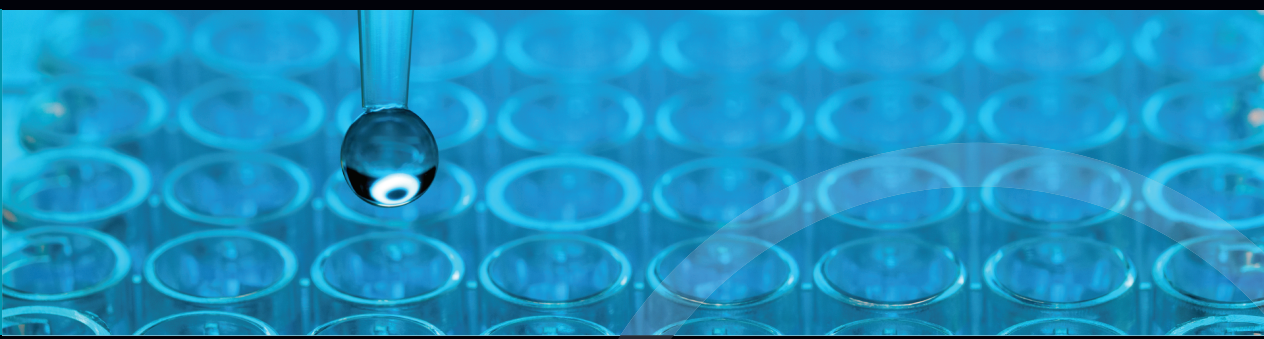
CLINICAL BIOCHEMISTRY

Lecture Notes



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9th Edition

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Clinical Biochemistry

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Lecture Notes

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Contents

Preface

This is the ninth edition of the book that first appeared under the authorship of Professor Gordon Whitby, Dr Alistair Smith and Professor Iain Percy-Robb in 1975.

Changes to the medical teaching curriculum and pressures on teaching time have reduced or even abolished teaching courses that focus exclusively on clinical biochemistry. Instead, the discipline is integrated into systems-based teaching at all levels of the medical curriculum. Whilst this has many advantages in placing the material in a holistic, clinical context it is also very valuable to bring together teaching material on clinical biochemistry. This textbook attempts to do that. In one volume can be found a wealth of information on the biochemical basis of many diseases, the selection of biochemical diagnostic tests and their interpretation. To that end, the book is highly relevant to the medical student throughout the whole training period and as a reference for the qualified doctor. Moreover, other health professionals, such as nurses who take on specialist roles in defined clinical areas, should also find the book helpful. In addition, we believe it would be of value to specialist registrars, clinical scientists and biomedical scientists who are studying for higher qualifications to pursue a career in clinical biochemistry and metabolic medicine.

In this edition, the number of clinical cases has been increased and these have been integrated into the text rather than collected at the end of each chapter. The order of chapters has been kept the same but we have taken the opportunity to update the material and to try to present it more clearly. The MCQs that featured at the end of the last edition have been gathered on-line and a detailed commentary provided on the reasons for the 'true' and 'false' answers to each question. An on-line resource also collects together the key points for each chapter.

As with previous editions, we are indebted to our colleagues for contributing to this latest revision. We would particularly like to thank Maria Squires, Mike Crane, Neil Syme and Neil Squires for reading and commenting on some of the chapters in this new edition. Dr Allan Deacon kindly helped with his views on the investigation of porphyria. We would also like to express our thanks to the staff at Wiley for their continued interest and support towards this title since its appearance in 1975.

Simon Walker
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List of abbreviations

ABP	androgen-binding protein
A&E	accident and emergency
ACE	angiotensin-converting enzyme
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
AFP	α-fetoprotein
AI	angiotensin I
AII	angiotensin II
AIP	acute intermittent porphyria
ALA	aminolaevulinic acid
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMP	adenosine 5-monophosphate
ANP	atrial natriuretic peptide
API	α_1-protease inhibitor
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BChE	butylcholinesterase
BMI	body mass index
BMR	basal metabolic rate
BNP	B-type natriuretic peptide
CAH	congenital adrenal hyperplasia
cAMP	cyclic adenosine monophosphate
CBG	cortisol-binding globulin
CCK-PZ	cholecystokinin-pancreozymin
CDT	carbohydrate-deficient transferrin
CEA	carcinoembryoinic antigen
ChE	cholinesterase
CK	creatine kinase
CKD	chronic kidney disease
CNS	central nervous system
CoA	coenzyme A
COC	combined oral contraceptive
COHb	carboxyhaemoglobin
CRH	corticotrophin-releasing hormone
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	computed tomography
DDAVP	1-deamino,8-D-arginine vasopressin
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DHCC	dihydrocholecalciferol
DHT	dihydrotestosterone
DIT	di-iodotyrosine
DKA	diabetic ketoacidosis
DPP-4	dipeptidyl peptidase-4
DVT	deep venous thrombosis
ECF	extracellular fluid
ECG	electrocardiogram/electrocardiography
EDTA	ethylenediamine tetraacetic acid
eGFR	estimated glomerular filtration rate
ERCP	endoscopic retrograde cholangiopancreatography
ESR	erythrocyte sedimentation rate
FAD	flavin–adenine dinucleotide
FAI	free androgen index
FBHH	familial benign hypocalciuric hypercalcaemia
FOB	faecal occult blood
FSH	follicle-stimulating hormone
FT3	free tri-iodothyronine
FT4	free thyroxine
GAD	glutamic acid decarboxylase
Gal-1-PUT	galactose-1-phosphate uridylyl-transferase
GC–MS	gas chromatography–mass spectrometry
GFR	glomerular filtration rate
GGT	γ-glutamyltransferase
GH	growth hormone
GHD	growth hormone deficiency
GHRH	growth hormone-releasing hormone
GI	gastrointestinal
GIP	glucose-dependent insulinotrophic peptide
GLP-1	glucagon-like polypeptide-1
GnRH	gonadotrophin-releasing hormone
GP	general practitioner
GSA	glucocorticoid-suppressible hyperaldosteronism
GTT	glucose tolerance test

Hb	haemoglobin
HC	hereditary coproporphyria
HCC	hydroxycholecalciferol
hCG	human chorionic gonadotrophin
HDL	high-density lipoprotein
HDU	high dependency unit
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
5-HIAA	5-hydroxyindoleacetic acid
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HMG-CoA	β-hydroxy-β-methylglutaryl-coenzyme A
HNF	hepatic nuclear factor
HPA	hypothalamic–pituitary–adrenal
HPLC	high-performance liquid chromatography
HRT	hormone replacement therapy
hsCRP	high sensitive C-reactive protein
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
ICF	intracellular fluid
ICU	intensive care unit
IDL	intermediate-density lipoprotein
IFCC	International Federation for Clinical Chemistry
IFG	impaired fasting glycaemia
Ig	immunoglobulin
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor-binding protein
IGT	impaired glucose tolerance
IM	intramuscular
INR	international normalised ratio
IV	intravenous
LCAT	lecithin cholesterol acyltransferase
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LH	luteinising hormone
LHRH	luteinising hormone-releasing hormone
Lp (a)	lipoprotein (a)
LSD	lysergic acid diethylamide
MCAD	medium chain acyl-CoA dehydrogenase
MCV	mean cell volume
MDRD	Modification of Diet in Renal Disease
MEGX	monoethylglycinexylidide
MEN	multiple endocrine neoplasia
MGUS	monoclonal gammopathy of unknown significance
MIH	Mullerian inhibitory hormone
MIT	mono-iodotyrosine
MODY	maturity onset diabetes of the young
MOM	multiples of the median
MRI	magnetic resonance imaging
MSAFP	maternal serum α-fetoprotein
NAD	nicotinamide–adenine dinucleotide
NADP	NAD phosphate
NAFLD	nonalcoholic fatty liver disase
NASH	nonalcoholic steatohepatitis
NICE	National Institute for Health and Clinical Excellence
NTD	neural tube defect
NTI	nonthyroidal illness
OGTT	oral glucose tolerance test
PAPP-A	pregnancy-associated plasma protein A
PBG	porphobilinogen
PCOS	polycystic ovarian syndrome
PCT	porphyria cutanea tarda
PE	pulmonary embolism
PEM	protein-energy malnutrition
PKU	phenylketonuria
POCT	point of care testing
POP	progestogen-only pill
PP	pyridoxal phosphate
PRA	plasma renin activity
PRPP	5-phosphoribosyl-1-pyrophosphate
PSA	prostate-specific antigen
PT	prothrombin time
PTC	percutaneous transhepatic cholangiography
PTH	parathyroid hormone
PTHrP	PTH-related protein
RDA	recommended dietary allowance
RF	rheumatoid factor
ROC	receiver operating characteristic
SAH	subarachnoid haemorrhage
SD	standard deviation
SHBG	sex hormone-binding globulin
SI	Système International
SIADH	inappropriate secretion of ADH
SUR	sulphonylurea receptor
T3	tri-iodothyronine

T4	thyroxine
TBG	thyroxine-binding globulin
TDM	therapeutic drug monitoring
TIBC	total iron-binding capacity
TPMT	thiopurine methyltransferase
TPN	total parenteral nutrition
TPOAb	thyroid peroxidase antibody
TPP	thiamin pyrophosphate
TRAb	thyrotrophin receptor antibody
TRH	thyrotrophin-releasing hormone
TSH	thyroid-stimulating hormone
TSI	thyroid-stimulating immunoglobulin
tTG	tissue transglutaminase
U&Es	urea and electrolytes
UFC	urinary free cortisol
VIP	vasoactive intestinal peptide
VLDL	very low density lipoprotein
VMA	vanillylmandelic acid
VP	variegate porphyria
WHO	World Health Organization

How to use your textbook

Features contained within your textbook

‘Learning outcomes’ give a quick introduction to the topics covered in a chapter.

Learning objectives

To understand:

- ✓ how sample handling, analytical and biological factors can affect test results in health and disease and how these relate to the concept of a test reference range.
- ✓ the concepts of accuracy, precision, test sensitivity, test specificity in the quantitative assessment of test performance.

‘Case studies’ give further insight into specific conditions and topics.

CASE 1.4

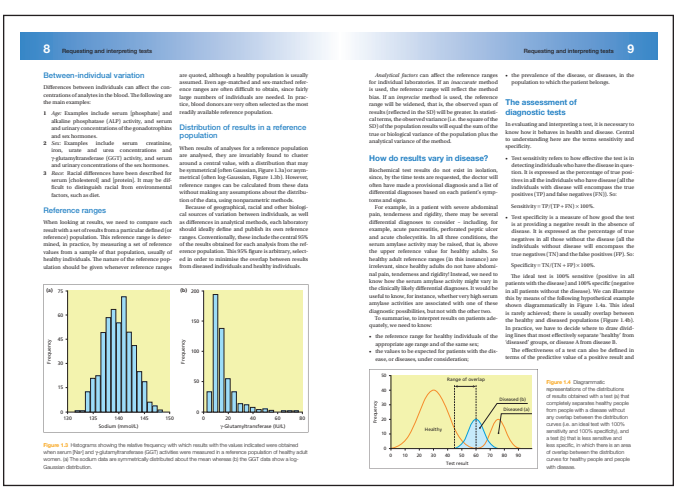
The following set of results was obtained on a young man admitted with a fractured femur after a motorcycle accident. He appeared stable and had no previous past medical history of note. The houseman was at a loss to explain the results but remembered that he had topped up the sample shortfall in the Biochemistry tube from the haematology full blood count tube. Can you account for the results?

Serum	Result	Reference range
Urea	6.4	2.5–6.6 mmol/L
Sodium	138	135–145 mmol/L
Potassium	16.1	3.6–5.0 mmol/L
Total CO_2	32	22–30 mmol/L

Serum	Result	Reference range
Bilirubin	14	3–16 μmol/L
ALT	40	10–50 U/L
ALP	38	40–125 U/L
Total protein	75	60–80 g/L
Calcium	0.6	2.1–2.6 mmol/L
Albumin	32	35–50 g/L

Comments: This particular case illustrates the importance of using the correct blood sample tube. In transferring some of the blood from the Haematology tube to the Biochemistry tube, the doctor had not appreciated that the anti-coagulant in the Haematology (pink) tube was potassium EDTA. This explains the high potassium and the low calcium since the EDTA chelates the calcium, leading to a low result on analysis.

Your textbook is full of photographs, illustrations and tables.



Between-individual variation

Differences between individuals can affect the concentrations of analytes in the blood. The following are the main examples:

1 *Age:* Examples include serum [phosphate] and alkaline phosphatase (ALP) activity, and serum and urinary concentrations of the gonadotrophins and sex hormones.
2 *Sex:* Examples include serum creatinine, iron, urate and urea concentrations and γ-glutamyltransferase (GGT) activity, and serum and urinary concentrations of the sex hormones.
3 *Race:* Racial differences have been described for serum [cholesterol] and [protein]. It may be difficult to distinguish racial from environmental factors, such as diet.

Reference ranges

When looking at results, we need to compare each result with a set of results from a particular defined (or reference) population. This reference range is determined, in practice, by measuring a set of reference values from a sample of that population, usually of healthy individuals. The nature of the reference population should be given whenever reference ranges are quoted, although a healthy population is usually assumed. Even age-matched and sex-matched reference ranges are often difficult to obtain, since fairly large numbers of individuals are needed. In practice, blood donors are very often selected as the most readily available reference population.

Distribution of results in a reference population

When results of analyses for a reference population are analysed, they are invariably found to cluster around a central value, with a distribution that may be symmetrical (often Gaussian, Figure 1.3a) or asymmetrical (often log-Gaussian, Figure 1.3b). However, reference ranges can be calculated from these data without making any assumptions about the distribution of the data, using nonparametric methods.

Because of geographical, racial and other biological sources of variation between individuals, as well as differences in analytical methods, each laboratory should ideally define and publish its own reference ranges. Conventionally, these include the central 95% of the results obtained for each analysis from the reference population. This 95% figure is arbitrary, selected in order to minimise the overlap between results from diseased individuals and healthy individuals.

Figure 1.3 Histograms showing the relative frequency with which results with the values indicated were obtained when serum [Na+] and γ-glutamyltransferase (GGT) activities were measured in a reference population of healthy adult women. (a) The sodium data are symmetrically distributed about the mean whereas (b) the GGT data show a log-Gaussian distribution.

Analytical factors can affect the reference ranges for individual laboratories. If an inaccurate method is used, the reference range will reflect the method bias. If an imprecise method is used, the reference range will be widened, that is, the observed span of results (reflected in the SD) will be greater. In statistical terms, the observed variance (i.e. the square of the SD) of the population results will equal the sum of the true or biological variance of the population plus the analytical variance of the method.

How do results vary in disease?

Biochemical test results do not exist in isolation, since, by the time tests are requested, the doctor will often have made a provisional diagnosis and a list of differential diagnoses based on each patient's symptoms and signs.

For example, in a patient with severe abdominal pain, tenderness and rigidity, there may be several differential diagnoses to consider – including, for example, acute pancreatitis, perforated peptic ulcer and acute cholecystitis. In all three conditions, the serum amylase activity may be raised, that is, above the upper reference value for healthy adults. So healthy adult reference ranges (in this instance) are irrelevant, since healthy adults do not have abdominal pain, tenderness and rigidity! Instead, we need to know how the serum amylase activity might vary in the clinically likely differential diagnoses. It would be useful to know, for instance, whether very high serum amylase activities are associated with one of these diagnostic possibilities, but not with the other two.

To summarise, to interpret results on patients adequately, we need to know:

- the reference range for healthy individuals of the appropriate age range and of the same sex;
- the values to be expected for patients with the disease, or diseases, under consideration;
- the prevalence of the disease, or diseases, in the population to which the patient belongs.

The assessment of diagnostic tests

In evaluating and interpreting a test, it is necessary to know how it behaves in health and disease. Central to understanding here are the terms sensitivity and specificity.

- Test sensitivity refers to how effective the test is in detecting individuals who have the disease in question. It is expressed as the percentage of true positives in all the individuals who have disease (all the individuals with disease will encompass the true positives (TP) and false negatives (FN)). So:

  Sensitivity = TP/(TP + FN) × 100%.

- Test specificity is a measure of how good the test is at providing a negative result in the absence of disease. It is expressed as the percentage of true negatives in all those without the disease (all the individuals without disease will encompass the true negatives (TN) and the false positives (FP). So:

  Specificity = TN/(TN + FP) × 100%.

The ideal test is 100% sensitive (positive in all patients with the disease) and 100% specific (negative in all patients without the disease). We can illustrate this by means of the following hypothetical example shown diagrammatically in Figure 1.4a. This ideal is rarely achieved; there is usually overlap between the healthy and diseased populations (Figure 1.4b). In practice, we have to decide where to draw dividing lines that most effectively separate 'healthy' from 'diseased' groups, or disease A from disease B.

The effectiveness of a test can also be defined in terms of the predictive value of a positive result and

Figure 1.4 Diagrammatic representations of the distributions of results obtained with a test (a) that completely separates healthy people from people with a disease without any overlap between the distribution curves (i.e. an ideal test with 100% sensitivity and 100% specificity), and a test (b) that is less sensitive and less specific, in which there is an area of overlap between the distribution curves for healthy people and people with disease.

About the companion website

This book is accompanied by a companion website:

www.lecturenoteseries.com/clinicalbiochemistry

The website includes:

- Interactive multiple-choice questions
- Key revision points for each chapter

1

Requesting and interpreting tests

Learning objectives

To understand:

- ✓ how sample handling, analytical and biological factors can affect test results in health and disease and how these relate to the concept of a test reference range;
- ✓ the concepts of accuracy, precision, test sensitivity, test specificity in the quantitative assessment of test performance.

Introduction

Biochemical tests are crucial to modern medicine. Most biochemical tests are carried out on blood using plasma or serum, but urine, cerebrospinal fluid (CSF), faeces, kidney stones, pleural fluid, etc. are sometimes required. Plasma is obtained by collecting blood into an anticoagulant and separating the fluid, plasma phase from the blood cells by centrifugation. Serum is the corresponding fluid phase when blood is allowed to clot. For many (but not all) biochemical tests on blood, it makes little difference whether plasma or serum is used.

There are many hundreds of tests available in clinical biochemistry but a core of common tests makes up the majority of tests requested in clinical biochemistry. These core tests are typically available over a 24 h period. Tests are sometimes brought together in profiles, especially when a group of tests provides better understanding of a problem than a single test (e.g. the liver function test profile). Many of the other more specialist tests are restricted to larger laboratories or specialist centres offering a national or regional service.

In dealing with the large number of routine test requests, the modern clinical biochemistry laboratory depends heavily on automated instrumentation linked to a laboratory computing system. Test results are assigned to electronic patient files that allow maintenance of a cumulative patient record. Increasingly, test requests can be electronically booked at the ward, clinic or in General Practice via a terminal linked to the main laboratory computer. Equally, the test results can be displayed on computer screens at distant locations, even negating the need for issuing printed reports.

In this first chapter, we set out some of the principles of requesting tests and of the interpretation of results. The effects of analytical errors and of physiological factors, as well as of disease, on test results are stressed. Biochemical testing in differential diagnosis and in screening is discussed.

Collection of specimens

Test requests require unambiguous identification of the patient (patient's name, sex, date of birth and, increasingly, a unique patient identification number), together with the location, the name of the requesting doctor and the date and time of sampling. Each test request must specify the analyses requested and provide details of the nature of the specimen itself and relevant clinical diagnostic information. This may be

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Table 1.1 Some more common causes of pre-analytical errors arising from use of the laboratory.

Error	Consequence
Crossover of addressograph labels between patients	This can lead to two patients each with the other's set of results. Where the patient is assigned a completely wrong set of results, it is important to investigate the problem in case there is a second patient with a corresponding wrong set of results.
Timing error	There are many examples where timing is important but not considered. Sending in a blood sample too early after the administration of a drug can lead to misleadingly high values in therapeutic monitoring. Interpretation of some tests (e.g. cortisol) is critically dependent on the time of day when the blood was sampled.
Sample collection tube error	For some tests the nature of the collection tube is critical, which is why the Biochemistry Laboratory specifies this detail. For example, using a plasma tube with lithium–heparin as the anti-coagulant invalidates this sample tube for measurement of a therapeutic lithium level! Electrophoresis requires a serum sample; otherwise, the fibrinogen interferes with the detection of any monoclonal bands. Topping up a biochemistry tube with a haematology (potassium ethylenediamine tetraacetic acid (EDTA) sample) will lead to high potassium and low calcium values in the biochemistry sample.
Sample taken from close to the site of an intravenous (IV) infusion	The blood sample will be diluted so that all the tests will be correspondingly low with the exception of those tests that might reflect the composition of the infusion fluid itself. For example, using normal saline as the infusing fluid would lead to a lowering of all test results, but with sodium and chloride results that are likely to be raised.

through a traditional request form and labelled specimen or be provided electronically in which case only the sample itself need be sent to the laboratory with its own unique identifier (typically a bar code which links it to the electronic request).

Because of the large number of samples that are processed by most clinical biochemistry laboratories, every step needs to be taken to avoid errors. Regrettably, errors do rarely occur and these can be divided according to the error source:

- Pre-analytical. These arise prior to the actual test measurement and can happen at the clinical or laboratory end. Most errors fall into this category (see Table 1.1).
- Analytical. Laboratory based analytical errors are rare but may occur e.g. reagent contamination, pipetting errors related to small sample volumes, computing errors.
- Post-analytical. These are increasingly rare because of electronic download of results from the analyser but include, for example, transcription errors when entering results from another laboratory into the computer manually; results misheard when these are telephoned to the clinician.

On the scale of the requesting of biochemical tests, errors are fortunately rare. However, occasional blunders do arise and, if very unexpected results are obtained, it is incumbent on the requesting doctor to contact the laboratory immediately to look into the possibility that a blunder may have occurred.

CASE 1.1

A new test is marketed which claims to diagnose heart failure. The test has a specificity of 70% and a sensitivity of 95% at the manufacturer's recommended cut-off for diagnosis. The Admissions Unit decides to use the test as part of an admission profile on breathless patients admitted for further assessment over the age of 65 years in order to exclude heart failure. Assuming a prevalence of 20% for heart failure in this population, calculate how many false negatives would be recorded after the first 1000 patients meeting the testing criteria had passed through the unit. Given that other tests can be used to establish a diagnosis of heart failure, do you think that the cut-off selected is sensible? (Prevalence figures are for illustrative purposes only.)

Comment: This is best examined by constructing a table as follows:

	Positive results	Negative results	Totals
Heart failure present	190 TP	10 FN	200
Heart failure absent	240 FP	560 TN	800
Total	430	570	1000

Because the test has a relatively high sensitivity, the table shows that it identifies the majority of patients with heart failure which is what is required in a test to rule out heart failure. Because the test lacks specificity, it can also be seen from the table that it identifies a considerable number of patients with positive results who do not have heart failure. In fact, the test is positive on more occasions in patients who do not have heart failure than in those with heart failure. Because other tests are available to the clinician, the false-positive patients can be separated from the true-positive patients on the basis of these further investigations. The 560 patients where the result is a true negative would then not need to go through more expensive further investigations. In this example, the test has been valuable in ruling out patients who would not require further investigation but ruling in those who would benefit. Clearly, it is not a perfect test but would potentially prevent costly further investigations in a significant number of patients and, provided that the test itself is not too expensive, ultimately be worthy of consideration in terms of health economics.

The use of clinical biochemistry tests

Biochemical tests are most often *discretionary*, meaning that the test is requested for defined diagnostic purposes. Tests may also be requested to screen for a disease, without there being any specific indication of its presence in the individual, or to assess the risk of a particular disease or disease prognosis in the individual. The justification for discretionary testing is well summarised by Asher (1954):

1. Why do I request this test?
2. What will I look for in the result?
3. If I find what I am looking for, will it affect my diagnosis?
4. How will this investigation affect my management of the patient?
5. Will this investigation ultimately benefit the patient?

Discretionary testing is the more common reason for biochemical tests to be requested. The main reasons for this type of testing are summarised in Table 1.2. Tests may also be used to help evaluate the future risk of disease (e.g. total cholesterol and HDL-cholesterol levels contribute to assessment of an individual's risk of cardiovascular disease) or in disease prognosis (e.g. biochemical tests to asses prognosis in acute pancreatitis or liver failure).

Table 1.2 Test selection for the purposes of discretionary testing.

Category	Example
To confirm a diagnosis	Serum [free T4] and [thyroid-stimulating hormone, (TSH)] in suspected hyperthyroidism
To aid differential diagnosis	To distinguish between different forms of jaundice
To refine a diagnosis	Use of adrenocorticotrophic hormone (ACTH) to localise Cushing's syndrome
To assess the severity of disease	Serum [creatinine] or [urea] in renal disease
To monitor progress	Plasma [glucose] and serum [K^+] to follow treatment of patients with diabetic ketoacidosis (DKA)
To detect complications or side effects	Alanine aminotransferase (ALT) measurements in patients treated with hepatotoxic drugs
To monitor therapy	Serum drug concentrations in patients treated with anti-epileptic drugs

Screening may take several forms:

- In well-population screening a spectrum of tests is carried out on individuals from an apparently healthy population in an attempt to detect

Table 1.3 Requirements for well-population screening.

The disease is common or life-threatening
The tests are sensitive and specific
The tests are readily applied and acceptable to the population to be screened
Clinical, laboratory and other facilities are available for follow-up
Economics of screening have been clarified and the implications accepted

pre-symptomatic or early disease. It is easy to miss significant abnormalities in the 'flood' of data coming from the laboratory, even when the abnormalities are 'flagged' in some way. For these and other reasons, the value of well-population screening has been called into question and certainly should only be initiated under certain specific circumstances that are listed in Table 1.3.

- In case-finding screening programmes appropriate tests are carried out on a population sample known to be at high risk of a particular disease. These are inherently more selective and yield a higher proportion of useful results (Table 1.4).

Table 1.4 Examples of tests used in case-finding programmes.

Programmes to detect diseases in	Chemical investigations
Neonates	
PKU	Serum [phenylalanine]
Hypothyroidism	Serum [TSH]
Adolescents and young adults	
Substance abuse	Drug screen
Pregnancy	
Diabetes mellitus in the mother	Plasma and urine [glucose]
Open neural tube defect (NTD) in the foetus	Maternal serum [α-fetoprotein]
Industry	
Industrial exposure to lead	Blood [lead]
Industrial exposure to pesticides	Serum cholinesterase activity
Elderly	
Malnutrition	Serum vitamin D levels
Thyroid dysfunction	Serum [TSH] and [thyroxine]

Point of care testing (POCT) (Table 1.5)

These are tests conducted close to the patient in the emergency department or an outpatient or general practitioner surgery, for example. The instrumentation used is typically small and fits on a desk or may even be handheld. This approach can be helpful where there is a need to obtain a result quickly (e.g. blood gas results in the emergency department in a breathless patient) or where a result can be used to make a real-time clinical management decision (e.g. whether to adjust someone's statin dose on the basis of a cholesterol result). A further attraction is the immediate feedback of clinical information to the patient. POCT can be used to monitor illness by the individual patient and help identify if a change in treatment is needed (e.g. blood glucose monitoring in a diabetic patient).The UK government, in outlining the future of the National Health Service, has indicated a desire to move laboratory testing from the hospital laboratory into the community setting. High street pharmacies have also taken up these opportunities. There is also an increasing number of urine test sticks that are sold for home use (e.g. pregnancy and ovulation testing by measuring human chorionic gonadotrophin (hCG) and luteinising hormone (LH), respectively). Table 1.5 shows examples of POCT tests in common use.

The introduction of POCT methodology requires attention to cost, ease of use, staff training, quality, health and safety as well as need. The advantages and disadvantages of POCT are summarised in Table 1.6.

Table 1.5 Examples of POCT that are in common use.

Common POCT in blood	Common POCT in urine
Blood gases	Glucose
Glucose	Ketones
Urea and creatinine	Red cells/haemoglobin
Na, K and Ca	Bilirubin
Bilirubin	Urobilinogen
Salicylate	pH
Paracetamol	Protein
Alcohol	hCG
Troponin	Drugs of abuse

Table 1.6 Advantages and Disadvantages of Point-of-Care Testing (POCT).

Advantages	Disadvantages
Rapid results on acutely ill patients	More expensive than centralised tests
Allows more frequent monitoring	Wide staff training may be needed
Immediate patient feedback	Nontrained users may have access with potential for errors
Available 24h if required	Calibration and quality control may be less robust
	Health and Safety may be less well monitored
	Results less often integrated into patient electronic record

Interpretation of clinical biochemistry tests

Most reports issued by clinical biochemistry laboratories contain numerical measures of concentration or activity, expressed in the appropriate units. Typically, the result is interpreted in relation to a reference range (see Chapter 1: Reference ranges) for the analyte in question.

This section discusses the interpretation of laboratory results and the factors that may cause them to vary, under the following main headings:

1 *Analytical factors* These cause errors in measurement.
2 *Biological and pathological factors* Both these sets of factors affect the concentrations of analytes in blood, urine and other fluids sent for analysis.

Sources of variation in test results

Analytical sources of variation

Systematic and random variation

Analytical results are subject to error, no matter how good the laboratory and no matter how skilled the analyst. These errors may be due to lack of accuracy, that is, always tend to be either high or low, or may be due to random effects and lack precision, that is, may be unpredictably high or low.

Accuracy

An accurate method will, on average, yield results close to the true value of what is being measured. It has no systematic bias.

Precision

A precise method yields results that are close to one another (but not necessarily close to the true value) on repeated analysis. If multiple measurements are made on one specimen, the spread of results will be small for a precise method and large for an imprecise one.

The 'dartboard' analogy is often used to illustrate the different meanings of the terms accuracy and precision, and this is illustrated in Figure 1.1.

The standard deviation (SD) is the usual measure of scatter around a mean value. If the spread of results is wide, the SD is large, whereas if the spread is narrow, the SD is small. For data that have a Gaussian distribution, as is nearly always the case for analytical errors, the shape of the curve (Figure 1.2) is completely defined by the mean and the SD, and these characteristics are such that:

- About 67% of results lie in the range mean ± 1 SD.
- About 95% of results lie in the range mean ± 2 SD.
- Over 99% of results lie in the range mean ± 3 SD.

Blunders

These are grossly inaccurate results that bear no constant or predictable relationship to the true value. They arise, for instance, from mislabelling of specimens at the time of collection, or transcription errors when preparing or issuing reports (see Table 1.1).

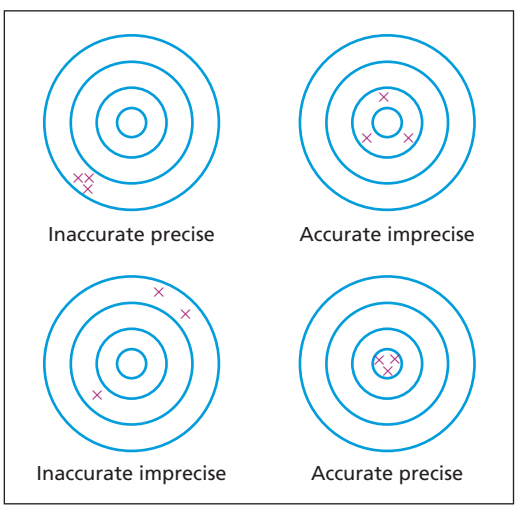

Figure 1.1 The 'dartboard' analogy can be used to illustrate accuracy and precision.

CASE 1.2

A 72-year-old man is admitted vaguely unwell with some nausea and associated vomiting, though not severe. He appears rather pale and wasted with a low blood pressure. He is on treatment with digoxin for his atrial fibrillation and the suspicion arises that his symptoms may arise from digoxin toxicity. This would also help explain the raised potassium result for which there is no other clear cause. The most recent digoxin dose had been taken just before his admission to the hospital. The house officer telephones to request an additional digoxin measurement on the admission sample and this is reported back as raised. On this basis, the digoxin is withheld and his condition monitored. Little improvement is noted and the nausea becomes worse, accompanied by a worsening of his atrial fibrillation. Further advice is sought.

Comment on this case with particular reference to the raised digoxin and the worsening of his atrial fibrillation.

Comment: The timing of a blood test is crucial to the interpretation of a number of drugs whose concentration in blood is monitored for therapeutic purposes. This is most certainly the case with digoxin where the blood sample should *not* be taken within 6 h of the most recent digoxin dose. The House Officer has requested digoxin as an additional test on the patient's admission sample, without reference to the exact time when the patient took his dose of digoxin prior to admission. In fact, the time elapsed between taking the drug and the blood sample was about 1 h. The raised digoxin concentration is uninterpretable and it may well be that the patient has digoxin levels within the therapeutic range or even on the low side. This turned out to be the case, explaining the worsening in his condition when the drug was inappropriately withheld.

An isolated raised potassium result can be a very important finding which reflects underlying pathology such as renal disease, DKA, etc. Although there was no immediate explanation for this man's raised potassium, it became evident what the problem was when the full blood count report was received. This showed a very high lymphocyte count consistent with chronic lymphocytic leukaemia. In this condition, the white cells are fragile and can lyse on blood sampling. With the high white cell count, it is then possible to measure a spuriously high potassium level in the corresponding biochemistry sample.

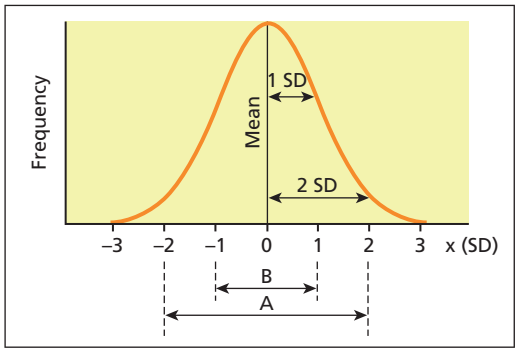

Figure 1.2 Diagram of a Gaussian (normal or symmetrical) distribution curve. The span (A) of the curve, the distance between the mean ± 2 SD, includes about 95% of the 'population'. The narrower span (B), the distance between the mean ± 1 SD, includes about 67% of the 'population'.

Serial results in the same patient

Doctors often have to interpret two or more sets of results for the same analysis or group of analyses performed on different occasions on the same patient. An important question is whether an analytical change is due mainly to laboratory imprecision or to a true change in the patient's clinical condition. Without elaborating on the statistical aspects of this, the following rule may be applied: if the results for analyses performed on specimens collected on different occasions, but under otherwise identical conditions, differ by more than 2.8 times the analytical SD then there is a chance of over 95% that a genuine change in concentration of the substance has occurred.

Biological causes of variation

As well as analytical variation, test results also show biological variation in both health and disease. Key questions are:

- How do results vary in health?
- How do results vary in disease?

How do results vary in health?

The concentrations of all analytes in blood vary with time due to diverse physiological factors *within* the individual. There are also differences *between* individuals.

Within-individual variation

The following may be important causes of within-individual variation:

1. *Diet:* Variations in diet can affect the results of many tests, including serum [triglyceride], the response to glucose tolerance tests and urinary calcium excretion.
2. *Time of day:* Several plasma constituents show diurnal variation (variation with the time of day), or a sleep/wake cycle. Examples include iron, adrenocorticotrophic hormone (ACTH) and cortisol concentrations.
3. *Posture:* Proteins and all protein-bound constituents of plasma show significant differences in concentration between blood collected from upright individuals and blood from recumbent individuals. Examples include serum calcium, cholesterol, cortisol and total thyroxine concentrations.
4. *Muscular exercise:* Recent exercise, especially if vigorous or unaccustomed, may increase serum creatine kinase (CK) activity and blood [lactate], and lower blood [pyruvate].
5. *Menstrual cycle:* Several substances show variation with the phase of the cycle. Examples include serum [iron], and the serum concentrations of the pituitary gonadotrophins, ovarian steroids and their metabolites, as well as the amounts of these hormones and their metabolites excreted in the urine.
6. *Drugs:* These can have marked effects on chemical results. Attention should be drawn particularly to the many effects of oestrogen-containing oral contraceptives on serum constituents (Chapter 10: Steroid contraceptives).

Even after allowing for known physiological factors that may affect plasma constituents and for analytical imprecision, there is still considerable residual individual variation (Table 1.7). The magnitude of this variation depends on the analyte, but it may be large and must be taken into account when interpreting successive values from a patient.

CASE 1.3

The following results were obtained on a 54-year-old woman after surgery for ovarian cancer. Can you account for the abnormalities found?

Serum	Result	Reference range
Urea	2.0	2.5–6.6 mmol/L
Sodium	147	135–145 mmol/L
Potassium	2.0	3.6–5.0 mmol/L
Total CO_2	10.0	22–30 mmol/L
Bilirubin	7.0	3–16 µmol/L
ALT	11.0	10–50 U/L
ALP	35.0	40–125 U/L
Total protein	42.0	60–80 g/L
Calcium	1.6	2.1–2.6 mmol/L

Comments: Many of these results are abnormal and, with the exception of the sodium result, are abnormally low. In a post-operative patient, a set of results like this should immediately raise the suspicion that the blood sample was taken close to the site of an IV infusion. The fluid infused would dilute the blood at the site of sampling, leading to a consequent lowering of the concentration of most of the analytes measured. If the IV infusion was normal saline, this would then account for the fact that only the sodium value is high while all the other values are low. When the Duty Biochemist contacted the House Officer on the ward, he did admit that he had had difficulty taking a blood sample from the patient and did recollect that he sampled from close to the site of the IV infusion. A repeat blood sample was requested from a site away from the infusion and confirmed the original error since all the results were within the reference range, apart from the sodium which was slightly low at 132 mmol/L.

Table 1.7 Residual individual variation of some serum constituents (expressed as the approximated day-to-day, within-individual coefficient of variation). CV = coefficient of variation.

Serum constituent	CV (%)	Serum constituent	CV (%)
Sodium	1	ALT activity	25
Calcium	1–2	AST activity	25
Potassium	5	Iron	25
Urea	10		

Between-individual variation

Differences between individuals can affect the concentrations of analytes in the blood. The following are the main examples:

1. *Age:* Examples include serum [phosphate] and alkaline phosphatase (ALP) activity, and serum and urinary concentrations of the gonadotrophins and sex hormones.
2. *Sex:* Examples include serum creatinine, iron, urate and urea concentrations and γ-glutamyltransferase (GGT) activity, and serum and urinary concentrations of the sex hormones.
3. *Race:* Racial differences have been described for serum [cholesterol] and [protein]. It may be difficult to distinguish racial from environmental factors, such as diet.

Reference ranges

When looking at results, we need to compare each result with a set of results from a particular defined (or reference) population. This reference range is determined, in practice, by measuring a set of reference values from a sample of that population, usually of healthy individuals. The nature of the reference population should be given whenever reference ranges are quoted, although a healthy population is usually assumed. Even age-matched and sex-matched reference ranges are often difficult to obtain, since fairly large numbers of individuals are needed. In practice, blood donors are very often selected as the most readily available reference population.

Distribution of results in a reference population

When results of analyses for a reference population are analysed, they are invariably found to cluster around a central value, with a distribution that may be symmetrical (often Gaussian, Figure 1.3a) or asymmetrical (often log-Gaussian, Figure 1.3b). However, reference ranges can be calculated from these data without making any assumptions about the distribution of the data, using nonparametric methods.

Because of geographical, racial and other biological sources of variation between individuals, as well as differences in analytical methods, each laboratory should ideally define and publish its own reference ranges. Conventionally, these include the central 95% of the results obtained for each analysis from the reference population. This 95% figure is arbitrary, selected in order to minimise the overlap between results from diseased individuals and healthy individuals.

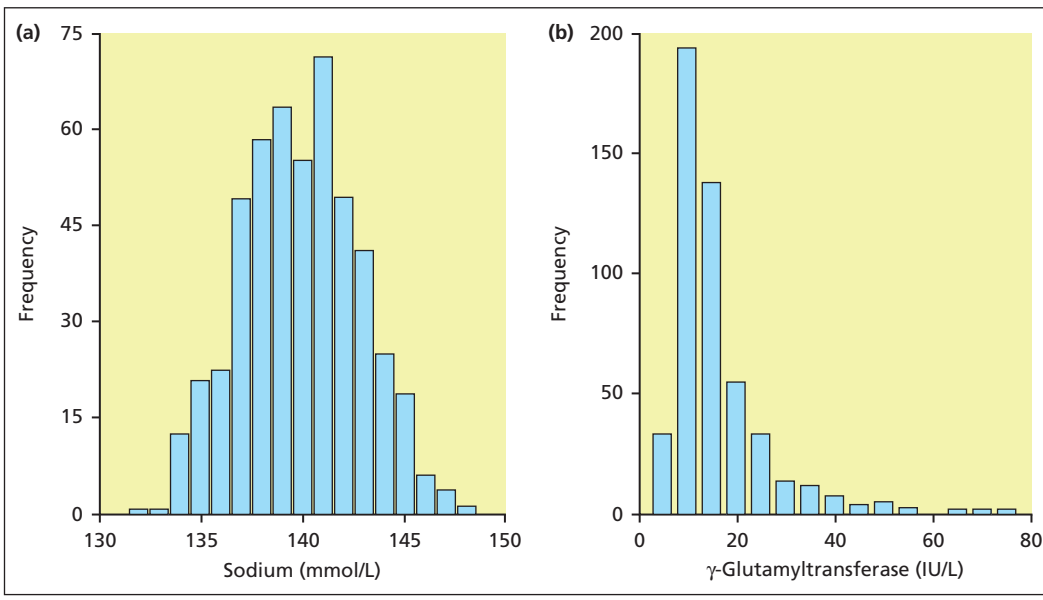

Figure 1.3 Histograms showing the relative frequency with which results with the values indicated were obtained when serum [Na^+] and γ-glutamyltransferase (GGT) activities were measured in a reference population of healthy adult women. (a) The sodium data are symmetrically distributed about the mean whereas (b) the GGT data show a log-Gaussian distribution.

Analytical factors can affect the reference ranges for individual laboratories. If an *inaccurate* method is used, the reference range will reflect the method bias. If an *imprecise* method is used, the reference range will be widened, that is, the observed span of results (reflected in the SD) will be greater. In statistical terms, the observed variance (i.e. the square of the SD) of the population results will equal the sum of the true or biological variance of the population plus the analytical variance of the method.

How do results vary in disease?

Biochemical test results do not exist in isolation, since, by the time tests are requested, the doctor will often have made a provisional diagnosis and a list of differential diagnoses based on each patient's symptoms and signs.

For example, in a patient with severe abdominal pain, tenderness and rigidity, there may be several differential diagnoses to consider – including, for example, acute pancreatitis, perforated peptic ulcer and acute cholecystitis. In all three conditions, the serum amylase activity may be raised, that is, above the upper reference value for healthy adults. So healthy adult reference ranges (in this instance) are irrelevant, since healthy adults do not have abdominal pain, tenderness and rigidity! Instead, we need to know how the serum amylase activity might vary in the clinically likely differential diagnoses. It would be useful to know, for instance, whether very high serum amylase activities are associated with one of these diagnostic possibilities, but not with the other two.

To summarise, to interpret results on patients adequately, we need to know:

- the reference range for healthy individuals of the appropriate age range and of the same sex;
- the values to be expected for patients with the disease, or diseases, under consideration;
- the prevalence of the disease, or diseases, in the population to which the patient belongs.

The assessment of diagnostic tests

In evaluating and interpreting a test, it is necessary to know how it behaves in health and disease. Central to understanding here are the terms sensitivity and specificity.

- Test sensitivity refers to how effective the test is in detecting individuals who have the disease in question. It is expressed as the percentage of true positives in all the individuals who have disease (all the individuals with disease will encompass the true positives (TP) and false negatives (FN)). So:

  $$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN}) \times 100\%.$$

- Test specificity is a measure of how good the test is at providing a negative result in the absence of disease. It is expressed as the percentage of true negatives in all those without the disease (all the individuals without disease will encompass the true negatives (TN) and the false positives (FP). So:

  $$\text{Specificity} = \text{TN}/(\text{TN} + \text{FP}) \times 100\%.$$

The ideal test is 100% sensitive (positive in all patients with the disease) and 100% specific (negative in all patients without the disease). We can illustrate this by means of the following hypothetical example shown diagrammatically in Figure 1.4a. This ideal is rarely achieved; there is usually overlap between the healthy and diseased populations (Figure 1.4b). In practice, we have to decide where to draw dividing lines that most effectively separate 'healthy' from 'diseased' groups, or disease A from disease B.

The effectiveness of a test can also be defined in terms of the predictive value of a positive result and

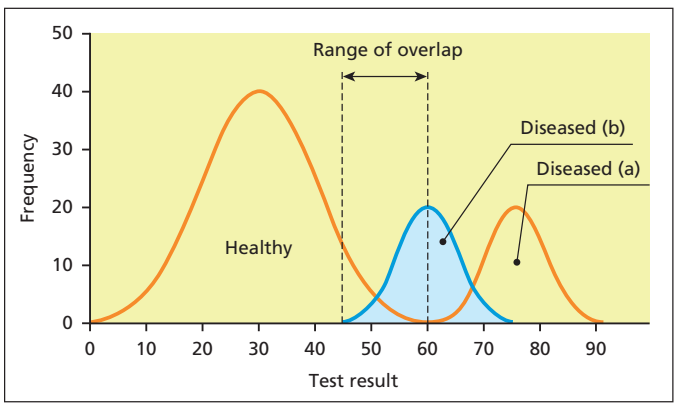

Figure 1.4 Diagrammatic representations of the distributions of results obtained with a test (a) that completely separates healthy people from people with a disease without any overlap between the distribution curves (i.e. an ideal test with 100% sensitivity and 100% specificity), and a test (b) that is less sensitive and less specific, in which there is an area of overlap between the distribution curves for healthy people and people with disease.

the predictive value of a negative result. The positive predictive value is:

$$TP/(TP + FP) \times 100\%.$$

A test with a high positive predictive value will, by definition, have few false positives. This would be important in a situation where a high number of false positives would otherwise lead to extensive and costly further investigation.

The negative predictive value is defined as follows:

$$TN/(TN + FN) \times 100\%.$$

A test with a high negative predictive value would, by definition, have few false negatives. This would be particularly important, for example, in a test which was used for a screening programme where it is essential not to miss a case of the disease in question.

In defining the presence or absence of a disease, a cut-off may be assigned to a test. Consider the situation where a high value for a particular test equates with the presence of a particular disease. A value above the cut-off would then define the presence of the disease and a value below the cut-off, the absence of disease. A cut-off which is set at a higher level will increase the test specificity at the expense of test sensitivity (more false negatives), whilst a cut-off set at a lower value will increase test sensitivity at the expenses of test specificity (more false positives).

In evaluating tests for decision making, it is clearly important to decide on the relative importance of sensitivity versus specificity in the context for which a test is used. To that end, it is helpful to be able to make comparisons of different tests with respect to sensitivity and specificity. This is often best carried out by plotting the test sensitivity against specificity and constructing a so-called receiver operating characteristic (ROC) curve. These curves will highlight which test is best suited to which requirement and will also help to define which cut-off to select in order to balance specificity versus sensitivity. This is illustrated in Figure 1.5.

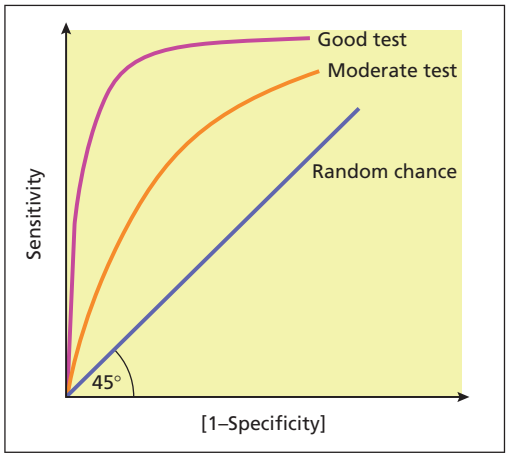

Figure 1.5 Schematic representation of a receiver operating characteristic (ROC) plot. A random test produces a straight line set at 45° to the axes. A discriminatory, good test produces a graph with a steep slope from the origin, displaying high sensitivity at high specificity. Less discriminatory tests produce curves at intermediate positions, as shown. (Adapted from: Roulston, J.E. and Leonard, R.F.C. (1993). *Serological Tumour Markers: An Introduction*. Reproduced with permission from Elsevier.)

In screening for diseases that are rare (e.g. phenylketonuria in neonates) tests of very high sensitivity and specificity are required. For readers who wish to read further this is covered in Appendix 1.1.

CASE 1.4

The following set of results was obtained on a young man admitted with a fractured femur after a motorcycle accident. He appeared stable and had no previous past medical history of note. The houseman was at a loss to explain the results but remembered that he had topped up the sample shortfall in the Biochemistry tube from the haematology full blood count tube. Can you account for the results?

Serum	Result	Reference range
Urea	6.4	2.5–6.6 mmol/L
Sodium	138	135–145 mmol/L
Potassium	16.1	3.6–5.0 mmol/L
Total CO_2	32	22–30 mmol/L
Bilirubin	14	3–16 μmol/L
ALT	40	10–50 U/L
ALP	38	40–125 U/L
Total protein	75	60–80 g/L
Calcium	0.6	2.1–2.6 mmol/L
Albumin	32	35–50 g/L

Comments: This particular case illustrates the importance of using the correct blood sample tube. In transferring some of the blood from the Haematology tube to the Biochemistry tube, the doctor had not appreciated that the anti-coagulant in the Haematology (pink) tube was potassium EDTA. This explains the high potassium and the low calcium since the EDTA chelates the calcium, leading to a low result on analysis.

Audit in clinical biochemistry

Audit is the process whereby the procedures involved in patient care are monitored in order to give high priority to the delivery of an efficient and cost-effective service. The measure of health outcome is benefit to the patient.

The value of audit can most readily be seen in those specialties concerned directly with patient care, but the principles are applicable to all clinical and investigational specialties (e.g. radiology), as well as laboratory-based specialties such as clinical biochemistry. For example, the monitoring of laboratory performance may identify that reports are arriving too late and too often at the wrong location. This would precipitate a review of the form printing and delivery process, implementation of a change in the arrangements and a re-monitoring of the delivery process to ensure that the original problem had been overcome.

The audit process

There is an essential sequence to auditing activities (Figure 1.6):

1. Identify an area of concern or interest, particularly if it is felt that there is room for improvement in the service, or if the same quality of service can be provided more economically.
2. Review and analyse the present procedures.
3. Identify specific aspects that might be capable of improvement.
4. Identify alternative procedures or standards that might lead to improvement.
5. Take the practical steps necessary to implement any changes proposed.
6. Compare the performance after the changes with those before them.

It must be emphasised that the final stage of analysis of the effects of any change is an integral part of the audit process; it is essential to know whether the measures taken have improved the service or made it more cost-effective. Sometimes, changes have no effect, or even adverse effects.

FURTHER READING

Asher, R. (1954) Straight and crooked thinking in medicine. *British Medical Journal* **2**: 460–2.

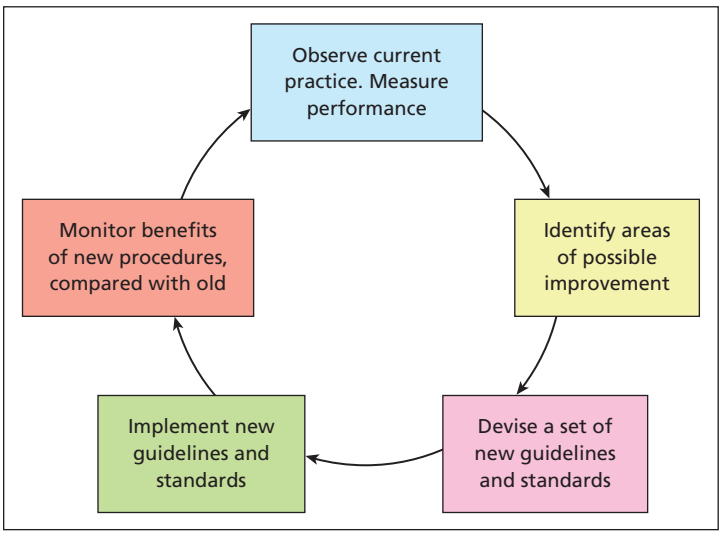

Figure 1.6 The audit cycle.

Appendix 1.1: Screening for rare diseases

For diseases that are rare, tests of extremely high sensitivity and specificity are required. To illustrate this, consider an inherited metabolic disorder with an incidence of 1:5000; this is similar to that of some of the more common, treatable, inherited metabolic diseases such as phenylketonuria (PKU) or congenital hypothyroidism. Assume that we have a test with a good performance, that is, a sensitivity and specificity each of 99.5% (Table 1.8).

Table 1.8 shows that for every neonate affected by the disorder who has a positive test result, there will be about 25 (4999/199) neonates who also have a positive test but who do not have the disease. Two important points emerge:

1. Tests with very high sensitivity and with very low false-positive rates are required when screening for rare disorders.
2. A heavy investigative load will result from the screening programme, since all the false positives will have to be followed up to determine whether or not they indicate the presence of disease.

The traditional 95% reference range (see above) is not relevant to screening for rare conditions, since the rate of false positives would be far too high. The cut-off value has to be altered to decrease the false-positive rate, at the probable expense of missing some patients who have the condition for which screening is being carried out.

Table 1.8 A hypothetical set of results of a screening test for a relatively common inherited metabolic disorder in neonates.

Diagnostic category	Positive results	Negative results	Total
Disease present	199	1	200
Disease absent	4999	994,801	999,800
Total	5198	994,802	1,000,000
Predictive value	3.8%	100%	

Assumptions: sensitivity of the test 99.5%, false-positive rate 0.5% (specificity 99.5%), prevalence of the disorder, 1:5000; 1 000 000 neonates screened.

Note that the prevalence of PKA and of hypothyroidism in the UK is about 1:5000 live births, and that about 800 000 neonates in the UK are screened annually.

2

Disturbances of water, sodium and potassium balance

Learning objectives

To understand:

- ✓ the distribution of water, Na^+ and K^+ in the different fluid compartments of the body, and their control by hormonal and other factors;
- ✓ the clinical effects and management of different types of loss, retention or redistribution of fluid;
- ✓ the causes of hypernatraemia, hyponatraemia, hyperkalaemia and hypokalaemia, and what further investigations might be useful.

Introduction

Fluid loss, retention or redistribution are common clinical problems in many areas of clinical practice. The management of these conditions is often urgent, and requires a rapid assessment of the history and examination, and of biochemical and other investigations. Both the internal and external balance of any substance must be considered. The internal balance is the distribution between different body compartments, while the external balance matches input with output.

Water and sodium balance

The movements of Na^+ and water that occur all the time between plasma and glomerular filtrate, or between plasma and gastrointestinal (GI) secretions, provide the potential for large losses, with consequent serious and rapid alterations in internal balance. For example, about 25 000 mmol of Na^+ are filtered at the glomerulus over 24 h, normally with subsequent reabsorption of more than 99%. Likewise, 1000 of mmol Na^+ enter the GI tract in various secretions each day, but less than 0.5% (5 mmol) is normally lost in the faeces.

Internal distribution of water and sodium

In a 70 kg adult, the total body water is about 42 L comprising about 28 L of intracellular fluid (ICF) and 14 L of extracellular fluid (ECF) water. The ECF water is distributed as 3 L of plasma water and 11 L of interstitial water. The total body Na^+ is about 4200 mmol and is mainly extracellular – about 50% is in the ECF, 40% in bone and 10% in the ICF.

Clinical Biochemistry Lecture Notes, Ninth Edition. S. Walker, G. Beckett, P. Rae and P. Ashby. Published 2013 by John Wiley & Sons, Ltd.

Two important factors influence the distribution of fluid between the ICF and the intravascular and extravascular compartments of the ECF:

- *Osmolality:* This affects the movement of water across cell membranes.
- *Colloid osmotic pressure:* Together with hydrodynamic factors, this affects the movement of water and low molecular mass solutes (predominantly NaCl) between the intravascular and extravascular compartments.

Osmolality and tonicity

The *osmolality* is the number of solute particles per unit weight of water, irrespective of the size or nature of the particles. Therefore, a given weight of low molecular weight solutes contributes much more to the osmolality than the same weight of high molecular weight solutes. The units are mmol/kg of water. This determines the osmotic pressure exerted by a solution across a membrane. Most laboratories can measure plasma osmolality, but it is also possible to calculate the approximate osmolality of plasma using a number of formulae of varying complexity. The following formula has the benefit of being easy to calculate and performs as well as more complex versions (all concentrations must be in mmol/L):

$$\text{Calculated osmolality} = 2[Na^+] + 2[K^+] + [\text{glucose}] + [\text{urea}]$$

This formula includes all the low molecular weight solutes contributing to plasma osmolality. Values for Na^+ and K^+ are doubled so as to allow for their associated anions, such as chloride. The formula is approximate and is not a complete substitute for direct measurement. Calculated osmolality is usually close to measured osmolality, but they may differ considerably for two different types of reason:

- There may be large amounts of unmeasured low molecular mass solutes (e.g. ethanol) present in plasma. These will contribute to the measured osmolality, but will obviously not be taken into account in the osmolality calculated from this formula. This will cause an 'osmole gap', with measured osmolality being greater than calculated osmolality.
- Alternatively, there may be a gross increase in plasma protein or lipid concentration, both of which decrease the plasma water per unit volume. This affects some methods of measurement of $[Na^+]$, giving an artefactually low result ('pseudohyponatraemia,' see Chapter 2: Other causes of hyponatraemia). This will result in an erroneously low calculated osmolality.

The osmolality of urine is usually measured directly, but is also linearly related to its specific gravity (which can be measured using urine dipsticks), unless there are significant amounts of glucose, protein or X-ray contrast media present.

Tonicity is a term often confused with osmolality. However, it should only be used in relation to the osmotic pressure due to those solutes (e.g. Na^+) that exert their effects across cell membranes, thereby causing movement of water into or out of the cells. Substances that can readily diffuse into cells down their concentration gradients (e.g. urea, alcohol) contribute to plasma osmolality but not to plasma tonicity, since after equilibration their concentration will be equal on both sides of the cell membrane. Tonicity is not readily measurable.

The tonicity of ICF and ECF equilibrate with one another by movement of water across cell membranes. An increase in ECF tonicity causes a reduction in ICF volume as water moves from the ICF to the ECF to equalise the tonicity of the two compartments, whereas a decrease in ECF tonicity causes an increase in ICF volume as water moves from the ECF to the ICF.

CASE 2.1

A 45-year-old man was brought into the A&E department late at night in a comatose state. It was impossible to obtain a history from him, and clinical examination was difficult, but it was noted that he smelt strongly of alcohol. The following analyses were requested urgently.

Why is his measured osmolality so high?

Serum	Result	Reference range
Urea	4.7	2.5–6.6 mmol/L
Na^+	137	132–144 mmol/L
K^+	4.3	3.6–5.0 mmol/L
Total CO_2	20	24–30 mmol/L
Glucose	4.2	mmol/L
Osmolality	465	280–290 mmol/kg

Comments: The osmolality can be calculated as 291.5, using the formula in Chapter 2: Osmolality and tonicity. The difference between this figure and the value for the directly measured osmolality (465 mmol/L) could be explained by the presence of another low molecular mass solute in plasma.

From the patient's history, it seemed that ethanol might be contributing significantly to the plasma osmolality, and plasma [ethanol] was measured the following day, on the residue of the specimen collected at the time of emergency admission. The result was 170 mmol/L, very close to the difference between the measured and calculated osmolalities.

Colloid osmotic pressure (oncotic pressure)

The osmotic pressure exerted by plasma proteins across cell membranes is negligible compared with the osmotic pressure of a solution containing NaCl and other small molecules, since they are present in much lower molar concentrations. In contrast, small molecules diffuse freely across the capillary wall, and so are not osmotically active at this site, but plasma proteins do not readily do so. This means that plasma [protein] and hydrodynamic factors together determine the distribution of water and solutes across the capillary wall, and hence between the intravascular and interstitial compartments (Figure 2.1).

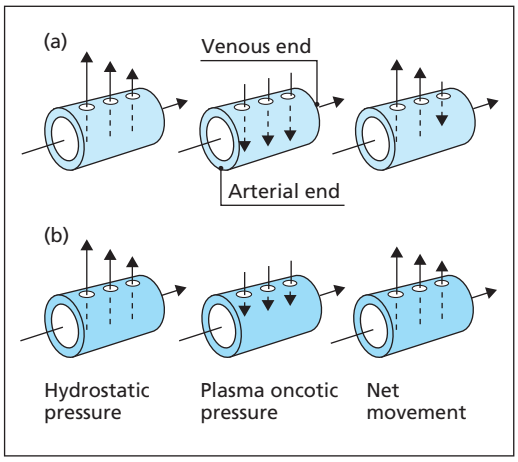

Figure 2.1 Movements of water and low molecular mass solutes across the capillary wall when the plasma [protein] is (a) normal and (b) low. The effects shown are: hydrostatic pressure, which drives water and low molecular mass solutes *outwards* and decreases along the length of the capillary; and plasma oncotic pressure, which attracts water and low molecular mass solutes *inwards* and is constant along the length of the capillary. The net movement of water and low molecular mass solutes across the capillary wall is governed by the net effect of hydrostatic and plasma oncotic pressures.

Regulation of external water balance

Typical daily intakes and outputs of water are given in Table 2.1. Water intake is largely a consequence of social habit and is very variable, but is also controlled by the sensation of thirst. Its output is controlled by the action of vasopressin, also known as antidiuretic hormone (ADH). In states of pure water deficiency, plasma tonicity increases, causing a sensation of thirst and stimulating vasopressin secretion, both mediated by hypothalamic osmoreceptors. Vasopressin then promotes water reabsorption in the distal nephron, with consequent production of small volumes of concentrated urine. Conversely, a large intake of water causes a fall in tonicity, suppresses thirst and reduces vasopressin secretion, leading to a diuresis, producing large volumes of dilute urine.

Table 2.1 Average daily water intake and output of a normal adult in the UK.

Intake of water	mL	Output of water	mL
Water drunk	1500	Urine volume	1500
Water in food	750	Water content of faeces	50
Water from metabolism of food	250	Losses in expired air and insensible perspiration	950
Total	2500	Total	2500

Secretion of vasopressin is normally controlled by small changes in ECF tonicity, but it is also under tonic inhibitory control from baroreceptors in the left atrium and great vessels on the left side of the heart. Where haemodynamic factors (e.g. excessive blood loss, heart failure) reduce the stretch on these receptors, often without an accompanying change in ECF tonicity, a reduction in tonic inhibitory control stimulates vasopressin secretion. The resulting water retention causes hyponatraemia, and is relatively ineffective in expanding the intravascular compartment, since water diffuses freely throughout all compartments (Figure 2.2).

Regulation of external sodium balance

Dietary intakes of Na^+ (and Cl^-) are very variable worldwide. A typical 'Western' diet provides

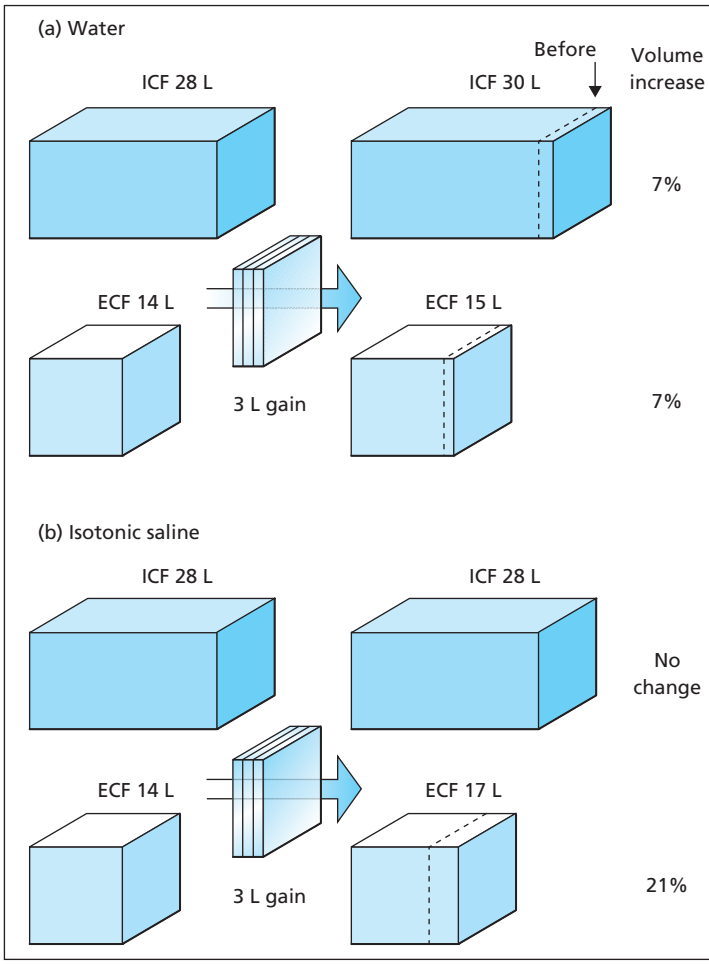

Figure 2.2 Different effects on the body's fluid compartments of fluid gains of 3 L of (a) water and (b) isotonic saline. The volumes shown relate to a 70 kg adult.

100–200 mmol of both Na^+ and Cl^- daily, but the total body Na^+ can be maintained even if intake is less than 5 mmol or greater than 750 mmol daily. Urinary losses of Na^+ normally closely match intake. There is normally little loss of these ions through the skin or in the faeces, but in disease the GI tract can become a major source of Na^+ loss.

The amount of Na^+ excreted in the urine controls the ECF volume since, when osmoregulation is normal, the amount of extracellular water is controlled to maintain a constant concentration of extracellular Na^+. A number of mechanisms are important regulators of Na^+ excretion:

- *The renin–angiotensin–aldosterone system:* Renin is secreted in response to a fall in renal afferent arteriolar pressure or to a reduction in supply of Na^+ to the distal tubule. It converts angiotensinogen in plasma to angiotensin I (AI), which in turn is converted to angiotensin II (AII) by angiotensin-converting enzyme (ACE). Both AII and its metabolic product angiotensin III (AIII) are physiologically active, and stimulate the release of aldosterone from the adrenal cortex. Aldosterone acts on the distal tubule to promote Na^+ reabsorption in exchange for urinary loss of H^+ or K^+. Since Na^+ cannot enter cells freely, its retention (with iso-osmotically associated water) contributes solely to ECF volume expansion, unlike pure water retention (Figures 2.2 and 2.3). Although the renin–angiotensin–aldosterone system causes relatively slow responses to Na^+ deprivation or Na^+ loading, evidence suggests that this is the main regulatory mechanism for Na^+ excretion.
- *The glomerular filtration rate (GFR):* The rate of Na^+ excretion is often related to the GFR. When the