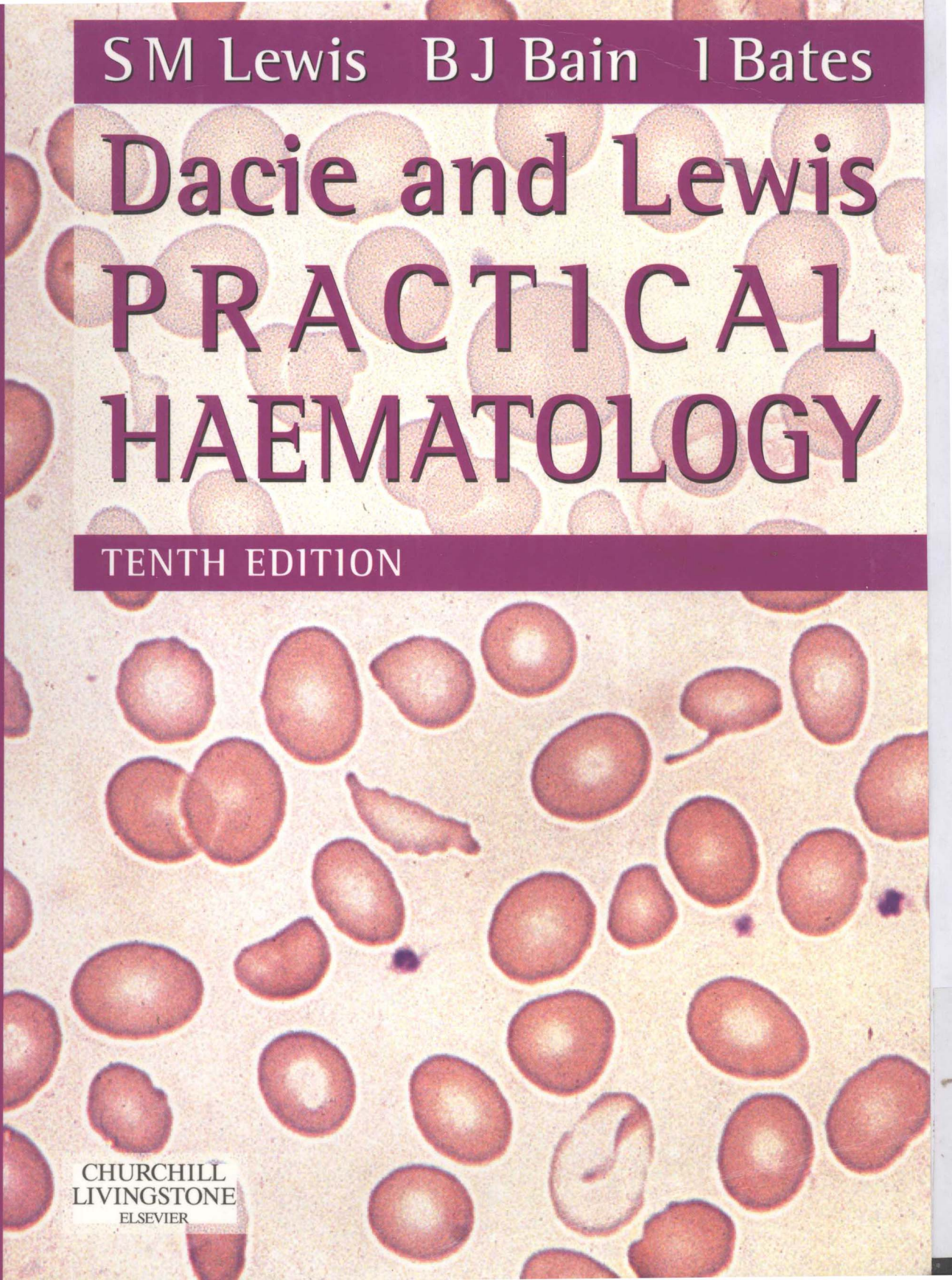
S M Lewis B J Bain I Bates

Dacie and Lewis PRACTICAL HAEMATOLOGY

TENTH EDITION



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Dacie and Lewis PRACTICAL HAEMATOLOGY

Tenth Edition

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PRACTICAL HAEMATOLOGY

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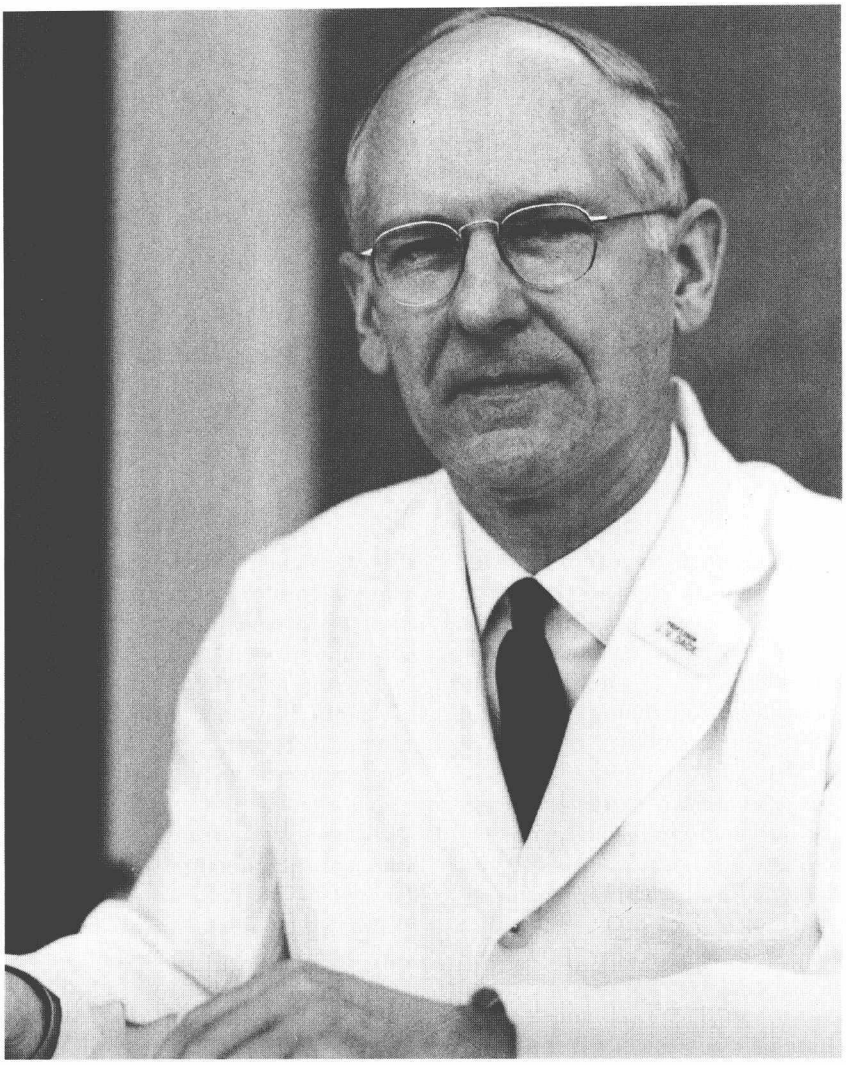
Preface

This 10th edition celebrates the 55th year of *Practical Haematology*. The first edition by J.V. Dacie was published in 1950. This work, and subsequent editions with Mitchell Lewis as co-author, were based on the haematology course for the London University Diploma of Clinical Pathology (DCP) and subsequently the MSc in Haematology at the then Royal Postgraduate Medical School.

Medical science has expanded exponentially in the last half century, but no discipline has expanded more than haematology in both its clinical and laboratory aspects. Originally laboratory haematology was virtually restricted to the "blood count" with a counting chamber and a microscope. The blood count remains an essential test as it provides an important component for diagnosis and patient management, but today automated instruments analyse the physico-chemical properties of individual blood cells and provide precise metrological data on the red cells, leucocytes, and platelets at a speed that would have been beyond imagination in those early days. Sophisticated technology enables complex analyses to be performed routinely in many laboratories; these include DNA studies, immunophenotyping for leukaemia classification, diagnosis of megaloblastic anaemias, and radio isotope measurements. However, we recognize that many of the more sophisticated tests are not readily available in all laboratories, and a chapter is devoted to the essential tests in under-resourced laboratories and health centres. We also take account of the increasing use of commercially available ready-to-use kits for many laboratory tests and the trend for some tests to be performed at point-of-care; advice is given on the supporting role of the laboratory in order to ensure the reliability of this latter practice.

Another advance with enormous impact is the internet. Virtually every topic is to be found, often with an overwhelming amount of information, from a single key word. We have indicated a few important Websites that are relevant to haematology, including those of manufacturers of specialized devices and reagents.

Biomedical scientists are increasingly responsible for laboratory practice as medically qualified haematologists become more concerned with clinical care of patients. But both groups should be aware of the importance of effective laboratory organization and management, the need to maintain a high technical standard in the laboratory, with well-established quality control, and an understanding of the clinical relevance of haematological investigations. The principles for good laboratory practice were established by Dacie in his first edition, when he wrote that "all those concerned with laboratory work should understand what is the significance of the tests that they carry out, the relative value of haematological investigations and the order in which they should be undertaken." We have attempted to maintain his approach in context and style, albeit appropriately updated to meet present-day practices.



Sir John V Dacie, MD, FRCPath, FRS
1912–2005

We are grateful to all our contributors whose names are listed on pages vii–viii, and also to other colleagues who gave us learned advice on specific topics. These include, especially, Michel Deenmamode (Radioisotopes), Mark Griffin (Health & Safety), Julia Howard (Cytogenetics), John Meek (Clinical Chemistry), Andrew Osei-Bimpong (General Haematology).

As a fifty-five-year tribute, this edition is dedicated to Sir John Dacie, to his students from many countries whose subsequent distinguished careers were inspired by him, and to the centre of excellence that he created in the former Royal Postgraduate Medical School, University of London, now Faculty of Medicine of Imperial College at Hammersmith Hospital.

S. Mitchell Lewis
Barbara J. Bain
Imelda Bates

Abbreviations

2,3 DPG	2,3-diphosphoglycerate
2-ME	2-mercaptoethanol
ABC	antibody binding capacity
ACD	acid-citrate dextrose
ACRES	amplification created restriction enzyme site
ADA	adenosine deaminase
ADP	adenosine-diphosphate
AET	2-aminoethyl-*iso*-thiouronium
AIHA	autoimmune haemolytic anaemia
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
ANAE	alpha-naphthyl acetate esterase
ANB	alpha-naphthyl butyrate
APAAP	alkaline phosphatase anti-alkaline phosphatase
APTT	activated partial thromboplastin time
ARMS	amplification refractory mutation system
ASOH	allele-specific oligonucleotide hybridization
AT	antithrombin
ATLL	adult T-cell leukaemia/lymphoma
ATP	adenosine triphosphate
BCSH	British Committee for Standards in Haematology
bp	base pair
B-PLL	B-cell prolymphocytic leukaemia
BM	bone marrow
BSA	bovine serum albumin
C	complement
C3, C3d, C3sg	complement components
CAE	naphthol AS-D chloroacetate esterase
CD	cluster of differentiation
CHAD	cold haemagglutinin disease
CLL	chronic lymphocytic leukaemia
CLL/PL	chronic lymphocytic leukaemia with increased prolymphocytes
CML	chronic myeloid leukaemia
CPD	cirtate–phosphate–dextrose
CTP	cytosine triphosphate
CV	coefficient of variation
Cy	cytoplasmic
DAB	diaminobenzidine
DAT	direct antiglobulin test
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EGIL	European Group for the Immunological Classification of Leukaemias
ELISA	enzyme-linked immunosorbent assay
FAB	French-American-British (classification)
FDPs	fibrin/fibrinogen degratation products
FVL	factor V Leiden
FITC	fluorescein isothiocyanate
FSc	forward light scatter
g	either gram or relative centrifugal force, as appropriate
G6PD	glucose-6-phosphate dehydrogenase
GPI	glycosylphosphatidylinositol
Hb	haemoglobin or haemoglobin concentration
HbCO	carboxyhaemoglobin
HCL	hairy cell leukaemia
HCT	haematocrit
HDW	haemoglobin distribution width

HEMPAS	hereditary erythroblastic multinuclearity with positive acidified-serum test
Hi	methaemoglobin
HiCN	cyanmethaemoglobin
HIV	human immunodeficiency virus
HPFH	hereditary persistence of fetal haemoglobin
HPLC	high performance liquid chromatography
HVR	hypervariable region
IAT	indirect antiglobulin test
ICSH	International Council (formerly Committee) for Standardization in Haematology
IEF	isoelectric focusing
Ig	immunoglobulin
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	immunoperoxidase
iu	international units
kb	kilobase
LISS	low ionic strength saline
McAb	monoclonal antibody
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean cell volume
MDS	myelodysplastic syndrome or syndromes
MESF	molecules equivalent soluble fluorochrome
MGG	May-Grünwald-Giemsa
MoAb	monoclonal antibody
MPO	myeloperoxidase
MPV	mean platelet volume
NAP	neutrophil alkaline phosphatase
NEQAS	national external quality assessment
NISS	normal ionic strength saline
NRBC	nucleated red blood cell
NSE	non-specific esterase
PA	plasminogen activator
PAS	periodic acid-Schiff
PB	peripheral blood
PBS	phosphate-buffered saline
PBS-azide-BSA	phosphate-buffered saline containing sodium azide and bovine serum albumin
PCH	paroxysmal cold haemoglobinuria
PCR	polymerase chain reaction
PCV	packed cell volume
PDW	platelet distribution width
PE	phycoerythrin
pI	isoelectric point
PK	pyruvate kinase
PML	protein encoded by *PML* gene
PNH	paroxysmal nocturnal haemoglobinuria
PT	prothrombin time
PVP	polyvinyl pyrrolidine
QSC	quantum simply cellular
RID	radial immunodiffusion
RE	restriction enzyme
RBC	red blood cell count
RDW	red cell distribution width
RFLP	restriction fragment-length polymorphism
RNA	ribonucleic acid
RT	reverse transcriptase
RT-PCR	reverse transcriptase-polymerase chain reaction
SD	standard deviation
SDS	sodium dodecyl sulphate
SHb	sulphhaemoglobin
SLVL	splenic lymphoma with villous lymphocytes
SmIg	surface immunoglobulin
SOP	standard operating procedure
SSc	sideways light scatter
SSCP	single-strand conformation polymorphism
TAE	Tris acetate EDTA (buffer)
TBE	Tris borate EDTA (buffer)
TBS	Tris-buffered saline
TCR	T-cell receptor
TE	Tris–EDTA (buffer)
TEB	Tris–EDTA-borate (buffer)
TNCC	total nucleated cell count
t-PA	tissue plasminogen activator
TT	thrombin time
UIBC	unsaturated iron-binding capacity
UV	ultraviolet
vol	volume
v/v	volume/volume
WBC	white cell count
WHO	World Health Organization
w/v	weight/volume

EDITORIAL NOTE

In keeping with recommendations from the International Organization for Standardardization (ISO), the World Health Organization (WHO), and other international authorities, we have used the Système International (SI) for expressing quantities and units (see p. 696). Concentration of solutions is expressed either in mol/l (for substance concentration) or g/l (for mass concentration), whichever is more appropriate. While we are aware that in some countries g/dl is still in common use for expressing haemoglobin concentration, in keeping with the internationally agreed convention we have expressed Hb in g/l. To convert this to g/dl divide the quantity by 10.

We have indicated the source of a reagent, kit, or device if there is a single manufacturer or if a specific make is recommended. If no source is indicated, suitable material or equipment will generally be available from different suppliers. Catalogues from these manufacturers and detailed information on the use of their unique devices can be found on their Websites.

Contents

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1 Collection and handling of blood

S. Mitchell Lewis and Noriyuki Tatsumi

In investigating physiological function and malfunction of blood, accurate and precise methodology is essential to ensure, as far as possible, that tests do not give misleading information because of technical errors. Obtaining the specimen is the first step toward analytic procedures. It is important to use appropriate blood containers and to avoid faults in specimen collection, storage, and transport to the laboratory. Venous blood generally is used for most haematological examinations and for chemistry tests; capillary skin puncture samples can be almost as satisfactory for some purposes if a free flow of blood is obtained (see p. 4), but in general this procedure should be restricted to children and to some "point-of-care" screening tests that require only a drop or two of blood. Bone marrow aspirates are described in Chapter 6.

BIOHAZARD PRECAUTIONS

Special care must be taken to avoid risk of infection from various pathogens during all aspects of laboratory practice, and the safety procedures described in Chapter 25 must be followed as far as possible when collecting blood. The operator should wear disposable plastic or thin rubber gloves. It is also desirable to wear a protective apron or gown and, if necessary, glasses or goggles. Care must be taken to prevent injuries, especially when handling syringes, needles, and lancets.

Disposable sterilized syringes, needles, and lancets should be used if at all possible, and they should never be reused. Reusable items must, after use, always be sterilized in an autoclave or hot-air oven at 160°C for 1 hour (see p. 652).

*Some individuals may have an allergic reaction to either plastic or rubber gloves (see p. 650).

STANDARDIZED PROCEDURE

The constituents of the blood may be altered by a number of factors, which are listed in Table 1.1. It is important to have a standard procedure for the collecting and handling of blood specimens. Recommendations for standardizing the procedure have been published.[1–3]

VENOUS BLOOD

It is now common practice for specimen collection to be undertaken by specially trained phlebotomists,

Table 1.1 Causes of Misleading Results from Discrepancies in Specimen Collection
Precollection
Toilet within 30 min; food or water intake within 2 hours
Smoking
Physical activity (including fast walking) within 20 min
Stress
Drugs or dietary supplement administration within 8 hours
During Collection
Different times (diurnal variance)
Posture: lying, standing, or sitting
Haemoconcentration from prolonged tourniquet pressure
Excessive negative pressure when drawing blood into syringe
Incorrect type of tube
Capillary vs. venous blood
Handling of Specimen
Insufficient or excess anticoagulant
Inadequate mixing of blood with anticoagulant
Error in patient and/or specimen identification
Inadequate specimen storage conditions
Delay in transit to laboratory

and there are published guidelines that set out an appropriate training programme.[1,4]

Phlebotomy Tray

It is convenient to have a tray that contains all the requirements for blood collection (Table 1.2).

Disposable Plastic Syringes and Disposable Needles

The needles should not be too fine, too large, or too long; those of 19 or 21G* are suitable for most adults, and 23G (especially with a short shaft of about 15 mm) is suitable for children. It may be helpful to collect the blood by means of a winged ("butterfly") needle connected to a length of plastic tubing, which can be attached to the nozzle of the syringe or to a needle for entering the cap of an evacuated container (see later discussion).

*The International Organization for Standardization has established a standard (ISO 7864) that relates the following diameters for the different gauges: 19G = 1.1 mm, 21G = 0.8 mm, 23G = 0.6 mm.

Table 1.2 Phlebotomy Tray
Syringes and needles
Tourniquet
Specimen containers (or evacuated tube system)—plain and with various anticoagulants
Request form
70% isopropyl alcohol swabs or 0.5% chlorhexidine
Sterile gauze swabs or cellulose pads
Adhesive dressings
Self-sealing plastic bags
Rack to hold specimens upright during process of filling
(A puncture-resistant disposal container should also be available.)

Specimen Containers

The common containers for haematology tests are available commercially with dipotassium, tripotassium, or disodium ethylenediaminetetra-acetic acid (EDTA) as anticoagulant, and they are marked at a level to indicate the correct amount of blood to be added. Containers are also available containing trisodium citrate, heparin, or acid citrate dextrose, and there are containers with no additive, which are used when serum is required. Design requirements and other specifications for specimen collection containers have been described in a number of national and international standards (e.g., that of the International Council for Standardization in Haematology[5]), and there is also a European standard (EN 14820). Unfortunately, there is not yet universal agreement regarding the colours for identifying containers with different additives; phlebotomists should familiarize themselves with the colours used by their own suppliers.

Evacuated tube systems, which are now in common use, consist of a glass or plastic tube/container (with or without anticoagulant) under defined vacuum, a needle, and a needle holder that secures the needle to the tube. The main advantage is that the cap can be pierced, so that it is not necessary to remove it either to fill the tube, or subsequently to withdraw samples for analysis, thus minimizing the risk of

aerosol discharge of the contents.[6,7] An evacuated system is useful when multiple samples in different anticoagulants are required. The vacuum controls the amount of blood that enters the tube, ensuring an adequate specimen for the subsequent tests and the correct proportion of anticoagulant when this is present. Silicone-coated evacuated tubes can be used for routine coagulation screening tests.

Phlebotomy Procedure

The phlebotomist should first check the patient's identity, making sure that it corresponds to the details on the request form, and also ensure that the phlebotomy tray contains all the required specimen containers.

Blood is best withdrawn from an antecubital vein or other visible veins in the forearm by means of either an evacuated tube or a syringe. It is usually recommended that the skin be cleaned with 70% alcohol (e.g., isopropanol) or 0.5% chlorhexidine and allowed to dry spontaneously before being punctured; however, some doubts have been expressed on the utility of this practice for preventing infection at the venepuncture site.[8] Care must also be taken when using a tourniquet to avoid contaminating it with blood, because infection risks have been reported during blood collection.[9] The tourniquet should be applied just above the venepuncture site and released as soon as the blood begins to flow into the syringe or evacuated tube—delay in releasing it leads to fluid shift and haemoconcentration as a result of venous blood stagnation.[4] It should be possible with practice to obtain venous blood even from patients with difficult veins (except for very young children). A butterfly needle is especially useful when a series of samples is required.

Successful venepuncture may be facilitated by keeping the subject's arm warm, applying to the upper arm a sphygmomanometer cuff kept at approximately diastolic pressure, and tapping the skin over the site of the vein a few times. After cleaning and drying the site and applying a tourniquet, ask the patient to make a fist a few times. Veins suitable for puncture will usually become apparent. If the veins are very small, a butterfly needle or 23G needle should enable at least 2 ml of blood to be obtained satisfactorily. In patients who are obese, it may be easier to use a vein on the dorsum of the hand, after warming it by immersion in warm water; however, this site is not generally recommended because vein punctures here tend to bleed into surrounding tissues more readily than at other sites. Venepuncture should not be attempted over a site of scarring or haematoma.

If a syringe is used for blood collection, the piston of the syringe should be withdrawn slowly and no attempt should be made to withdraw blood faster than the vein is filling. Anticoagulated specimens must be mixed by inverting the containers several times. Haemolysis can be avoided or minimized by using clean apparatus, withdrawing the blood slowly, not using too fine a needle, delivering the blood gently into the receiver, and avoiding frothing during the withdrawal of the blood and subsequent mixing with the anticoagulant. If the blood is drawn too slowly or inadequately mixed with the anticoagulant, some coagulation may occur. After collection, the containers must be firmly capped to minimize the risk of leakage.

If blood collection fails, it is important to remain calm and consider the possible cause of the failure. This includes poor technique, especially stabbing rather than holding the needle parallel to the surface of the skin as it enters; this may result in the needle passing through the vein. After two or three unsuccessful attempts, it may be wise to refer the patient to another operator after a short rest.

After obtaining the blood and releasing the tourniquet, remove the needle and then press a sterile swab over the puncture site, applying pressure on the swab. The arm should be elevated after withdrawal of the needle, and pressure should continue to be applied to the swab with the arm elevated for a few minutes before checking that bleeding has completely ceased. Then cover the puncture site with a small adhesive dressing.

Obtaining blood from an indwelling line or catheter is a potential source of error. Because it is common practice to flush lines with heparin, they must be flushed free from heparin before any blood is collected for laboratory tests. If intravenous fluids are being transfused into an arm, the blood sample should not be collected from that arm.

Postphlebotomy Procedure

The phlebotomist should again check the patient's identity and ensure that it corresponds to the details on the request form. In addition to the request form being labelled, it is essential that every specimen

is labelled with adequate patient identification immediately after the samples have been obtained. On the labels this should include at least surname and forename or initials, hospital number, date of birth, and date and time of specimen collection. The same information must be given on the request form, together with ward or department, name of requesting clinician, and test(s) requested. When relevant, a biohazard warning also must be affixed to the container and to the request form. If automated patient identification is available, both the label and the request form should be bar-coded with the relevant data.

Specimens should be sent in individual plastic bags separated from the request forms to prevent contamination of the forms in the event of leakage. Alternatively, the specimen tubes must be set upright in a holder or rack and placed in a carrier together with the request forms for transport to the laboratory.

Waste Disposal

Without separating the needle from the syringe, place both, together with the used swab and any other dressings, in a puncture-resistant container for disposal (see p. 653). If it is essential to dispose of the needle separately, it should be detached from the syringe only with forceps or a similar tool. Alternatively, the needle can be destroyed *in situ* with a special device (e.g., Sharp-X, Biomedical Disposal Inc., www.biodisposal.com).

CAPILLARY BLOOD

Skin puncture can be used for obtaining a small amount of blood either for direct use in an analytic process or for collecting into capillary tubes coated with heparin for packed cell volume or into a special anticoagulated microcollection device (p. 5). These methods are mostly used when it is not possible to obtain venous blood (e.g., in infants younger than 1 year, in cases of gross obesity, or for point-of-care blood tests).

Collection of Capillary Blood

Skin puncture is carried out with a needle or lancet. In adults and older children blood can be obtained from a finger; the recommended site is the distal digit of the third or fourth finger on its palmar surface, about 3–5 mm lateral from the nail bed. Formerly the ear lobe was commonly used, but it is no longer recommended because reduced blood flow renders it unrepresentative of the circulating blood.[1] In infants, satisfactory samples can be obtained by a deep puncture of the plantar surface of the heel in the area shown in Figure 1.1. Because the heel should be very warm, it may be necessary to bathe it in hot water. The central plantar area and the posterior curvature should not be punctured in small infants to avoid the risk of injury and possible infection to the underlying tarsal bones, especially in newborns.

Clean the area with 70% alcohol (e.g., isopropanol) and allow to dry. Puncture the skin to a depth of 2–3 mm with a sterile disposable lancet. Wipe away the first drop of blood with dry sterile gauze. If necessary, squeeze very gently to encourage a free flow of blood. Collect the second and following drops

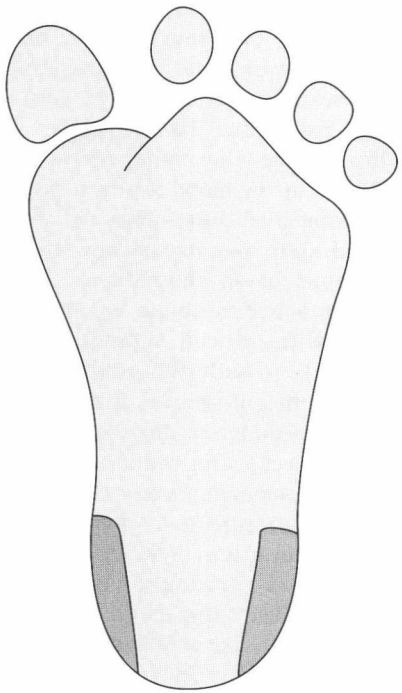

Figure 1.1 Skin puncture in infants. Puncture must be restricted to the outer medial and lateral portions of the plantar surface of the foot where indicated by the shaded area.

directly onto a reagent strip or by a 10 ml or 20 ml micropipette for immediate dispensing into diluent. A free flow of blood is essential, and only the very gentlest squeezing is permissible; ideally, large drops of blood should exude slowly but spontaneously. If it is necessary to squeeze firmly to obtain blood, the results are unreliable. If the poor flow is the result of the sampling site being cold and cyanosed, then the figures obtained for haemoglobin, red blood cell count (RBC), and leucocyte count are usually too high.

There are methods for collecting the blood into a capillary tube fixed into the cap of a microcontainer to allow the blood to pass by capillary action into the container (e.g., Microtainer*).[10] In another system (Unopette*), a calibrated capillary is completely filled with blood and linked to a premeasured volume of diluent.[11] An adequate puncture with a free flow of blood can also enable a larger volume to be collected, drop by drop, into a plastic or glass container.[12]

After use, lancets (and needles) should be placed in a puncture-resistant container for subsequent waste disposal. They must never be reused on another individual.

Blood Film Preparation

Ideally, blood films should be made immediately after the blood has been collected. Because blood samples are usually sent to the laboratory after a variable delay, there are advantages in preparing blood films when the phlebotomy is carried out. The phlebotomy tray might include some clean glass slides and spreaders, and phlebotomists should be given appropriate training for film preparation, as described in Chapter 4. An automated device for making smears is also available.[13] When films are not made on site, they should be made in the laboratory without delay as soon as the specimens have been received.

DIFFERENCES BETWEEN CAPILLARY AND VENOUS BLOOD

Venous blood and "capillary" blood are not quite the same. Blood from a skin puncture is a mixture of blood from arterioles, veins, and capillaries, and it contains some interstitial and intracellular fluid.[1,4,14] Although some studies have suggested that there are negligible differences when a free flow of blood has been obtained,[15] others have shown definite differences in composition between skin puncture and venous blood samples in neonates,[16] children,[17] and adults.[18] The differences may be exaggerated by cold with resulting slow capillary blood flow.[4]

The packed cell volume (PCV), RBC, and haemoglobin concentration (Hb) of capillary blood are slightly greater than in venous blood. The total leucocyte and neutrophil counts are higher by about 8%; the monocyte count is higher by about 12%, and in some cases by as much as 100%, especially in children. Conversely, the platelet count appears to be higher in venous than in capillary blood; this is on average by about 9% and in some cases by as much as 32%.[16–18] This may be the result of adhesion of platelets to the site of the skin puncture.

SERUM

The difference between plasma and serum is that the latter lacks fibrinogen and some of the coagulation factors. Blood collected to obtain serum should be delivered into sterile tubes with caps or commercially available, plain (nonanticoagulant), evacuated collection tubes and allowed to clot undisturbed for about 1 hour at room temperature.† Then the clot should be loosened gently from the container wall by means of a wooden stick or a thin plastic or glass rod. Rough handling will cause lysis. The tube should be closed with a cap/stopper. Some products contain a clot activator combined with a gel for accelerated separation of serum (e.g., serum separator tubes*).

The tubes, with or without a serum separator, are centrifuged for 10 min at about 1200 g. The supernatant serum then is pipetted into another tube and centrifuged again for 10 min at about 1200 g. The supernatant serum is transferred to tubes for tests or for storage. For most tests, serum should be kept at 4°C until used, but if testing is delayed, serum can be stored at −20°C for up to 3 months and at −40°C or less for long-term storage. Frozen specimens should be thawed on the bench or in a

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†Room temperature is usually taken as 18–25°C.

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