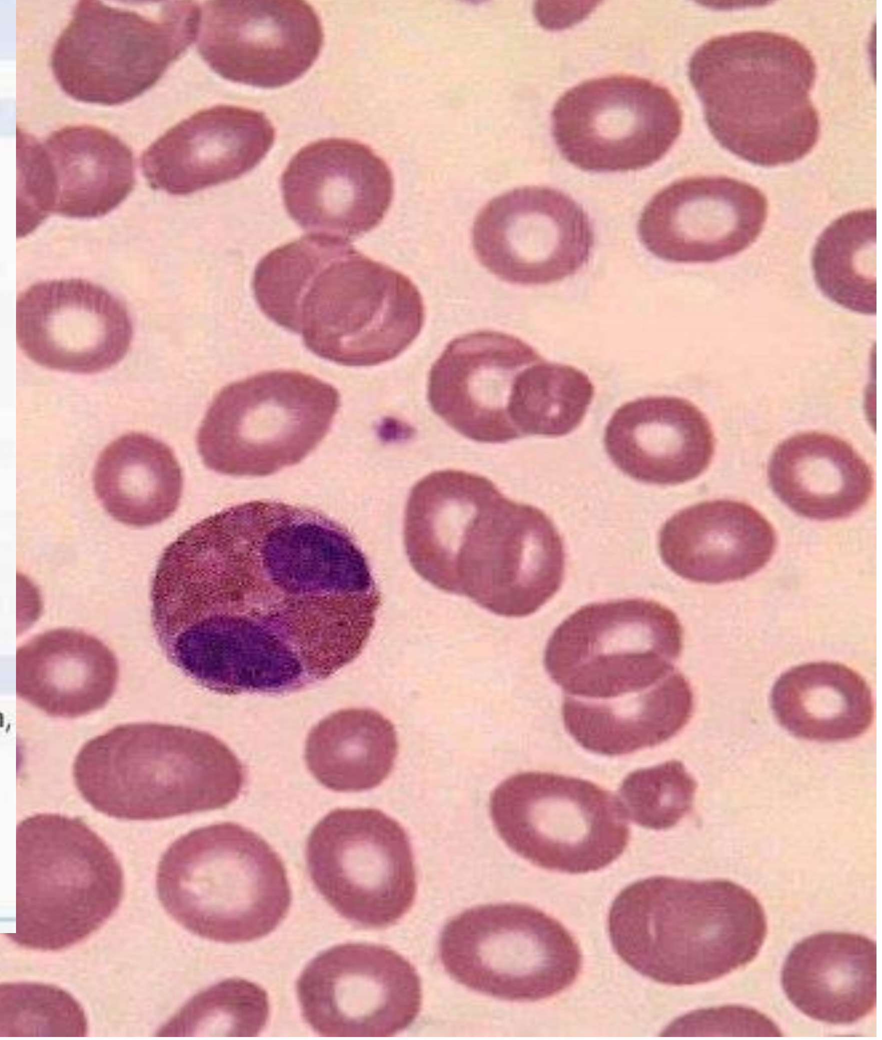


Table 30–4 Eosinophil Constituents and Functions

Eosinophil-specific granules
Larger granules
Major basic protein
Acid hydrolases
Peroxidase
Phospholipase
Cathepsin
Eosinophil cationic protein
Eosinophil-derived neurotoxin
Eosinophil protein X
Smaller granules
Arylsulfatase
Peroxidase
Acid phosphatase
Eosinophil functions
Anthelmintic activity – major basic protein, eosinophilic cationic protein, peroxidase
Phagocytosis
Allergic response
Dampen inflammatory reactions



BASOPHIL

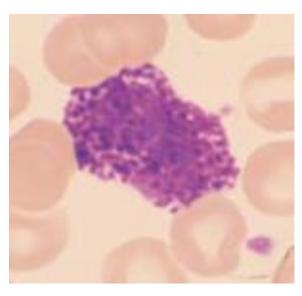


Table 30–5 Basophil Constituents and Functions

Basophil-specific granules
Histamine
Heparin
Peroxidase
Eosinophilic chemotactic factor-A
Other cellular constituents
Slow-reacting substance of anaphylaxis
Platelet-activating factor
Basophil functions
Immediate hypersensitivity reactions
Some delayed hypersensitivity reactions

MONOCYTE

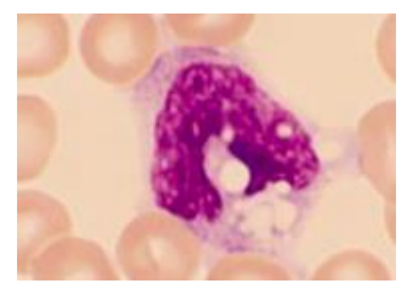
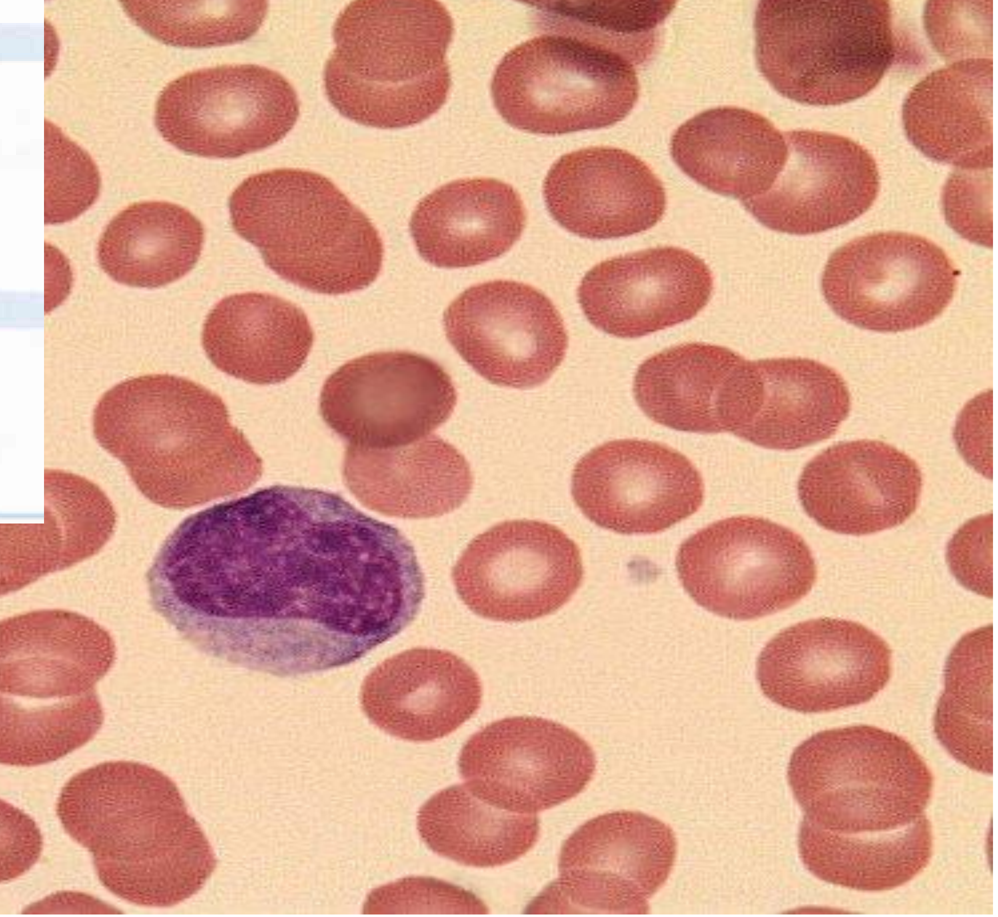


Table 30–6 Monocyte/Macrophage Constituents and Functions

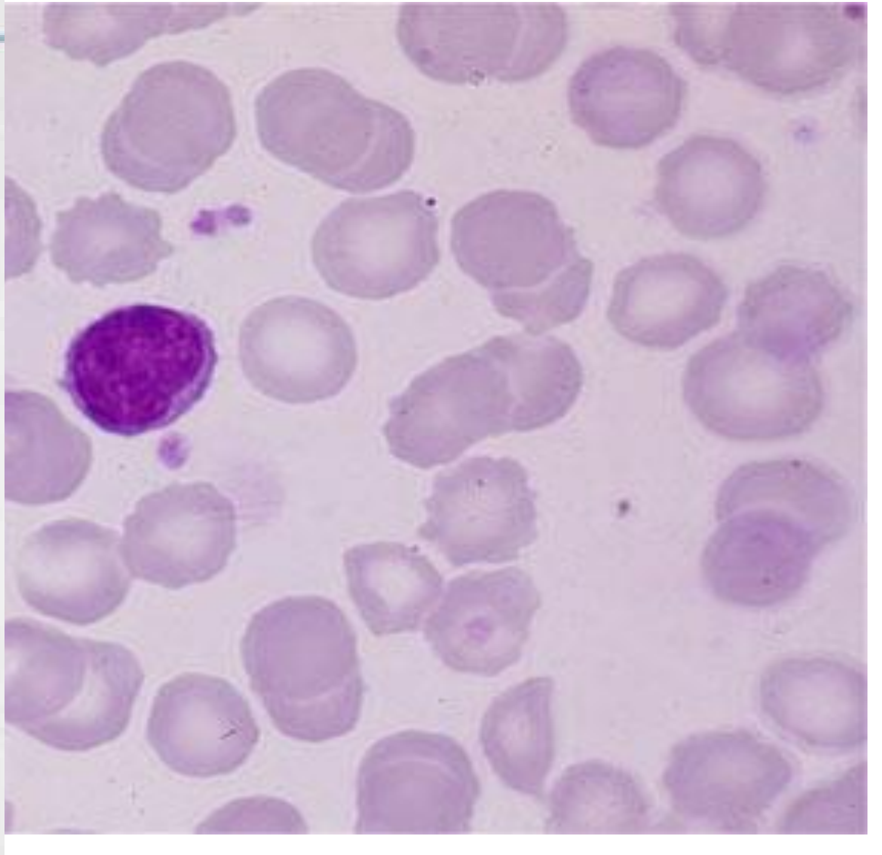
Granule and other constituents
Acid hydrolase
Arylsulfatase
Nonspecific esterase
Peroxidase
Acid phosphatase
Monocyte/macrophage functions
Phagocytosis – bacteria, cellular debris, senescent cells
Antigen processing
Cell-mediated immunity – antibody-dependent cellular cytotoxicity
Synthesis of bioactive molecules



LYMPHOCYTE

Table 30–8 Summary of T Cell Maturation

Stage	Maturation events	Cell surface markers
Pro-T cell	Migration from marrow to thymic cortex	CD2, CD44
α/β Pre-T cell	Migration from thymic cortex to medulla, elimination of self-recognizing T cells, α/β TCR rearrangement (for T cells destined for either the helper or suppressor subset)	TdT, CD1, CD2, CD3, CD4, CD5, CD7, CD8
γ/δ Pre-T cell	Migration from thymic cortex to medulla, elimination of self-recognizing T cells, γ/δ TCR rearrangement (for T cells destined for the cytotoxic subset)	TdT, CD1, CD2, CD3, CD7
Mature T cell	Loss of ability to make TdT, circulation in peripheral blood as helper, suppressor or cytotoxic subset	Helper T: CD2, CD3, CD4, CD5, CD7 Suppressor T: CD2, CD3, CD5, CD7, CD8 Cytotoxic T: CD2, CD3, CD7



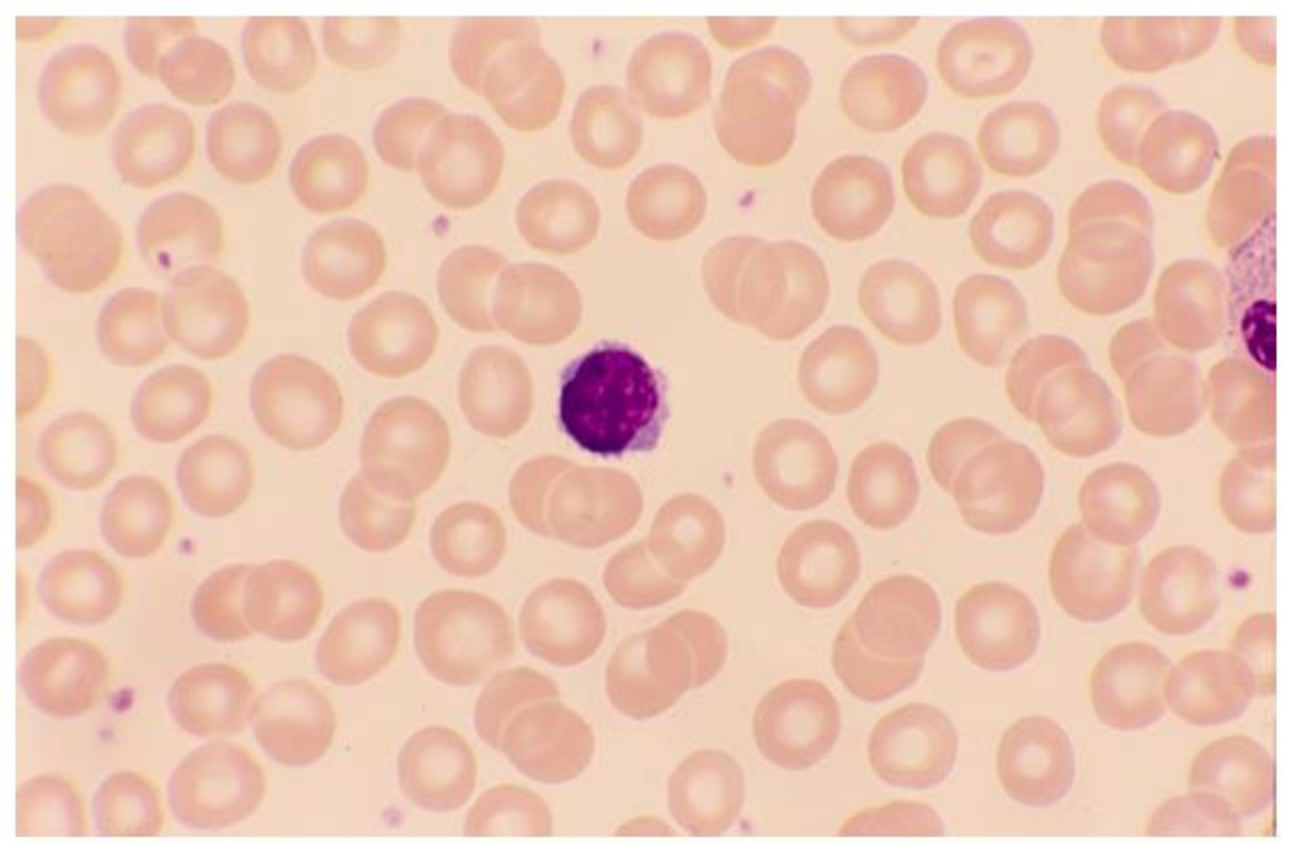


Figure 1–6 Resting lymphocyte. Note the small round nucleus and scant cytoplasm.

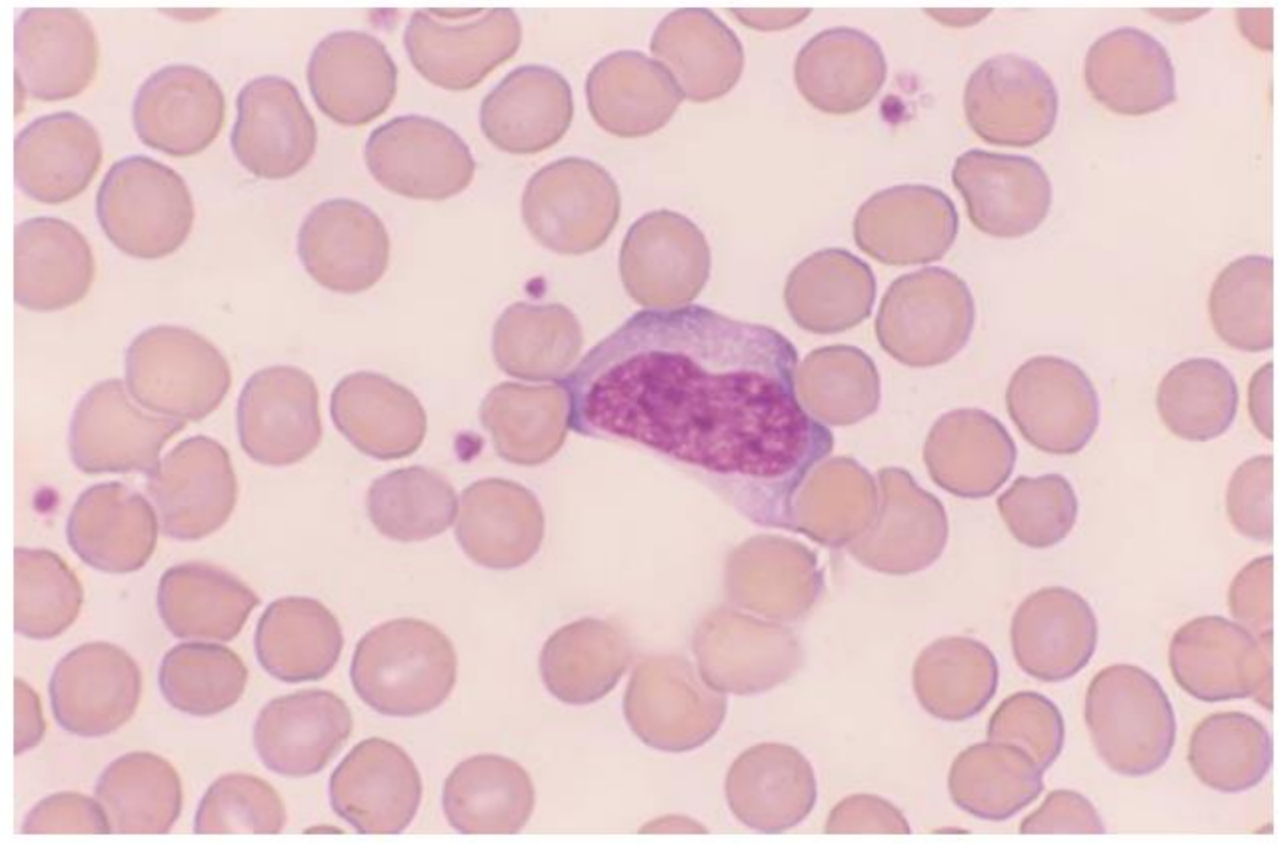


Figure 1–7 **Reactive lymphocyte.** Note the abundant cytoplasm that "hugs" erythrocytes.

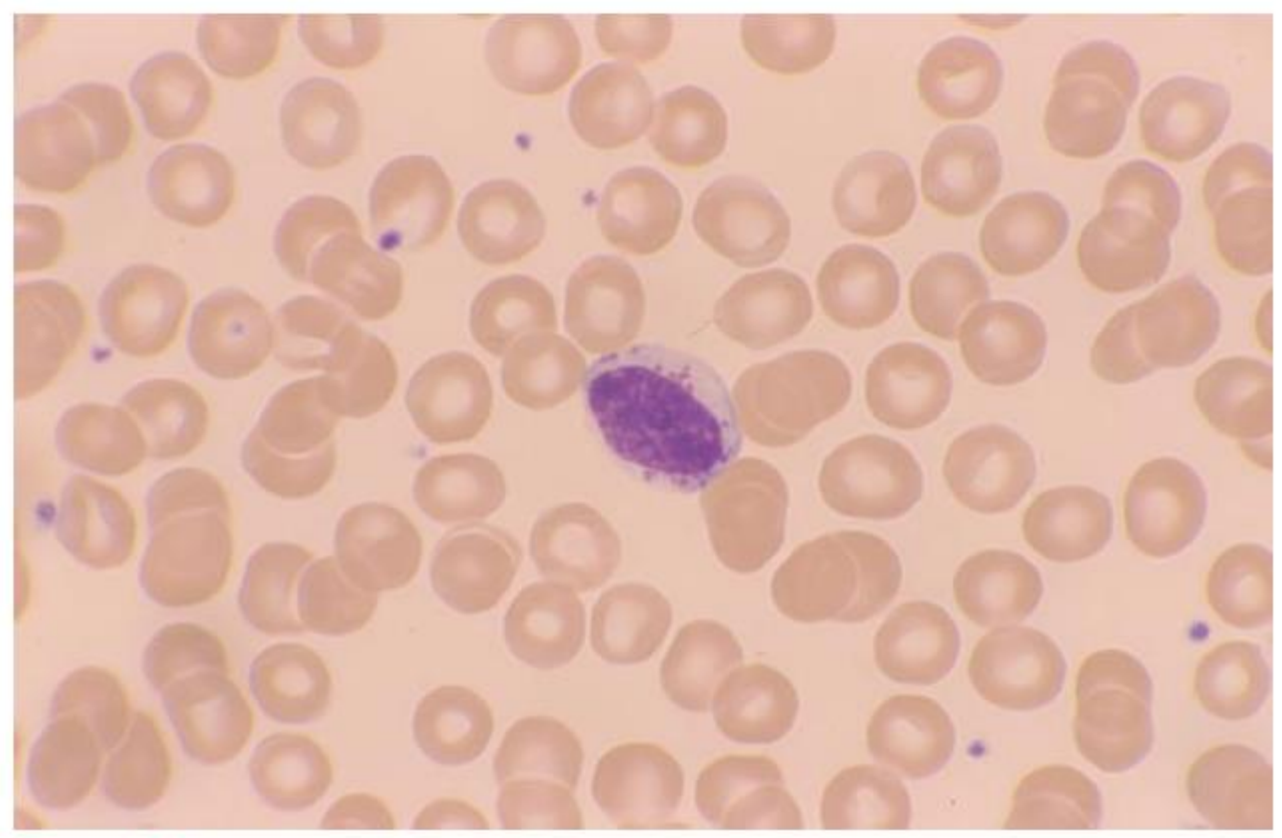
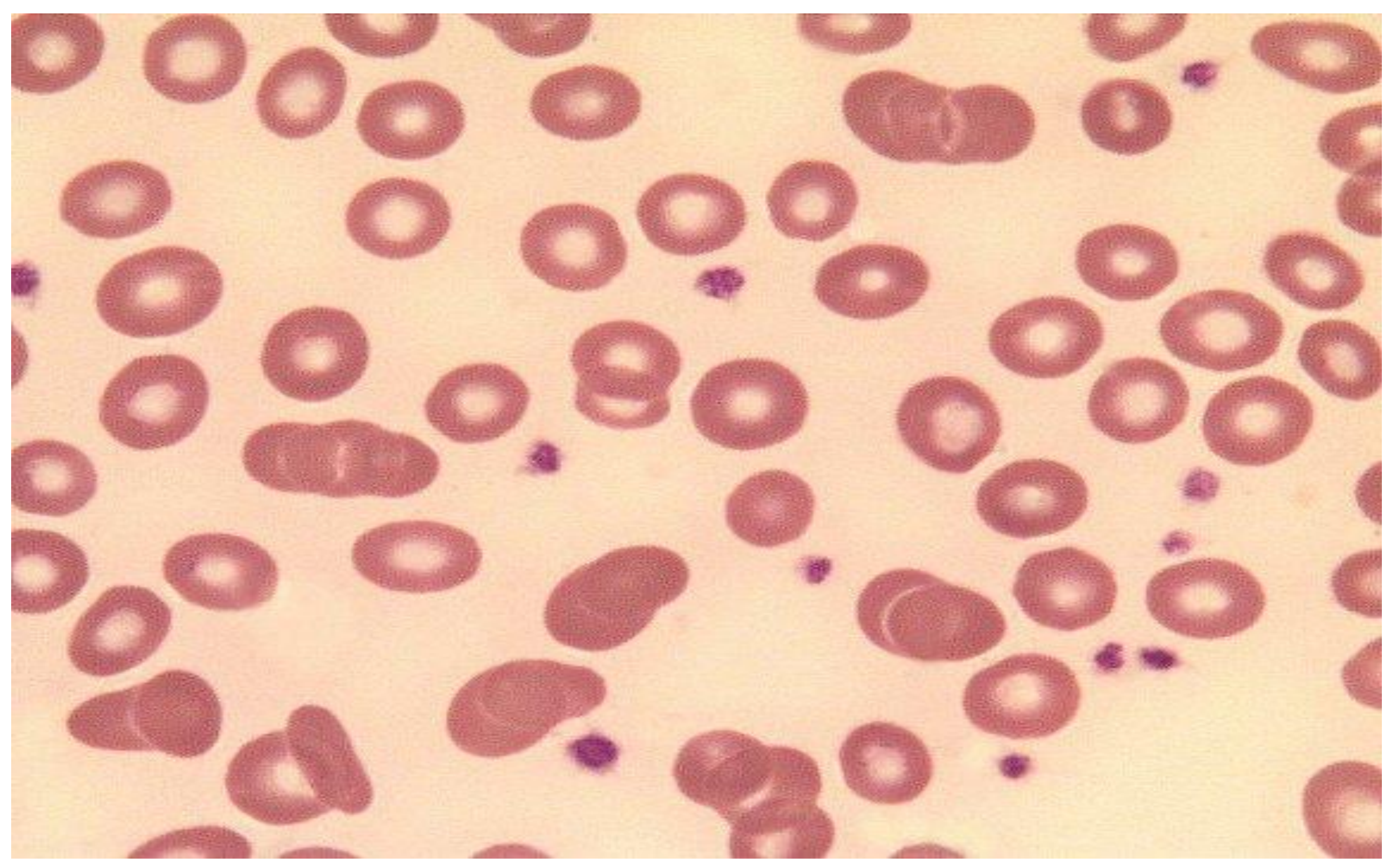


Figure 1–8 Large granular lymphocyte.

PLATELETS



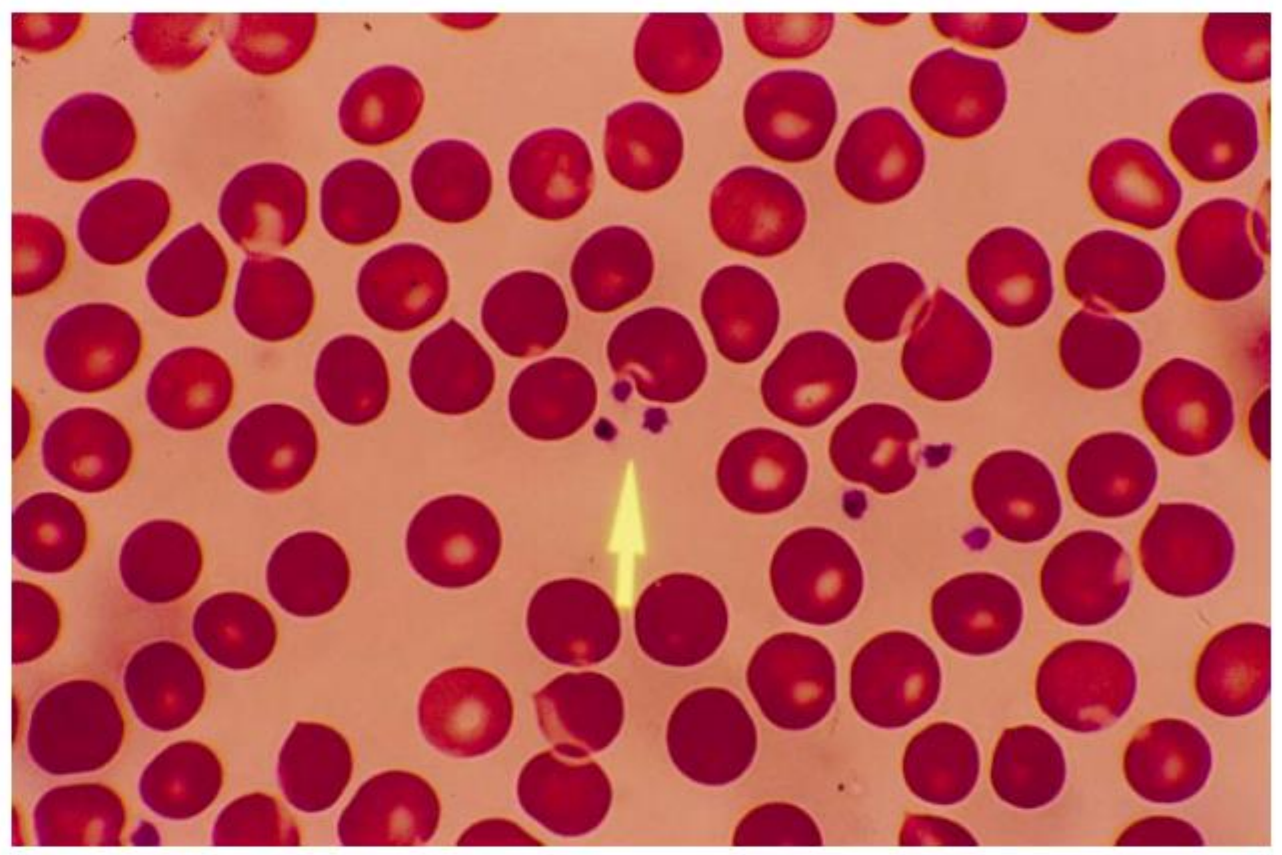


Figure 1–10 **Platelets** (*arrow*).

Table 1.1 Common Terms in Hematology

Name	Meaning
Leukocytosis	Benign or malignant increase in leukocytes
Leukopenia	Decrease in the total white cell count
Neutrophilia	Benign (usually) increase in neutrophils
Neutropenia	Decrease in neutrophils
Monocytosis	Benign (usually) increase in monocytes
Monocytopenia	Decrease in monocytes
Lymphocytosis	Benign or malignant increase in lymphocytes
Lymphopenia	Decrease in lymphocytes
Eosinophilia	Benign (usually) increase in eosinophils
Eosinopenia	Benign (usually) decrease in eosinophils
Basopenia	Decrease in basophils
Thrombocythemia	Malignant increase in platelets
Thrombocytosis	Benign increase in platelets
Thrombocytopenia	Decrease in platelets

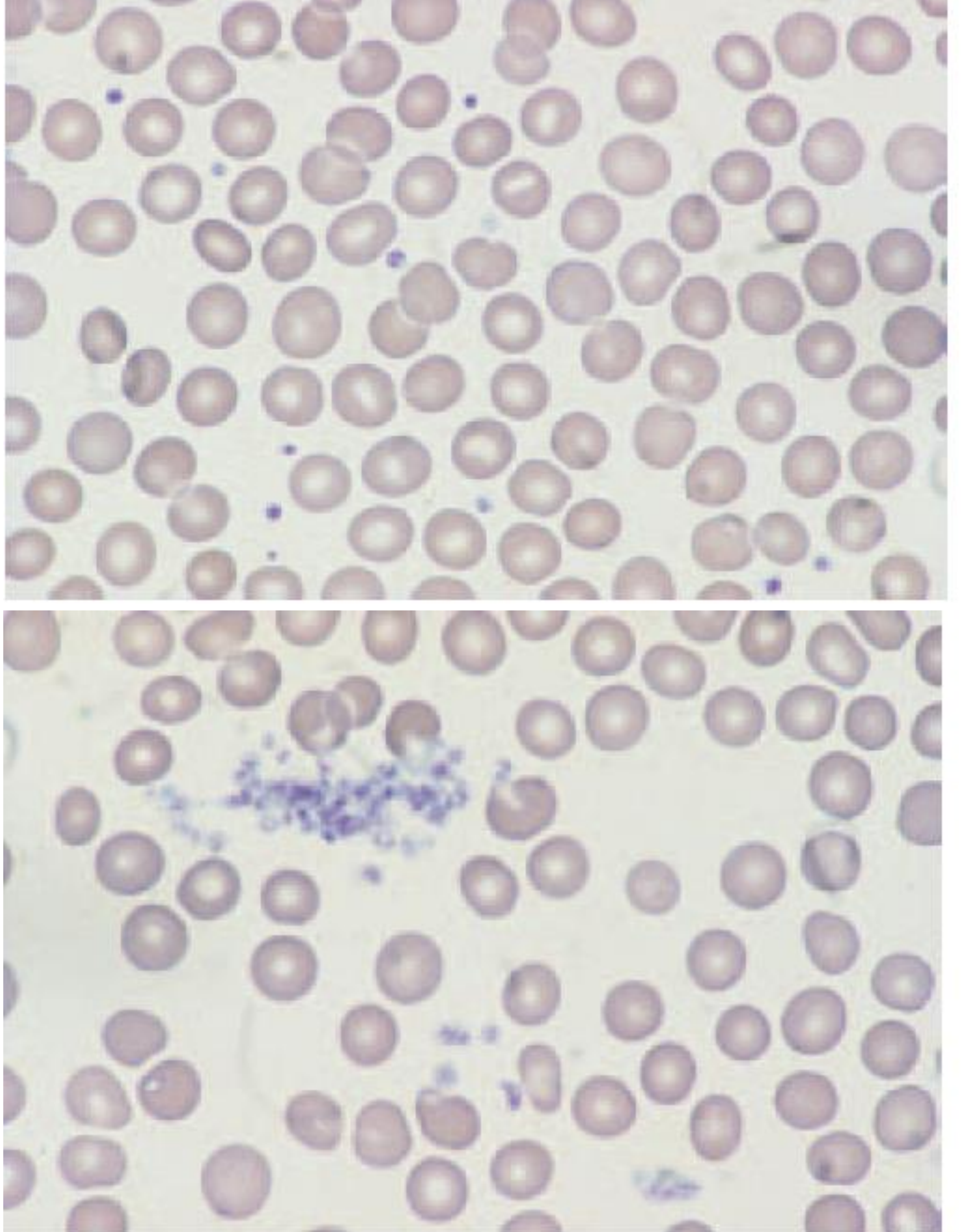


Fig. 1.6 A blood film from EDTA-anticoagulated blood showing an even distribution of platelets.

Fig. 1.7 A blood film from non-anticoagulated capillary blood showing the aggregation of platelets that usually occurs.

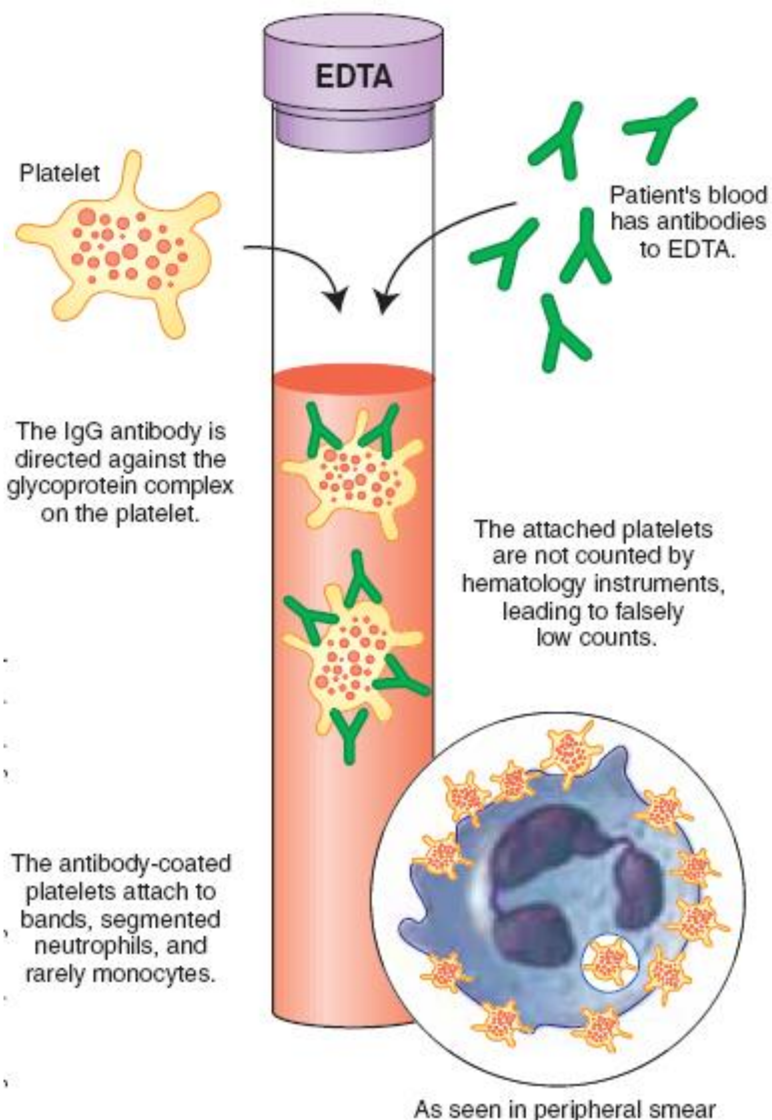


Figure 10.19 Platelet satellitism formation. An unexpected reaction of the patient to the EDTA causes the platelets to ring around segmented neutrophils.

Red cell morphology

Red cell abnormalities	Causes	Red cell abnormalities	Causes
Normal		Spherocyte	Hereditary spherocytosis autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage—e.g. G6PD deficiency, unstable haemoglobin
Ecchinocyte	Liver disease, post-splenectomy	Howell–Jolly body	Hyposplenism, post-splenectomy
Acanthocyte	Liver disease, abetalipoprotein-aemia, renal failure	Basophilic stippling	Haemoglobinopathy, lead poisoning, myelodysplasia, haemolytic anaemia
Sickle cell	Sickle cell anaemia	Malarial parasite	Malaria. Other intra-erythrocytic parasites include *Bartonella bacilliformis*, babesiosis
Microcyte	Iron deficiency, haemoglobinopathy	Siderotic granules (Pappenheimer bodies)	Disordered iron metabolism e.g. sideroblastic anaemia, post-splenectomy

TABLE 1-4 **CELL MORPHOLOGY IN ANEMIA**

Morphologic Characteristic	*Basis of Finding*	*Special Stain*	*Comment*
Howell-Jolly bodies	RBC nuclear remnants	N	• Increased with brisk hemolysis • Increased following splenectomy
Basophilic stippling	RNA remnants	N	• Impaired globin chain production (thalassemia; lead intoxication)
Pappenheimer bodies	Iron ferritin granules in cytoplasm	N	• Increased following splenectomy • Increased with transfusional iron overload
Heinz bodies	Hemoglobin aggregates	Y	• Unstable Hb • Enzymopathies • Hemoglobin H
Burr cells	Membrane perturbance	N	• Chronic renal failure • Common smear prep artifact
Acanthocytes (spur cells)	Membrane perturbance	N	• Severe hepatic insufficiency
Nucleated RBCs	Normoblast nuclei	N	• High with brisk hemolysis • Present with myelopthesis
Sickle cells	RBC distortion by hemoglobin polymers	N	• Sickle cell disease
Target cells	Low ratio of hemoglobin to red cell membrane; RBC dehydration	N	• Prominent in thalassemia • Present with iron deficiency
Spherocytes	Defective membrane protein	N	• Hereditary disorder • Immune hemolysis
Pseudo-Pelger-Huet cells	Neutrophils with bilobed nuclei	N	• Myelodysplasia

Figure 20.1 Standard microhematocrit centrifuge. Maximum packing time is dependent on a calibrated centrifuge.

Hematocrit

Newborn (1 to 7 days)	56 ± 2%
Adult (female)	42 ± 2%
Adult (male)	47 ± 2%

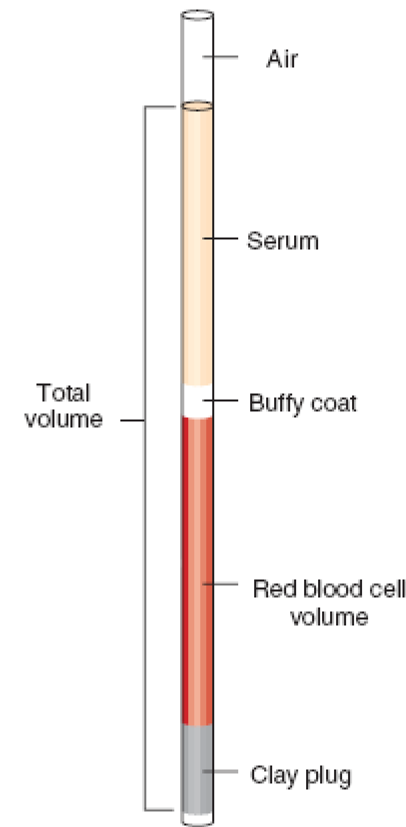


Figure 20.3 Capillary tubes. Note the distinct layers once blood sample has been spun.

CALCULATING RED BLOOD CELL INDICES

a. Mean corpuscular volume (MCV)

$$\text{MCV} = \frac{\text{Hematocrit \%} \times 10}{\text{Erythrocyte count}} = \text{fL}$$

$$\text{Example: } \frac{35\% \times 10}{4.0} = 87.5 \text{ fL}$$

b. Mean corpuscular hemoglobin (MCH)

$$\text{MCH} = \frac{\text{Hemoglobin} \times 10}{\text{Erythrocyte count}} = \text{pg}$$

$$\text{Example: } \frac{14.0 \times 10}{4.0} = 35 \text{ pg}$$

c. Mean corpuscular hemoglobin concentration (MCHC)

$$\text{MCHC} = \frac{\text{Hemoglobin} \times 100}{\text{Hematocrit}} = \%$$

$$\text{Example: } \frac{14 \times 100}{42} = 33.3\%$$

Normal Erythrocyte Indices Values

MCV 80 to 96 fL
MCH 27 to 32 pg
MCHC 32% to 36%

Figure 20.4 Sediplast ESR rack. The sample must be placed on a level surface with no vibration.

Normal Ranges

Men 0 to 15 mm/hr
Women 0 to 20 mm/hr

Manual Differential Counts

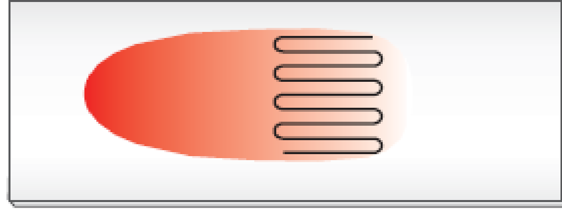


Figure 20.8 Zigzag method of performing differential.

Conditions Associated With...

Increased ESR

1. Kidney disease
2. Pregnancy
3. Rheumatic fever
4. Rheumatoid arthritis
5. Anemia
6. Syphilis
7. Systemic lupus erythematosus
8. Thyroid disease
9. Elevated room temperature

Decreased ESR

1. Congestive heart failure
2. Hyperviscosity
3. Decreased fibrinogen levels
4. Polycythemia
5. Sickle cell anemia

Table 20.3 Normal Differential Results in Adults and Infants

WBC Type	Adult	Infant
Segmented neutrophils	50% to 70%	37% to 67%
Bands	1% to 10%	4% to 14%
Lymphocytes	20% to 44%	18% to 38%
Monocytes	4% to 10%	1% to 12%
Eosinophils	0% to 4%	1% to 4%
Basophils	0% to 2%	0% to 2%

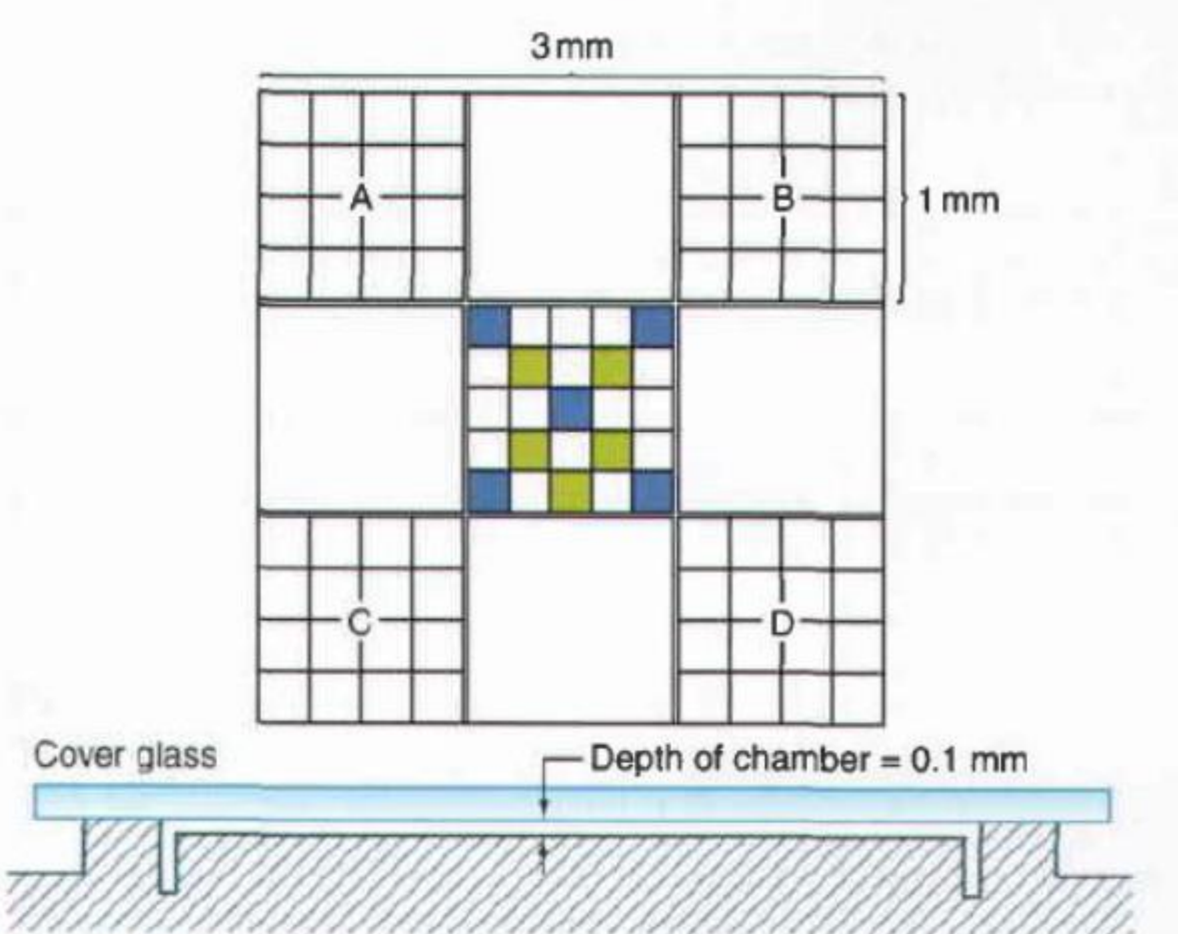


Figure 29–3 The upper figure is a diagram of the improved Neubauer ruling; this is etched on the surface of each side of the hemocytometer. The large corner squares, A, B, C, and D, are used for leukocyte counts. The five blue squares in the center are used for red cell counts or for platelet counts, and the 10 green plus blue squares for platelet counts. Actually, each of the 25 squares within the central sq mm has within it 16 smaller squares for convenience in counting. The lower figure is a side view of the chamber with the cover glass in place.

Table 20.10 • Normal Fluid Results

Normal Results	CSF Adult	CSF Neonate	Serous (Pleural, Pericardial, Peritoneal)	Synovial
Appearance	Clear and colorless	Clear and colorless	Pale yellow and clear	Pale yellow and clear
RBC	0 to 1/mm^3	0 to 3/mm^3	0 to 1/mm^3	0 to 1/mm^3
WBC	0 to 5/mm^3	0 to 30/mm^3	0 to 200/mm^3	0 to 200/mm^3
Neutrophils (includes bands)	2% to 6%	0% to 8%	<25%	<25%
Lymphs	40% to 80%	5% to 35%	<25%	<25%
Monocytes	5% to 45%	50% to 90%	Included with others	Included with others
Others (includes)	Rare	Rare	Monocytes and macrophages 65% to 75%	Monocytes and macrophages 65% to 75%

Specimen Collection and Storage

Cerebrospinal Fluid

1. Collected in the sterile plastic tubes from the spinal tray. The laboratory accepts tubes 1 and/or 4.
2. Tube 4 is the preferred tube because it is least likely to be contaminated with blood.
3. CSF cell counts should be performed within **1 hour** of receipt in the laboratory because cells lyse on prolonged standing and accurate counts become impossible.

Synovial and Serous Fluids (Pleural, Pericardial, and Peritoneal)

1. Fluids should be collected in a heparinized tube or EDTA tube.
2. Perform testing within 4 hours.
3. The addition of hyaluronidase to the fluid may reduce the viscosity of synovial fluid.

Table 20.14 • Color and Appearance in CSF

Color/appearance	CSF
Colorless	Normal
Cloudy	Infections
Straw	Excess protein
Yellow	Xanthochromia
Bloody	Traumatic tap CNS hemorrhage

Table 20.11 • Causes of Abnormal Cells in CSF

Abnormal Results	CSF	Abnormal Results	CSF
Increased neutrophils	Acute inflammation Early viral meningitis Bacterial meningitis (see Fig. 20.13)	Increased monocytes	Newborn infants Recovery phase of meningitis
Increased lymphocytes	Neurosyphilis Viral, fungal, and tubular meningitis Alzheimer's disease Multiple sclerosis Tumors Lymphocytic leukemias and lymphomas Reactive lymphocytes to include plasma cells in most of the above diseases, particularly in multiple sclerosis and viral meningitis	Increased macrophages	Siderophages present, indicating a CNS hemorrhage in past 48 hours Erythrophages present, indicating an active CNS bleed and if siderophages are also present Lipophages present in brain abscesses and cerebral infarctions
		Increased eosinophils	Parasitic infections Postmyelogram specimens
		Tumor/malignant cells	Acute and chronic leukemias Primary neurologic tumors
		Others	Choroid plexus and ependymal cells seen in post pneumoencephalogram specimens

Table 32.1 Laboratory tests of coagulation.

Screening test (normal range)	Abnormalities indicated (prolonged abnormal)	Most common cause of disorder
Prothrombin time (PT) (10–14 s)	Extrinsic and common coagulation pathways Deficiency/inhibition of factor VII, factors X, V, II and fibrinogen	Liver disease, Warfarin therapy, DIC
Activated partial thromblastin time (APTT or PTTK) (30–40 s)	Intrinsic and common coagulation pathways Deficiency/inhibition of one or more of factors XII, IX, VIII, X, V, II and fibrinogen	Liver disease, heparin therapy, haemophilia A and B, DIC
Thrombin time (14–16 s)	Deficiency or abnormality of fibrinogen; inhibition of thrombin by heparin or FDPs	DIC, heparin therapy, fibrinolytic therapy
Fibrin degradation products (<10 mg/mL)	Accelerated destruction of fibrinogen	DIC
Platelet aggregation tests	Abnormal platelet function	Drugs (e.g. aspirin), uraemia, von Willebrand's disease.
Euglobulin clot lysis time	Fibrinolytic pathway defect	Smoking

PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME:

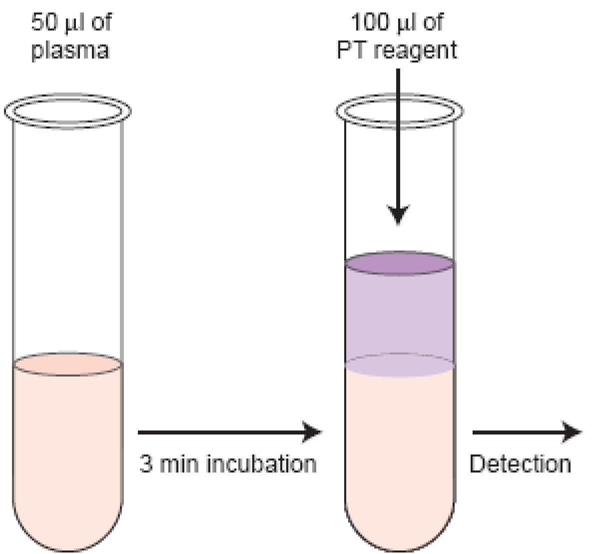


Figure 20.14 Prothrombin time procedure.

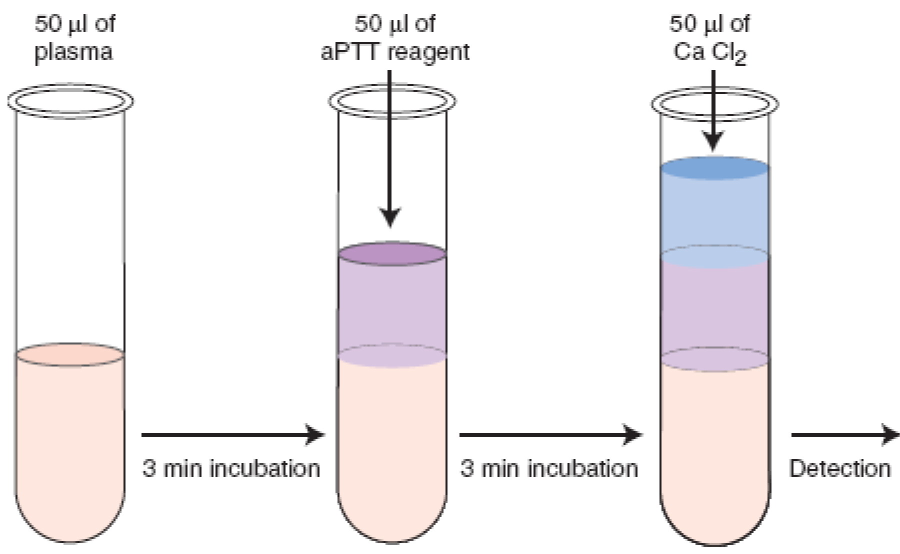


Figure 20.15 Activated partial thromboplastin time procedure.

Apheresis

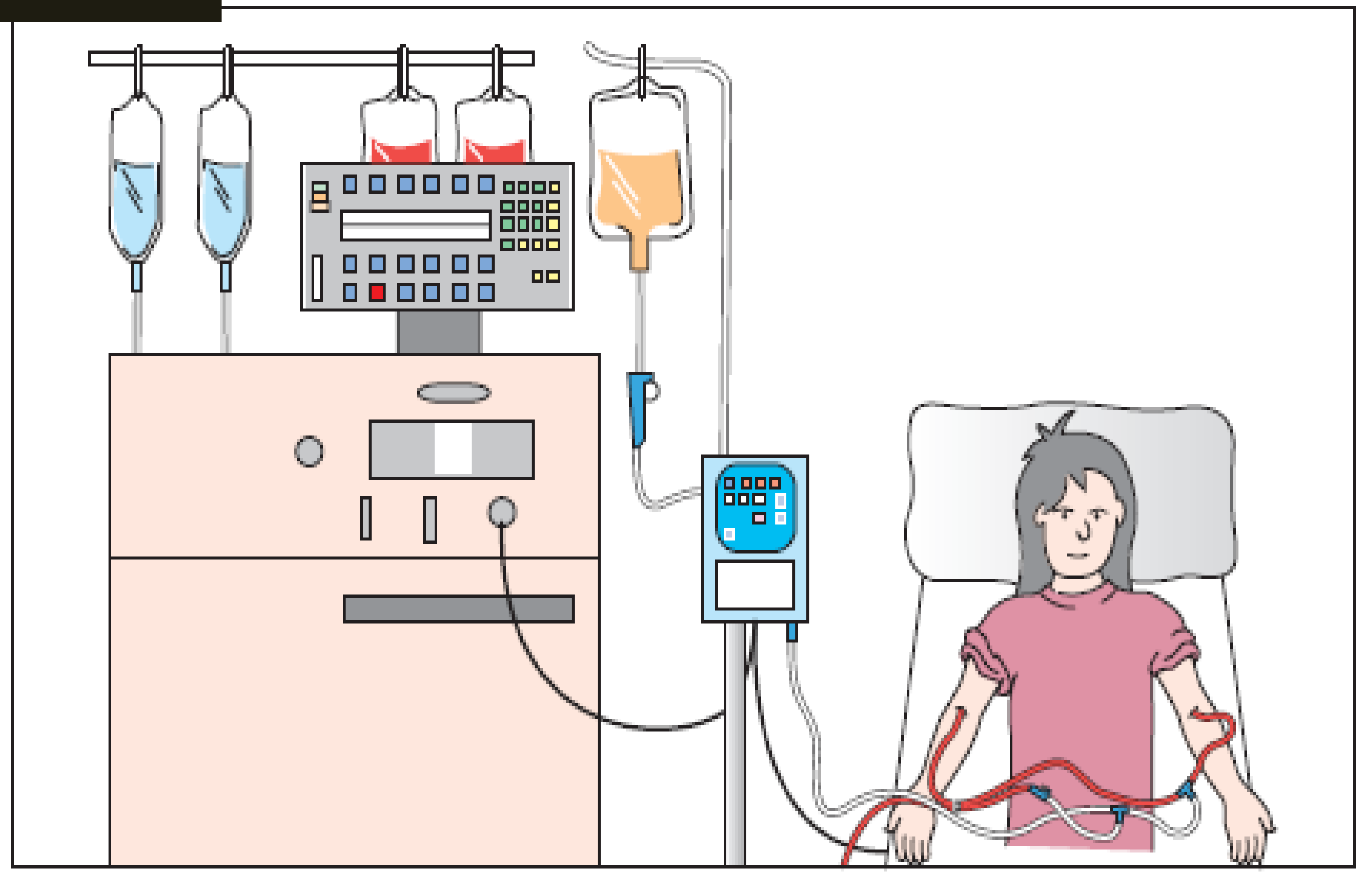


Table 29-4 Principles Used by Various Multichannel Instruments in the Clinical Laboratory

Method

Instrument	Impedance	Conductivity	Light Scatter	Cytochemistry
Abbott	X	X	X	
ABX	X		X	X
Bayer			X	X
Coulter	X	X	X	
Sysmex	X	X	X	

Table 29–5 Potential Causes of Erroneous Results With Automated Cell Counters

Parameter	Causes of spurious increase	Causes of spurious decrease
WBC	Cryoglobulin, cryofibrinogen Heparin Monoclonal proteins Nucleated red cells Platelet clumping Unlysed red cells	Clotting Smudge cells Uremia plus Immunosuppressants
RBC	Cryoglobulin, cryofibrinogen Giant platelets High WBC (> 50 000/μL)	Autoagglutination Clotting Hemolysis (in vitro) Microcytic red cells
Hemoglobin	Carboxyhemoglobin (> 10%) Cryoglobulin, cryofibrinogen Hemolysis (in vitro) Heparin High WBC (> 50 000/μL) Hyperbilirubinemia Lipemia Monoclonal proteins	Clotting Sulfhemoglobin (?)
Hematocrit (automated)	Cryoglobulin, cryofibrinogen Giant platelets High WBC (> 50 000/μL) Hyperglycemia (> 600 mg/dL)	Autoagglutination Clotting Hemolysis (in vitro) Microcytic red cells

Table 29–5 Potential Causes of Erroneous Results With Automated Cell Counters

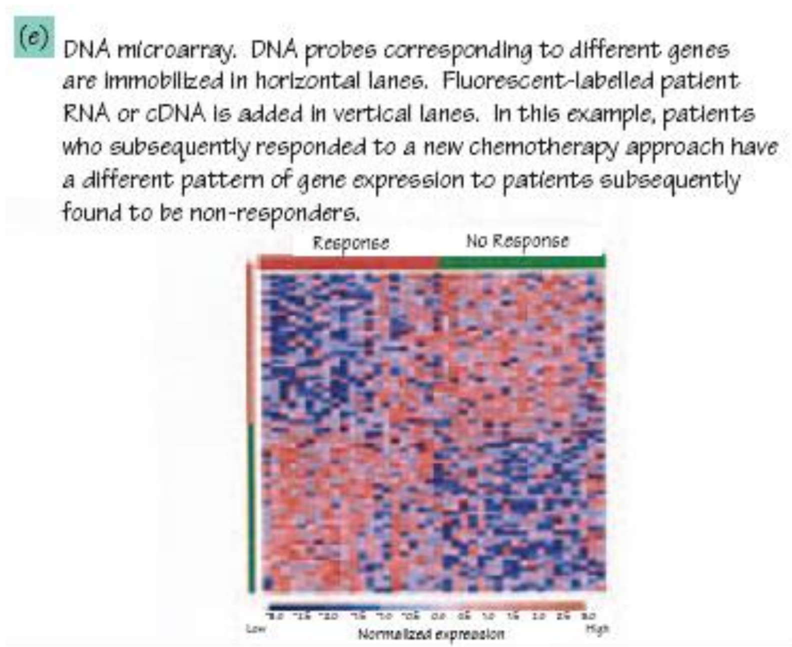
Parameter	Causes of spurious increase	Causes of spurious decrease
Hematocrit (microhematocrit)	Hyponatremia Plasma trapping	Excess EDTA Hemolysis (in vitro) Hypernatremia
MCV	Autoagglutination High WBC (> 50 000/μL) Hyperglycemia Reduced red cell deformability	Cryoglobulin, Cryofibrinogen Giant platelets Hemolysis (in vitro) Microcytic red cells Swollen red cells
MCH	High WBC (> 50 000/μL) Spuriously high Hb Spuriously low RBC	Spuriously low Hb Spuriously high RBC
MCHC	Autoagglutination Clotting Hemolysis (in vitro) Hemolysis (in vivo) Spuriously high Hb Spuriously low Hct	High WBC (> 50 000/μL) Spuriously low Hb Spuriously high Hct
Platelets	Cryoglobulin, cryofibrinogen Hemolysis (in vitro and in vivo) Microcytic red cells Red cell inclusions White cell fragments	Clotting Giant platelets Heparin Platelet clumping Platelet satellitosis

Laboratory assessment

THESE INCLUDE GENERAL AND SPECIAL TESTS

Special tests include:

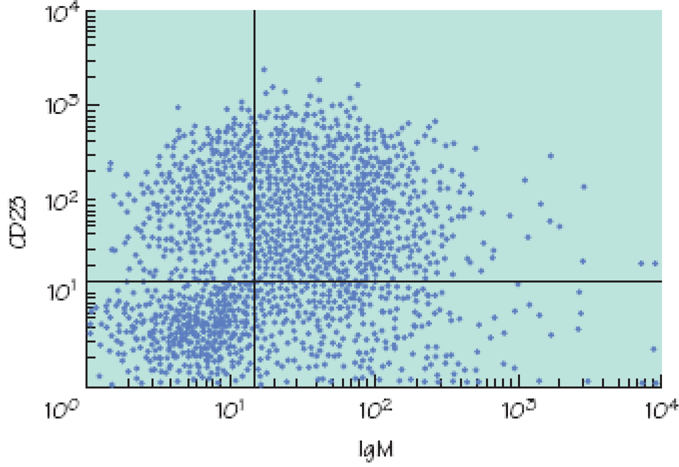
- *-Microarray*
- *-Flow cytometery*
- *-Southern blot*
- *-Polymerase chain reaction*
- *-FISH*



(e) DNA microarray. DNA probes corresponding to different genes are immobilized in horizontal lanes. Fluorescent-labelled patient RNA or cDNA is added in vertical lanes. In this example, patients who subsequently responded to a new chemotherapy approach have a different pattern of gene expression to patients subsequently found to be non-responders.

- DNA microarray analyses expression of multiple cellular genes (Fig. 7e). Fluorescent labelled cell RNA or cDNA is hybridized to DNA probes immobilized on a solid support. The pattern of mRNA expression is obtained and this is characteristic of the different leukaemia or lymphoma subtype and often gives important prognostic information.

(a) Flow cytometry. In this example, cells are simultaneously tested for expression of CD23 and IgM. The x and y scales indicate the number of cells detected expressing that antigen.

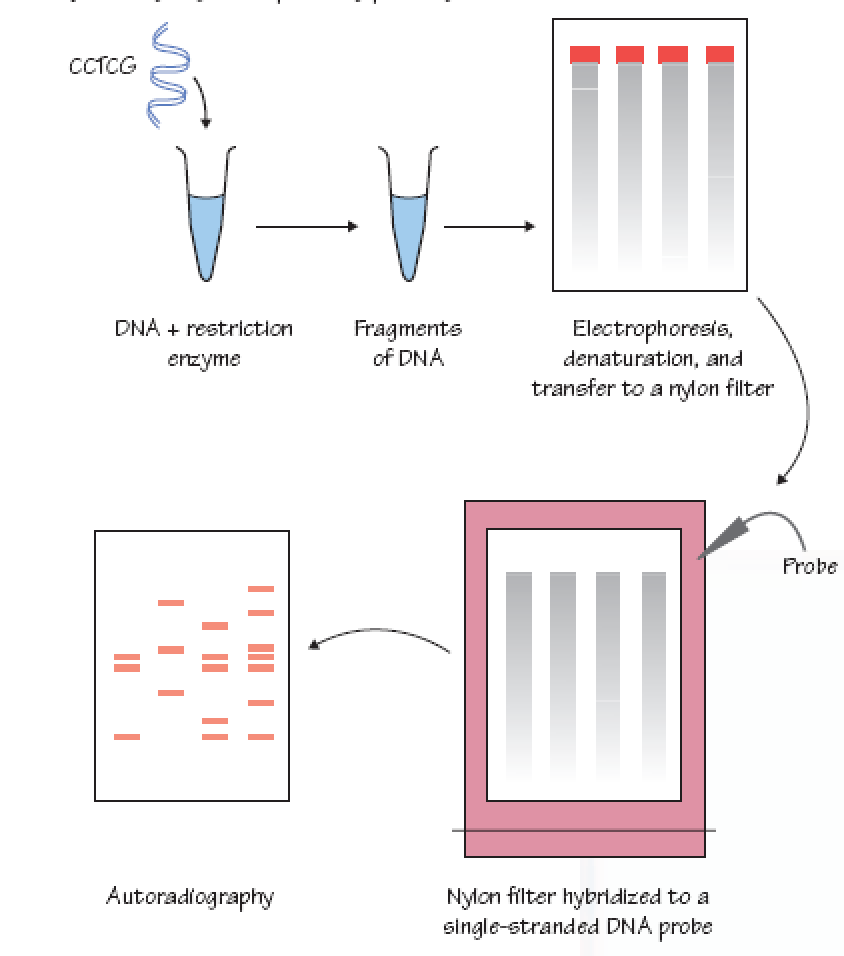


Flow cytometry

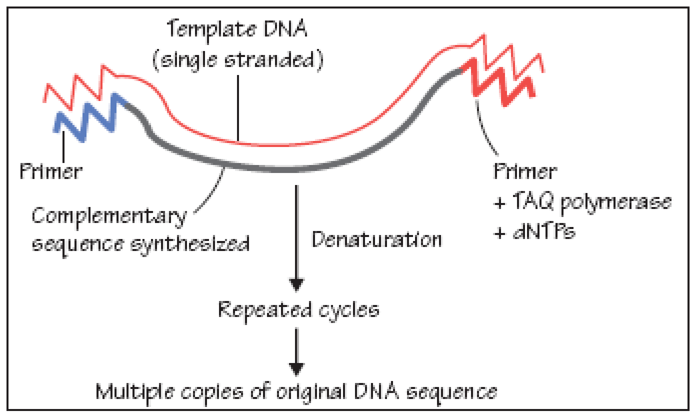
Flow cytometry (Fig. 7a) is an automated technique whereby a population of cells is incubated with specific monoclonal antibodies which are conjugated to a fluorochrome. The labelled cells are then passed in a fluid stream across a laser light source which allows quantitative analysis of antigen expression on the cell population. The technique is important in leukaemia diagnosis and assessment of residual malignant disease.

- Southern blotting (Fig. 7c), which allows assessment of deletion, rearrangement, inversion or duplication of DNA segments. Single base mutations will only be detected, however, if they alter the recognition sequence of a restriction enzyme.

(c) Southern blot technique. Restriction enzymes are bacterial enzymes which recognize specific 3–6 nucleotide sequences (e.g. CCTCG) and cleave DNA whenever that sequence occurs. The fragments of DNA are separated by gel electrophoresis. DNA is then denatured to make it single-stranded and transferred by capillary action to a nylon filter. The filter is then incubated with a single stranded probe, which will hybridize to those DNA fragments with which there is base-pair homology. The Northern blot technique is a way of analysing RNA species by probe hybridization.

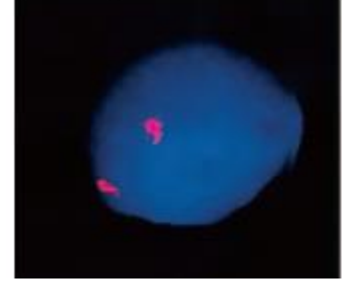


(d) The polymerase chain reaction (PCR) can be used to amplify a DNA segment (typically 0.1–2 kb). The template DNA is denatured and incubated with oligonucleotides (15–20 bp which hybridize specifically to sequences in template DNA and not anywhere else in the genome). The enzyme TAQ polymerase, in the presence of deoxynucleotides (dNTP), allows formation of a complete DNA chain complementary to the template sequence. This process is repeated 40 or so times. Modifications include amplifying a mRNA sequence into DNA and using nucleotides which only bind to a mutant and not a wild-type DNA sequence so that only mutant sequences are amplified.



• Polymerase chain reaction (PCR) (Fig. 7d) can be used to amplify a DNA segment which can then be sequenced or fractionated by size using gel electrophoresis. PCR can also be used to selectively amplify a particular sequence which may, for example, characterize a clone of malignant cells (minimal residual disease, see Chapter 20).

(b) Fluorescent *in situ* hybridization (FISH). Slides have been prepared from a cytogenetic preparation and hybridized with a fluorescent-labelled probe specific for chromosome 7. (a) Normal control showing two signals; (b) a patient with myelodysplasia who has monosomy 7



Chromosomal analysis

Normal individuals have 46 chromosomes: 44 autosomes (22 from each parent) and two sex chromosomes (46 XY = male, 46 XX = female). Chromosomal analysis is made initially by special stains of cells in division. Loss or gain of whole chromosomes, chromosome breaks, and loss, inversion or translocation of a part of a chromosome can be detected. *Fluorescent* in situ *hybridization* (FISH) is a sensitive technique for detecting chromosome abnormalities (Fig. 7b) which involves the use of a fluorescent DNA probe which hybridizes selectively to a particular chromosome segment, allowing sensitive detection of deletion, translocation and duplication of that segment, or fusion with another chromosome.

Introduction to Hematology and Basic Laboratory Practice

Summary Points

- Hematology is the study of blood in health and disease.
- Morphological and analytical skills are needed in the practice of hematology.
- Compound microscopes have a two-lens system to magnify the image.
- The objectives of the microscope are ×10, ×40, and ×100; a ×50 oil immersion lens may be added.
- Proper care of the microscope is essential for maintaining microscopic quality.
- Standard precautions involve behaviors that prevent contact with bodily fluids, aerosol contamination, or contaminated surfaces.
- PPE includes gloves, eyewear, laboratory coats, face shields, and fluid-resistant gowns.
- Handwashing is the most important element of standard precautions.
- Quality assurance is a set of laboratory practices that ensure reliable outcomes for patient results.
- Quality control is part of the quality assurance plan and consists of standards, controls, normal distribution, and statistical parameters.
- Accuracy and precision are measured by the mean standard deviation around a set of data points.
- Patient identification is the essential first step in ensuring the quality of laboratory results.
- Preanalytic variables refer to events or circumstances that occur to the unknown sample before analysis.
- Postanalytic variables refer to laboratory practice after the sample has been analyzed.
- Critical results are those results that exceed or are markedly decreased from the reference interval.