chapter 1:

THE NATURE AND INVESTIGATION OF DISEASES

OBJECTIVES

After studying this chapter you should be able to:

- define appropriate terms associated with health and disease;
- give a simple classification of diseases;
- list the ways pathological investigations help in the diagnosis, treatment and management of diseases;
- discuss the clinical and analytical evaluations of laboratory tests.

1.1 INTRODUCTION

Disease can be defined as any abnormality or failure of the body to function properly and this may require medical treatment (*Figure 1.1*). The scientific study of diseases is called **pathology**.

Every disease has a distinct set of features that include a cause, associated clinical symptoms and a characteristic progression, with associated morphological and functional changes in the patient. The presence of an abnormality on its own, however, does not necessarily indicate disease since the affected individual must also suffer from ill health. **Health** can be defined as an absence of signs and symptoms associated with any disease. This definition has limitations in that there are circumstances where individuals believe they are ill even though detectable indications of disease are not present. Conversely, there are individuals who believe they are healthy but on detailed examination are found to suffer from a serious disease. For this reason, the World Health Organization (WHO) devised the more appropriate definition of health as *a state of physical, mental and social well-being and not merely the absence of disease*. Currently there is considerable interest, particularly in developed countries, in promoting health by improving lifestyle and reducing mental and social factors associated with ill health.

Figure 1.1 A modern intensive care unit of a hospital.

Individuals differ in their physical appearance and also internally in the composition of the biological materials of which they are made. Differences

between individuals are due to biological variation. Variation in values for measurable body features or biological substances (**analytes**) occurs for a number of reasons. Differences in the genotypes of individuals ensure that no two individuals are the same (other than identical twins). Biological variation arises due to differences in lifestyle, experiences, dietary and other factors as well as genes. There are also differences in physiological processes arising from bodily control mechanisms. For example, the concentration of blood glucose varies between individuals with diet, time of day and physical activity.

1.2 CHARACTERISTIC FEATURES OF DISEASES

Every disease has a number of characteristic features. These features allow diseases to be categorized and allow a better understanding of the disease, its diagnosis and management. A correct diagnosis should mean that appropriate treatment is given.

ETIOLOGY

Etiology refers to the cause of a disease. Etiological agents can be **endogenous**, in other words originating from within the body, or **exogenous**, coming from outside the body. Endogenous agents include genetic defects and endocrine disorders, while exogenous agents include microorganisms such as viruses (*Figure 1.2*), bacteria (*Figure 1.3*) and fungi that cause infections, chemicals, physical trauma and radiation. Many diseases are said to be predictable and arise as a direct consequence of exposure to the causative agent. Other diseases are considered probable in that they may be a consequence of the causative agent but the development of illness is not inevitable. An individual can be infected with a pathogenic microorganism but the outcome of the disease may depend on other factors such as the nutritional and immune status of the affected person.

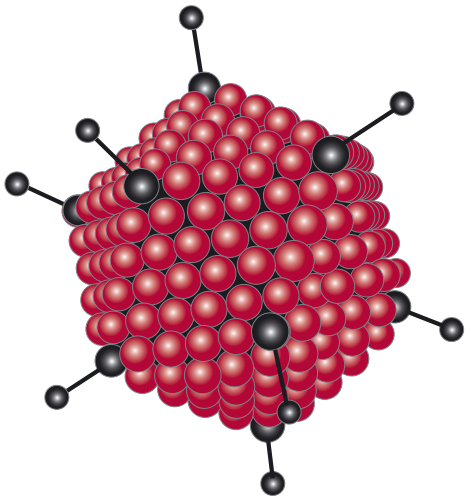

Figure 1.2 Schematic of an adenovirus (diameter about 70nm), which can cause respiratory infections.

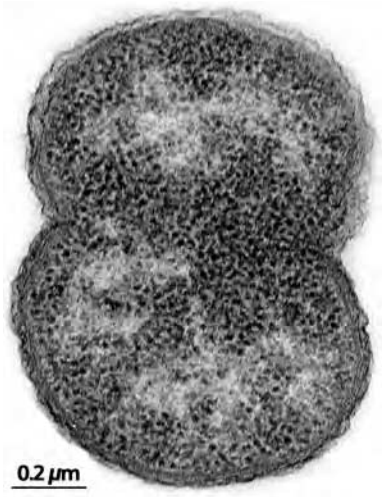

Figure 1.3 Electron micrograph of the bacterial pathogen, ***Neisseria meningitidis***, a causative organism of meningitis (***Chapters 2*** and ***3***). Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

Some diseases have more than one etiological agent and may, indeed, be caused by a range of factors. Such diseases are said to be multifactorial in origin. Diabetes mellitus type 2, a disorder of carbohydrate, fat and protein metabolism, is believed to have a multifactorial origin involving several genetic, dietary and environmental factors (*Chapter 7*). Many diseases are of unknown cause and are said to be **idiopathic**. An example of this is hypertension, where more than 90% of cases are of unknown cause. The treatment of idiopathic diseases is restricted to alleviating the symptoms. Some conditions are caused by the effects of treatment and are called **iatrogenic** diseases (from the Greek word *iatros,* doctor). The treatment of some cancers with cytotoxic drugs, for example, can cause a severe iatrogenic anemia although they may be curing the cancer.

Occasionally a disease of unknown etiology is more commonly found in populations with certain dietary, occupational or lifestyle conditions called **risk factors.** Smoking is a significant risk factor in the development of heart disease and lung cancer (*Chapters 14* and *17*). Some risk factors may be important in the development of the disease whereas others may make the individual more susceptible to disease. **Predisposing factors** are conditions or situations that make an individual more susceptible to disease. They include age, sex, heredity and environmental factors. For example, the immune system in a newborn is not fully developed and, as a consequence, babies are more susceptible to infections. However, during aging the immune system undergoes a progressive decline in function making the elderly also more susceptible to infections. Sex may also be a predisposing factor: men are more likely to suffer from gout than women whereas osteoporosis is more common in the latter.

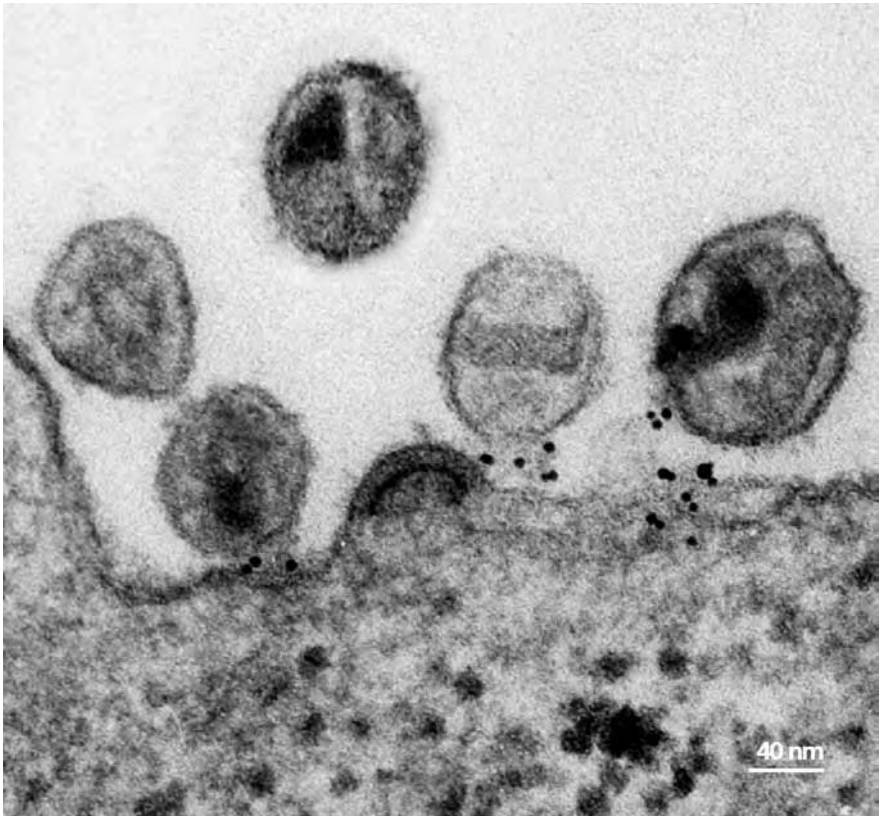

Figure 1.4 Electron micrograph of HIV particles escaping from a cultured human cell. Courtesy of Dr. D. Robertson and Professor R.A. Weiss, Institute for Cancer Research, Royal Cancer Hospital, UK.

Some diseases increase the risk of developing others. Thus some conditions increase the risk of someone developing cancer and are said to be **premalignant**. This is seen in ulcerative colitis, an inflammatory condition affecting the large intestine that increases the probability of developing bowel cancer in sufferers. Some diseases predispose the patient to other conditions by allowing infectious agents, not normally pathogenic, to cause disease. This is seen in **opportunistic infections** where a decline in the immunological functions of an individual makes them susceptible to infections by microorganisms that are normally nonpathogenic. In acquired immunodeficiency syndrome (AIDS), the individuals infected with the human immunodeficiency virus (HIV), (*Figure 1.4*) have little resistance to infections by microorganisms, such as those responsible for causing pneumonia, as well as microorganisms such as the yeast *Candida*, which are part of the normal flora of the body (*Chapter 2*).

Diseases are often described as being **primary** or **secondary**. Primary may refer to a disease of unknown cause or **idiopathic**, whereas secondary is used to refer to a condition that arises from an existing disease. However, these terms are also often used to describe the stages of a disease. For example, in cancer the primary tumor is the initial tumor whereas secondary tumors arise following metastasis of the primary tumor to other tissues (*Chapter 17*).

PATHOGENESIS

Every disease has a pathogenesis that describes the development of the disease or, more specifically, how the etiological agent(s) acts to produce the clinical and pathological changes characteristic of that disease. Some examples of how diseases undergo pathogenesis include inflammatory reactions in response to harmful agents and carcinogenesis where the formation of tumors occurs as a result of exposure to carcinogens (cancer-inducing substances).

Diseases have 'natural histories' that describe the typical patterns of how each disease usually progresses, its effects and its duration. The effects of the disease on the patient are referred to as **morbidity**. Occasionally the morbidity of a disease may cause disability that, in turn, may restrict the activities of the patient. The **mortality** of a disease describes its possibility of causing death. This is usually expressed as a percentage. Some diseases have a rapid, often severe onset that is described as **acute**. However, other diseases have a **chronic** onset and develop gradually over a relatively longer time. Thus acute renal failure is characterized by rapid deterioration of kidney function over a matter of days, while chronic renal failure develops over months or even years (*Chapter 8*).

Diseases rarely occur immediately following exposure to an etiological agent. In most cases, a period of time, the **incubation period**, must elapse before the disease becomes apparent. In carcinogenesis, this period may last several decades and is referred to as the **latent period**. With infectious diseases, the time between exposure and development of the disease is often characteristic of the infectious agent involved.

MANIFESTATIONS OF DISEASES

The etiology of a disease and its pathogenesis produce **clinical manifestations** that include signs and symptoms of the disease. A **symptom** is an indication that a disease is present and something of which the patient complains, for example, nausea, malaise or pain (*Table 1.1*). A **sign** is something that the clinician specifically looks or feels for, such as redness or swelling of the skin, when examining the patient. Some diseases present with a **subclinical** stage where these signs and symptoms are not apparent, even though the disease is established and characteristic biochemical and cellular changes that are detectable by laboratory analysis of, for example blood or urine have taken place.

Clinical signs and symptoms are often accompanied by structural or functional abnormalities, called **lesions**, in affected tissues that are responsible for ill health and usually cause the signs and symptoms of disease. Lesions may be biochemical in nature, such as defective hemoglobin in patients with hemoglobinopathies (*Chapter 13*). Alternatively, a lesion may include deposition of abnormal substances in cells, tissues and organs such as deposition of amyloid in the brain in patients with Alzheimer's disease (*Chapter 18*). Loss of healthy surface tissue, for example, in gastric ulceration, may also

Symptom	Cause
Pain	stimulation of nerve endings by trauma, chemicals and heat
Swelling	increase in number or size of cells or an accumulation of tissue fluid
Fever	actions of interleukin-1 and prostaglandins stimulate thermoregulatory center in the brain
Weight loss	decreased intake of food or a catabolic state stimulated by release of factors from tumors
Diarrhea	inadequate absorption of food by the GIT leads to an osmotic retention of water and production of watery stools (*Chapter 11*)
Cough	release of neuropeptides following irritation of the respiratory mucosa
Cyanosis	reduced supply of oxygenated hemoglobin to the skin

Table 1.1 Disease symptoms and their causes

be a feature of some diseases. Diseases may involve functional abnormalities such as an inappropriate secretion of hormones. Examples of these include the excessive production of thyroid hormones in hyperthyroidism or the inadequate secretion of insulin in type 1 diabetes mellitus. Other functional defects might include impaired nerve conduction and muscular contraction.

The term **syndrome** is often applied to describe certain diseases that are characterized by multiple abnormalities that form a distinct clinical picture. For example, Cushing's syndrome (*Chapter 7*) occurs when an excess of the hormone cortisol produces a combination of clinical features that include **hirsutism** (excessive growth of facial hair), obesity, hypertension and characteristic facial and body features.

Some diseases frequently present with complications, that is, new or separate processes secondary to, and a consequence of, the initial disease. Diabetic cataracts, retinopathy and nephropathy are all chronic or long-term complications of diabetes mellitus.

The manifestations of a disease in a given person are not static and are affected by compensatory mechanisms in the body as well as by environmental influences and responses to treatment. Diseases often have a range of manifestations and their presence and severity may vary from patient to patient. In addition to differences between individuals, differences occur within an individual at different stages of development, from infancy to old age. *Figure 1.5* outlines the key features of some diseases in terms of their etiology, pathogenesis and manifestations.

OUTCOME OF A DISEASE

The **prognosis** of a disease is its likely outcome. Prognoses can vary considerably between different diseases and, of course, can be influenced by treatment. Hence when giving a prognosis, it is necessary to clarify if the disease is following its natural course or whether there is, or needs to be, medical or surgical intervention. A viral disease such as German measles (rubella) will normally resolve of its own accord, whereas a broken leg or a heart attack needs treatment. With some diseases, especially some cancers, patients may go through a period of good health with a reduction or disappearance of the symptoms and the disease is said to be in **remission**. However, a **relapse** may occur with a return of the disease symptoms following this period of apparent recovery. Diseases with a tendency towards remission and relapse include acute lymphoblastic leukemia and ulcerative colitis (*Chapters 17* and *11*).

	Etiology	Pathogenesis	Morphological and functional features	Complications and sequelae
Local skin infection *(Chapters 2 & 3)*	*Staphylococcus aureus*	acute inflammation	local skin infection visible	septicemia
Neoplasms, for example lung cancer *(Chapter 17)*	smoking	mutation *(Chapter 15)*	tumor of the lung	metastases leading to secondary tumors
Cirrhosis of the liver *(Chapters 2 & 12)*	hepatitis B virus	immune reaction to virus-infected cells *(Chapter 4)*	cirrhosis of the liver	liver failure

Figure 1.5 Characteristics of some diseases showing relationship between etiology, pathogenesis, morphological and functional features and complications and sequelae.

Class of disease
Infectious
Immunological
Endocrine
Homeostatic
Nutritional
Toxic
Genetic
Congenital
Neoplastic
Traumatic
Degenerative
Psychogenic
Iatrogenic
Idiopathic

Table 1.2 A classification of diseases

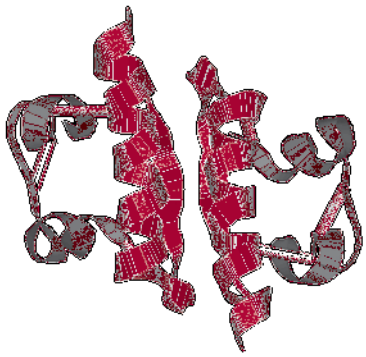

Figure 1.6 Model of a dimer (two molecules) of insulin. The represent disulfide bonds. PDB file 1ZEH.

1.3 CLASSIFICATION OF DISEASES

Some diseases share common features and can be grouped together in a classification system. One way of classifying diseases is on the basis of their cause (*Table 1.2*). This is by no means perfect as some diseases have multiple causes and there is likely to be an overlap between the different categories.

Infectious diseases are caused when microorganisms such as viruses, bacteria, fungi, protozoa and helminths enter and spread within the body (*Chapters 2* and *3*).

Immunological diseases (*Chapters 4* and *5*) occur in circumstances in which the immune system can cause damage to the body's own tissues. In autoimmune conditions, for example autoimmune thyroiditis, antibodies are produced that attack the body's own tissues. Alternatively, there are diseases associated with immunodeficiency that increase the susceptibility of the patient to infectious agents. This occurs in severe combined immunodeficiency (SCID) and in AIDS.

Endocrine diseases arise from the over- or underproduction of hormones or from resistance to a particular hormone perhaps because the cellular receptor is absent as the result of a mutation (*Chapter 7*). Thus, for example, acromegaly is caused by the overproduction of growth hormone in adults, whereas type 2 diabetes mellitus is a consequence of insulin resistance, when the appropriate target cells fail to respond to the hormone (*Figure 1.6*).

Homeostatic diseases arise when mechanisms for controlling homeostasis are disrupted. For example, in the syndrome of 'inappropriate ADH secretion' (*Chapter 8*) diminished urine production leads to an increase in body fluids.

Nutritional diseases result from an inadequate intake of nutrients, such as proteins (which supply essential amino acids), carbohydrates, essential fatty acids, vitamins or trace elements. Inadequate nutrition is a major cause of disease, particularly in developing countries. Such deficiencies may be generalized, as in protein-energy malnutrition (*Chapter 10*) where there is simply not enough food, or there may be a lack of a specific nutrient, for example, vitamin A (*Figure 1.7*) leading to several disorders including night blindness. In contrast, in many developed countries an excessive intake of energy combined with a lack of exercise is responsible for a worrying increase in obesity.

Toxic diseases (*Chapter 12*) are caused by the ingestion of a variety of poisons that may be encountered in the environment. Ingestion may be accidental or deliberate. Carbon monoxide can be inhaled from car exhausts or faulty gas fires or water heaters, causing tissue hypoxia and death.

Genetic diseases arise due to defects in the genes or chromosomes of individuals (*Chapter 15*). A defective gene may result in the inadequate production of a key enzyme, such as phenylalanine hydroxylase in phenylketonuria. Down syndrome is an example of a disorder which arises due to an abnormal chromosome complement. Some genetic disorders are not inherited from parents and may arise from a new genetic mutation in the offspring, as in the disease progeria. Congenital diseases are present at birth and may or may not have been inherited. They may arise due to a developmental defect of known or unknown cause. Thus, a newborn may suffer from fetal alcohol syndrome, a congenital condition arising as a consequence of excessive alcohol intake by the mother during pregnancy.

Neoplastic diseases are characterized by the uncontrolled and abnormal growth of cells. These cells form benign or malignant tumors (*Chapter 17*). Malignant neoplasms are a major cause of death in many developed countries. Moreover, their incidence is increasing as people live longer.

Traumatic diseases are caused by physical injury and include mechanical trauma, extremes of heat or cold, electrical shock and radiation. Apart from the obvious problems caused by extensive damage to tissues, traumatic diseases may render an individual more prone to infection by compromizing the immune system (*Chapter 4*).

Degenerative diseases involve the progressive loss of body tissues and impairment of their functions usually associated with aging (*Chapter 18*). Examples include neurodegenerative diseases, such as the relatively common Alzheimer's disease and muscular dystrophy.

Psychogenic diseases originate in the mind. They may have a significant psychological or emotional component as seen, for example, in schizophrenia.

Iatrogenic diseases arise as a consequence of treatment. For example, patients who are receiving drugs, such as thiazide diuretics to control their blood pressure, may suffer from low serum K^+ (hypokalemia, see *Chapter 8*) caused by an excessive renal loss. If untreated, hypokalemia may, in turn, cause cardiac arrhythmias (*Chapter 14*). Finally, **idiopathic diseases** are those of unknown cause.

1.4 EPIDEMIOLOGY OF DISEASE

Epidemiology is the study of how diseases spread in populations in relation to their causal factors. Consequently, epidemiology is largely concerned with the collection and interpretation of data about diseases in groups of people rather than in individuals. The types of data collected in epidemiological studies provide information about the etiology of the diseases, whether there is a need for screening or the introduction of other preventative measures and whether health care facilities are appropriate.

The **prevalence** of a disease refers to the proportion of people in a population affected at a specific time. The **incidence rate** is the number of new cases of a disease in a population occurring within a specified period of time.

Epidemiological studies can often provide information about the cause(s) of diseases. Thus if a disease has a high incidence in a particular region or population, then the disease may have a genetic origin or it may be caused by environmental factors peculiar to that area. Epidemiological studies of migrant populations are especially useful since they can provide valuable information on the etiology of a disease. A case in point might be where a migrant population has a high incidence of a particular disease and then moves to another geographical area where the incidence of the same disease is low. If the incidence of disease in the migrant population remains high, then it is likely that the disease has a genetic basis. If, however, the incidence in the migrant population decreases to the level of the new geographical region, then environmental factors probably play a role in its etiology.

The data on the incidence of some diseases are very reliable. This is especially so for some infectious diseases and cancers that are **notifiable**. Clinicians are legally required to supply details of all new cases of diseases on the notifiable list to a central register. However, obtaining data on the incidence of other diseases can be difficult. For most diseases, the data obtained refer to mortality rates for that disease based on the causes listed on death certificates. This method of obtaining data has the major limitation of underestimating the incidence if the disease does not have a fatal outcome.

The incidence of certain diseases changes with time and also can vary considerably from one country to another and even within different regions of the same country. These differences are particularly marked between

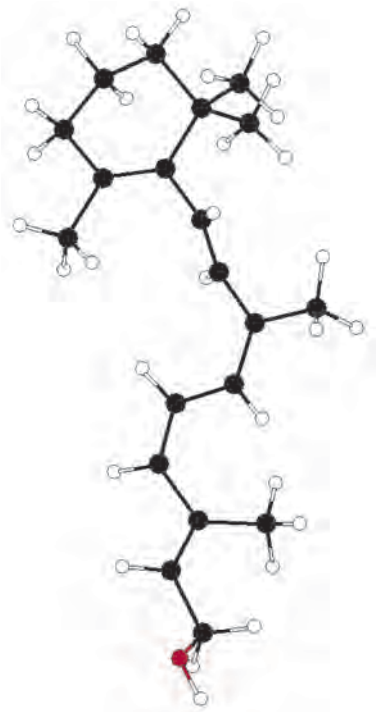

Figure 1.7 Computer generated model of vitamin A (retinol).

developing and developed countries. Infectious diseases and malnutrition are still more prevalent in developing countries, while in the developed world, the incidence of many infectious diseases has been reduced dramatically in the last 100 years. The infant mortality rate is often used as a measure of health related to socioeconomic status. In general, the infant mortality rate is higher in developing compared with developed countries.

The decreased incidence of many diseases in developed countries may reflect changes in exposure to causative agents as well as the effects of preventative measures. For example, the reduction of diseases such as cholera is associated, in a large part, with improved public health measures. Improvements in sanitation, sewage and hygiene have had a considerable impact in reducing the incidence of many infectious diseases. Mass immunization against infectious diseases, such as polio, has had enormous beneficial effects in reducing disease in the population as a whole. Unfortunately the reduction in infectious diseases has been accompanied by an increasing incidence of other diseases, such as cardiovascular diseases, diabetes, several types of cancers and psychiatric diseases. All are associated with aging and, to a certain extent, this may reflect the increased life expectancy in the developed countries: people are not killed by infectious diseases and live longer. Some evidence does suggest that the increased incidence of these diseases is also due to changes in diet such as increased consumption of saturated fats and other lifestyle factors, for instance a lack of exercise. **Intervention studies** aimed at changing diet and lifestyle factors in an attempt to reduce the incidence of these diseases are already proving beneficial.

Socioeconomic factors can also influence the incidence of many diseases. Poverty tends to be associated with an increased incidence of malnutrition and malnourished individuals are more susceptible to infectious diseases. Overcrowding is known to promote the spread of infectious diseases resulting in epidemics.

Some diseases have a high incidence in populations associated with certain occupations. For example, coal workers have a high incidence of pneumoconiosis caused by inhalation of coal dust and, in the past, workers with asbestos faced a high risk of asbestosis, and of developing mesothelioma of the lung. Occupational hazards need to be identified and minimized to reduce the incidence of these diseases.

1.5 INVESTIGATING DISEASES

For the majority of diseases, the clinical outcome is likely to be improved if treatment is started at an early stage. Consequently the proper investigation of disease is necessary to ensure a rapid and accurate diagnosis and to allow appropriate treatment to be initiated as soon as possible. The procedure for investigating a disease is outlined in *Figure 1.8*. It starts with the affected person presenting symptoms and visiting his or her physician when feeling unwell or after a period of ill health. The examination usually begins with the clinician asking the patient about his or her current and past medical histories, current and previous medications, use of alcohol and tobacco, any family history of disease and possibly occupational history. This is usually followed by a clinical examination to look for signs of any abnormality. This may involve visual examinations of the skin, eyes, tongue, throat, nails and hair to detect abnormalities together with tests to assess cardiovascular, respiratory, gastrointestinal, genitourinary, nervous and musculoskeletal functions. Since diseases typically present with recognizable signs and symptoms, the clinician may make a diagnosis of the disease based on the clinical history and the examination and then initiate treatment. Sometimes this may not be possible, given that many clinical symptoms and signs are not specific to any

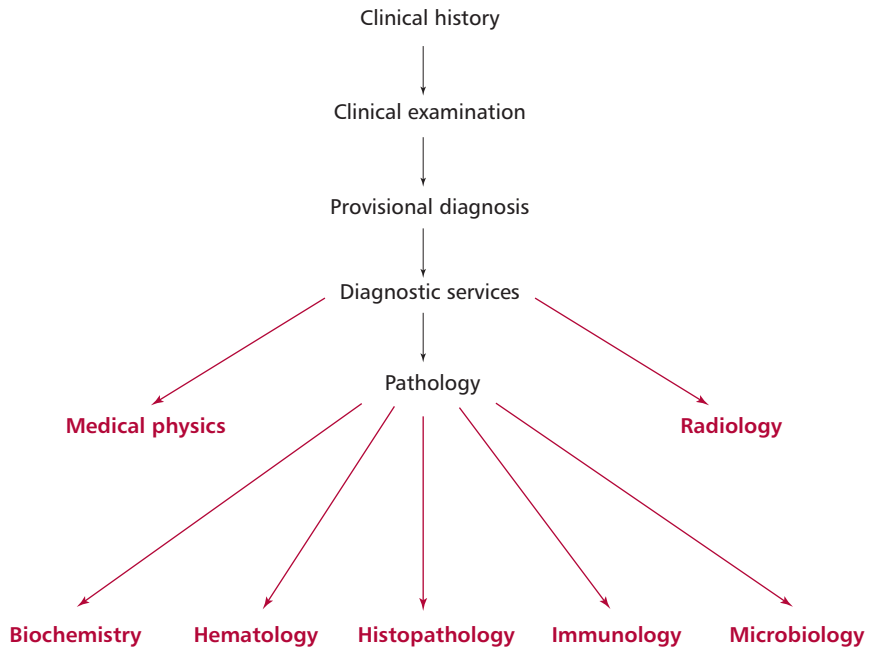

Figure 1.8 An outline of the steps involved in investigating diseases.

one disease. However, a range of diagnostic services is also available to the clinician in modern health care systems. These include imaging techniques, physiological function tests, radiographic examinations (X-rays) and pathology laboratory investigations that can be applied to confirm, reject or distinguish between the various provisional diagnoses. The clinician may only be able to make a provisional diagnosis or a shortlist of possible diagnoses and then request additional investigations that rely on the diagnostic services available at the surgery, clinic or hospital.

1.6 TYPES OF PATHOLOGY LABORATORIES

The function of hospital pathology laboratories (*Table 1.3*) is to make scientific investigations of disease. The typical pathology service offered by hospitals has six main branches: medical microbiology, immunology, clinical biochemistry, hematology, histopathology and clinical genetics.

Medical microbiology is concerned primarily with the detection and identification of pathogenic microorganisms. For clinical purposes, these consist of viruses, bacteria, protozoa, fungi and helminths (worms). Microorganisms are detected directly in specimens obtained from the patient or on swabs (for example throat, nasal) that are cultured in growth medium to increase the number of microorganisms and allow their easier detection. The presence of microorganisms may also be determined indirectly by detecting antibodies produced by the patient in response to the infection. Medical microbiology laboratories also investigate the responses of pathogenic microorganisms to antibiotics.

Immunology laboratories are concerned with studying the body's immune response in both healthy and diseased states. Immune responses are 'cell-mediated' or 'humoral'. The former involves T lymphocytes, the latter the production of antibodies by specialized B lymphocytes. The presence or absence of antibodies in plasma can be determined, for example, by serum electrophoresis (*Figure 1.9*) to assess generalized immunodeficiencies and other diseases. However, of more diagnostic value during the investigation

Types of pathology laboratories
Medical microbiology
Immunology
Clinical biochemistry
Hematology
Histopathology
Clinical genetics

Table 1.3 Types of pathology laboratories

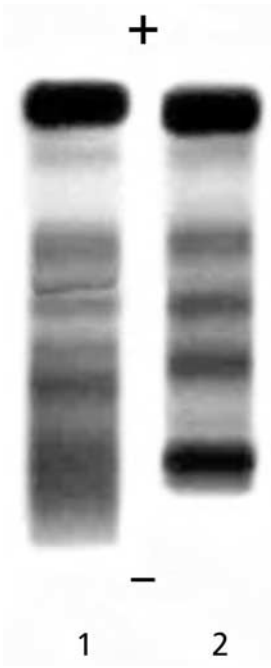

Figure 1.9 Excessive immunoglobulin (*Chapters 4* and *5*) is produced in multiple myeloma, a tumor of the B lymphocytes; the white blood cells that produce immunoglobulins. The proteins in samples of serum can be separated by electrophoresis and stained with dye. Lane 1 shows the separated proteins from normal serum. The most abundant protein is serum albumin which shows as the strongly staining band near the positive end. Lane 2 shows a myeloma serum sample with a second dense band at the negative end. This band shows the enormous amount of a single type of immunoglobulin, produced by the tumor. In multiple myeloma, the serum protein concentration increases making the blood thick and difficult to pump around, putting a strain on the heart and kidneys. In addition, so much effort is put into synthesizing one type of useless antibody that the concentration of other antibody molecules decreases and patients become prone to infection.

Figure 1.10 A typical automated analyzer in a hospital clinical chemistry laboratory capable of performing most of the major investigations. Courtesy of the Department of Clinical Biochemistry, Manchester Royal Infirmary, UK.

of immune diseases may be the measurement of specific antibodies that are produced in response to a particular antigen (which may be an infectious agent or an autoantigen). The number of cells involved in immunity, such as T-cells, B-cells, T-helper and T-suppressor cells, are often determined as this can provide valuable information about the immune status of an individual.

Clinical biochemistry is concerned with investigating the biochemical changes associated with diseases. A wide range of substances or **analytes** are measured in clinical biochemistry laboratories. Some of these analyses are carried out routinely on all samples (blood, urine) coming into the laboratory using automated methods (*Figure 1.10*); others need to be requested specially. Analyses include those for proteins, enzymes, hormones, lipids, tumor markers, blood gases, sugars and inorganic ions to investigate a variety of disorders, including those associated with abnormal renal, respiratory, metabolic, bone and endocrine function. In addition, analytes are measured during investigations of genetic disorders both to diagnose and to monitor the effectiveness of therapies.

Hematology is concerned with the study of disorders of blood cells, including blood clotting (coagulation) defects. Hematological investigations can involve determining the concentrations of blood proteins, such as hemoglobin, to aid in the diagnosis of diseases. The microscopic examination of blood films, thin layers of blood spread out on a microscope slide and stained (*Figure 1.11*) and marrow removed from bone cavities by aspiration (*Figure 1.12*) may also be helpful. Some hematology laboratories may also be involved in the provision of blood and blood products for transfusion services, but these are often run as separate services.

Histopathology is concerned with the investigation of disease by examining cells and tissues. This involves the macro- and microscopic investigation of body tissues for the identification of disease. Amongst other things, histopathology laboratories are usually involved in the diagnosis of malignancies and can also provide information on how far a tumor has progressed ('staging') and therefore can suggest a likely prognosis. In addition, histopathology laboratories may also assist with investigation of a range of infectious and inflammatory conditions affecting body tissues.

Clinical genetics is a growing area in the investigation of diseases. A major focus of clinical genetics laboratories is the identification of genetic abnormalities (*Chapter 15*). This could include, for example, identifying the number and form of chromosomes (*Figure 1.13*) in blood films to identify any numerical and structural abnormalities.

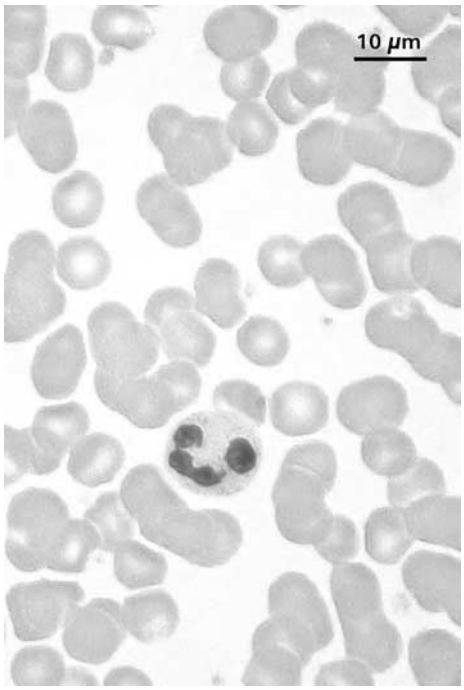

Figure 1.11 Blood film showing a single white blood cell surrounded by erythrocytes (*Chapter 13*). Courtesy of Dr L. Seal, School of Biology, Chemistry and Health Science, Manchester Metropolitan University, UK.

1.7 ROLE OF HOSPITAL LABORATORY TESTS

Tests performed by the pathology laboratory can assist clinicians in investigating disease. The tests may only give a subjective assessment, such as when a pathologist assesses the types of cells obtained from a fine needle aspirate of a suspected breast tumor when investigating breast cancer. However, tests may provide quantitative information, such as the concentration of thyroid hormones in the serum, that can then be compared with a **normal** value. Unfortunately, the term normal is often difficult to define in clinical terms. To alleviate this problem, **reference ranges** have been widely adopted. Numerical reference limits are based on the mean value plus or minus two standard deviations against which test results can be compared. The uses of reference ranges are explored more thoroughly later in the chapter. The term **normal range** is still used synonymously with reference range.

In general, the roles of laboratory tests include:

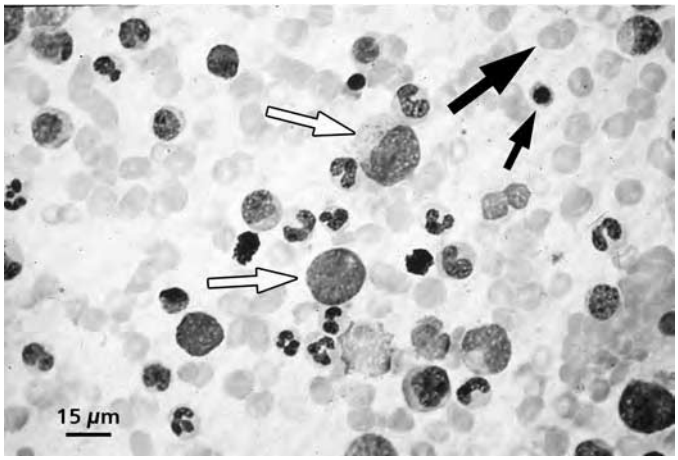

Figure 1.12 The bone marrow site is the site of blood cell formation. This light micrograph shows its normal cells with a range of immature erythrocytes (smaller solid arrow) and leukocytes (open arrow). The large proportion of mature erythrocytes (larger, solid arrow) in the background is due to unavoidable contamination with peripheral blood during sample collection. Courtesy of J. Overfield, School of Biology, Chemistry and Health Science, Manchester Metropolitan University, UK.

Figure 1.13 A spread of human chromosomes from a female (*Chapter 15*).

- the diagnosis, to identify the disease;
- monitoring of treatment;
- screening and assessment of risk;
- the prognosis, to inform the physician and the patient of the likely outcome;
- detection of complications.

The results of laboratory investigations are used in conjunction with the patient's clinical history and examination to determine the nature of the disease affecting the patient. Thus a low value for the concentration of glucose in the blood of a patient can confirm hypoglycemia and the clinician can start palliative treatment, even though the cause of the hypoglycemia may be unknown at this stage.

Laboratory tests may be used to monitor the course of an illness or the effects of its treatment. For example, the concentration of glycated hemoglobin in erythrocytes is measured in diabetics on a regular basis. The higher the concentration of glucose in the serum, the more readily the sugar reacts with proteins in a nonenzymic reaction to form glycated hemoglobin, in which sugar molecules are covalently attached to the protein. Thus the amount of glycated hemoglobin is an indicator of average glycemia in such patients over a period of days or months. Diabetics who are not complying with their treatment by not taking their insulin regularly or giving themselves the wrong dose, or whose treatment is ineffective, can be identified because poor control of blood glucose gives rise to higher concentrations of glycated hemoglobin than is normal or even found in well-controlled diabetics (*Figure 1.14*).

Laboratory tests may be used to detect a disease *before* it presents clinically. This is referred to as screening. For example, the concentration of phenylalanine in the serum of all newborn babies in the UK is measured to detect phenylketonuria (*Chapter 15*). Affected children have a high concentration of serum phenylalanine (hyperphenylalaninemia) and metabolites of phenylalanine, such as phenylpyruvic acid are usually present. If untreated, this condition leads to irreversible brain damage but if caught early and treated by diet, individuals develop normally. Other examples of screening tests include smears taken from the lining of the uterine cervix (*Margin Note 1.1* and *Figure 1.15*). Screening tests may also be

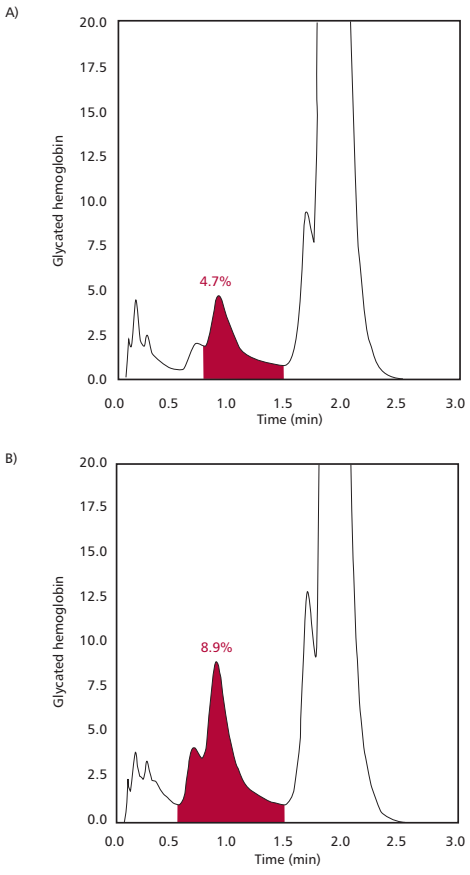

Figure 1.14 A chromatogram glycated hemoglobin determined by HPLC. The results are shown for (A) a normal person and (B) a patient with diabetes mellitus. Courtesy of Department of Clinical Biochemistry, Manchester Royal Infirmary, UK.

BOX 1.1 Clinical specificity and sensitivity

If a test for a particular disease gives a positive result in affected patients, the result is referred to as a true positive (TP). However, if a positive result is obtained in an individual who does not have the disease, this is referred to as a false positive (FP). In individuals without the disease, the test results should be a true negative (TN) but occasionally a negative result is obtained in a patient who has the disease and this is referred to as a false negative (FN). The ability of a test to discriminate between diseased and healthy states is described by its *clinical* specificity and sensitivity.

The specificity of a test is the measure of the incidence of negative results in individuals free of the disease, and defined as:

$$Specificity = TN \times 100 / TN + FP$$

A test with a specificity of 90% means that, on average, 90 out of 100 individuals without the disease would give a negative test. Conversely, 10 of these individuals would give a positive result even though they do not have any disease.

The sensitivity of a clinical test is a measure of the incidence of positive results in individuals affected by the disease. Sensitivity can be expressed as:

$$Sensitivity = TP \times 100 / TP + FN$$

A test of 90% sensitivity means that, on average, 90% of individuals with the disease will give a positive test while the remaining 10% of individuals with the disease would give a negative result.

Ideally, a test should have 100% specificity and sensitivity, that is, it should give a negative result in all individuals without disease and a positive result in all patients affected by the disease. Such a test would discriminate completely between the diseased and healthy states. Unfortunately, such perfection rarely occurs and tests almost always have some degree of overlap (*Figure 1.16(A)*). Indeed, factors that increase the specificity of a test often decrease its sensitivity and *vice versa*.

When using a clinical test, it should also be appreciated that its ability to detect a disease is influenced by the prevalence of that disease in the population being studied. This ability is described by the predictive values of the test. The predictive value of a positive test is defined as:

$$Predictive\ value\ of\ positive\ test = TP \times 100 / TP + FP$$

and of a negative test as:

$$Predictive\ value\ of\ negative\ test = TN \times 100 / TN + FN$$

The range of values obtained for any test in healthy individuals usually overlaps with those obtained from patients with the disease. Hence, some patients who are genuinely ill will give test results that imply they are healthy (FN), whereas others who are not ill appear to have the disease (FP). However, if extreme values are used in the test for comparison, then the number of FN results will be reduced or eliminated. The method will, however, detect more FP results (*Figure 1.16(B)*). Thus the test will have a high specificity but low sensitivity. If the cut-off value is reduced, then the number of FP results would be reduced but at the expense of increasing the number of FN results. Thus the test has a high sensitivity but only by decreasing its specificity (*Figure 1.16(C)*).

Whether the sensitivity or the specificity of a test should be increased depends on the disease under investigation and the

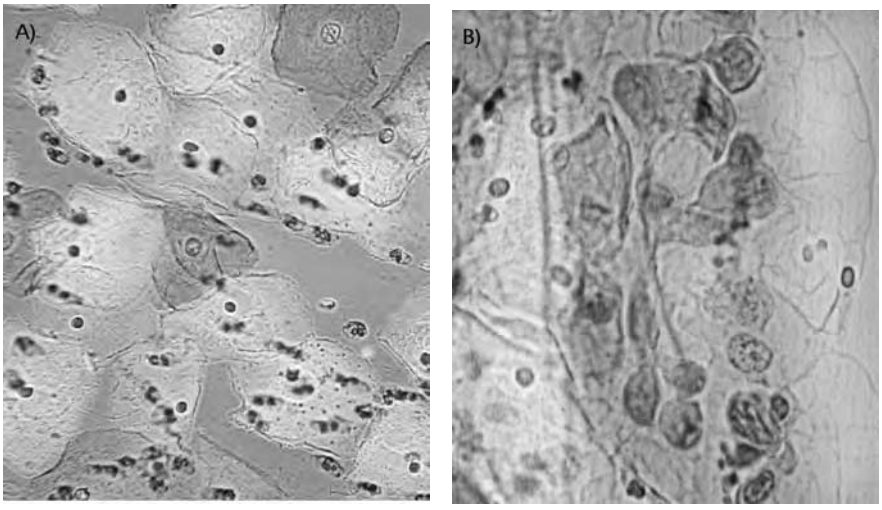

Figure 1.15 (A) Light micrograph showing normal squamous cells in a cervical smear from the superficial layer of the cervix and are from the layer of the cervical wall immediately below that of the squamous cells. The cells are healthy and have comparatively small nuclei. (B) Light micrograph showing abnormal cells from a different patient. Note the comparatively large nuclei compared with the healthy cells. Courtesy of H. Glencross, Manchester Cytology Centre, Manchester Royal Infirmary, UK.

consequences of making an incorrect diagnosis. Thus when screening for a disease with severe or fatal consequences, the test must have a high sensitivity. This ensures it will detect all results that are TP although some FP results will also be detected. Patients with positive results can then be investigated further to identify those with FP results. This test should also have a high predictive value for a negative test so that affected individuals are not missed when screening for the condition. Conversely, in some circumstances, it might be more important to have a test with high specificity. For example, if the purpose of the test is to identify and select patients for treatment with a new drug then it is necessary that the test has a high specificity. This will ensure that individuals without the disease are not selected and treated. This type of test should have a high predictive value for a positive result so that number of individuals with FP results are minimized and not subjected to any unnecessary treatment.

Physicians have to be very careful when interpreting the results of tests. It is obviously very unsatisfactory to tell a patient that he or she is suffering from cancer when this is not the case and *vice versa*. The wrong diagnosis may well lead to the wrong treatment being given.

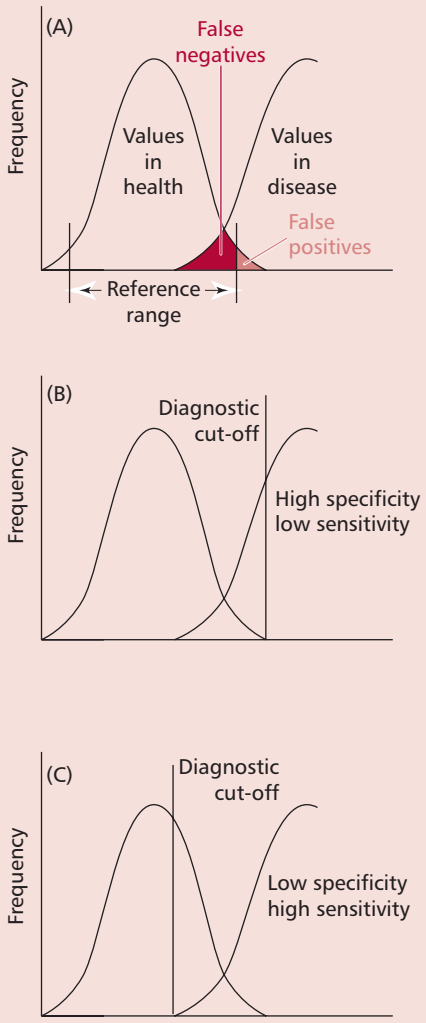

Figure 1.16 **(A) The range of results for tests in a healthy and diseased population overlap and so some patients with disease will have results within the reference range (false negatives) whereas others without disease will fall outside the reference range (false positives). (B) If the diagnostic cut-off value is set too high, then this will reduce false positives but increase the number of false negatives, that is, the test will have high specificity but low sensitivity. (C) If the diagnostic cut-off value is set too low then the number of false positives increase whereas number of false negatives decrease, that is, the test has low specificity and high sensitivity.**

used in certain groups of people to assess occupational exposure to harmful substances such as lead and radiation.

Laboratory tests can be used to indicate the risk of developing a disease. The risk of developing coronary artery disease increases with increasing concentration of blood cholesterol, more so if other risk factors, such as smoking, obesity or diabetes, are present.

Tests in pathology laboratories can indicate the likely outcome of a disease. Renal failure (*Chapter 8*) is a progressive disease that leads to a gradual build up of creatinine in the serum (*Figure 1.17*). Measurements of serum creatinine may therefore indicate end-stage renal disease when the patient may need dialysis to survive.

Vital information regarding the development and complications in a particular disease may be provided by laboratory tests. Urine is normally essentially free of protein; hence the presence of 30 to 200 mg dm^{-3} of serum albumin in the urine (microalbuminuria) of diabetic patients may indicate the development of nephropathy, a common secondary complication of diabetes.

Margin Note 1.1 Cervical smear screening

Cervical smear (*Figure 1.15*) testing is a screening that looks for abnormal changes in cells of the cervix, that is the neck of the uterus (*Chapter 17*). Some of these abnormal cells can develop into cancer over 10 or more years. The commonest cervical cancer, squamous cell carcinoma, is largely preventable given that treatment of the abnormal cells will remove them in more than 90% of cases although occasionally further treatment may be needed. However, no screening is 100% reliable and some abnormalities may go undetected, hence the importance of regular tests every three years if screening is to be effective in preventing cancer. The development of a precancerous state in the cervix is described and illustrated more fully in *Chapter 17*.

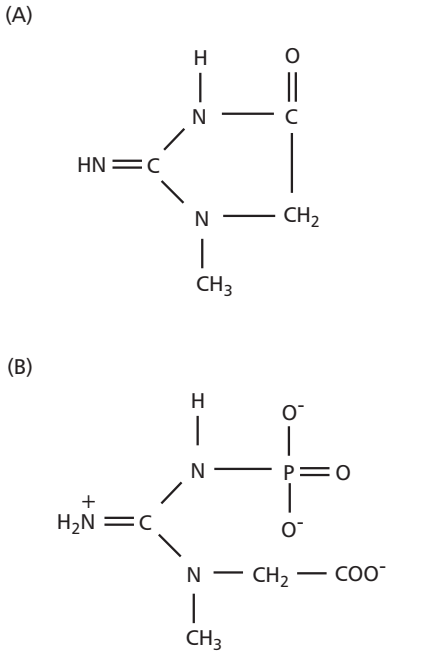

Figure 1.17 (A) Creatinine is formed from (B) creatine phosphate by the body at a relatively constant rate and excreted in urine. It is produced in amounts that are essentially proportional to muscle mass and so its concentration in blood is commonly used as an indicator of kidney function (*Chapter 8*).

1.8 HOSPITAL LABORATORY TESTS

Hospital laboratories routinely offer a wide range of clinical tests all of which must undergo a thorough evaluation for both analytical and clinical performance. The clinical demand for the test has to be established and its clinical relevance is subject to review. In addition to thorough evaluation of analytical methods, other aspects, such as the stability of samples, needs to be considered. For example, some samples must be assayed immediately while others can be stored at an appropriate temperature. All laboratory staff must be appropriately trained to ensure high analytical standards and produce valid data. This usually means that control urine and sera are run through the analyzers at regular intervals to check that the methods are working properly and reproducibly. Many of the companies that supply apparatus and reagents also supply standard sera for example, which are tested in laboratories all over the country, and the results are recorded in a nationwide database so that comparisons can be made and laboratories can check that their methods are all giving the same results.

Figure 1.18 illustrates the overall procedure routinely followed when a clinician requires a hospital test. Note that this procedure can be divided into a number of distinct steps:

- request for the test (a form; patient's details recorded);
- specimen collection, labeling, transport and storage (instructions);
- analysis (obtaining the results);
- interpretation (the results are often printed out with the range of values to help the doctor make an interpretation).

Prior to any test being requested, careful thought should be given as to whether the test is necessary and how its results will affect the management of the disease for the benefit of the patient. If this is not the case, then one has to consider the value in requesting and performing the test. Unfortunately clinicians sometimes request clinical tests that are unnecessary and will not be of benefit in treating the patient. This problem often occurs when using forms on which tests can be requested simply by ticking a box. Requesting unnecessary tests poses a number of problems for the patients, clinicians, the biomedical scientists (medical technologists in the USA) and the hospital. The test means that the patient is put to an inconvenience, as extra specimens are required. Unnecessary tests can be misleading and result in poor patient management while imposing a financial burden on the hospital. The increased workload for laboratory scientific staff may make the clinician in question rather unpopular! However, set against this is the fact that many of the machines used in the hospital for clinical analysis routinely test for a number of analytes, whether they are asked to or not, since it is easier to set up the machine in this way rather than to adjust them for individual patients.

Specimens are collected in a variety of ways (*Figure 1.19*) from the collection of blood using a simple thumbprick or, more usually a syringe, to surgery to obtain a **biopsy,** where a small piece of tissue is taken from the patient.

Blood needs to be collected with care (*Chapter 13*). If too many erythrocytes burst, this is known as **hemolysis**; the specimen will be unsuitable for the determination of some analytes: for example, the value obtained for 'serum K^+' will not be a true value since potassium is released from hemolyzed blood cells. Blood should not be collected from an arm that is receiving an infusion as a drip, since this will dilute the blood. Often, in such cases, the measured concentrations of electrolytes and glucose in the blood samples resemble those of the infusion fluid.

For some analytes, the blood must be collected into a tube containing an anticoagulant or preservative. Specimens of blood for glucose determination must be collected into a tube containing fluoride ions (F^-) since this inhibits glycolysis and prevents the utilization of serum glucose by blood cells. Occasionally blood is collected into the wrong tube and then decanted into the correct tube. This can cause a number of problems. For example, blood collected into a tube containing ethylene diamine tetraacetate (EDTA) will be unsuitable for the determination of serum Ca^{2+}, since EDTA is an anticoagulant, works by chelating and removing available Ca^{2+}.

The transport of specimens and their storage must be considered carefully since an inappropriate environment can influence the values of clinical test results. Swabs, for example, obtained during a microbiological investigation of an affected site, contain only a small volume of specimen and dry easily. They therefore need to be transported to the laboratory as quickly as possible

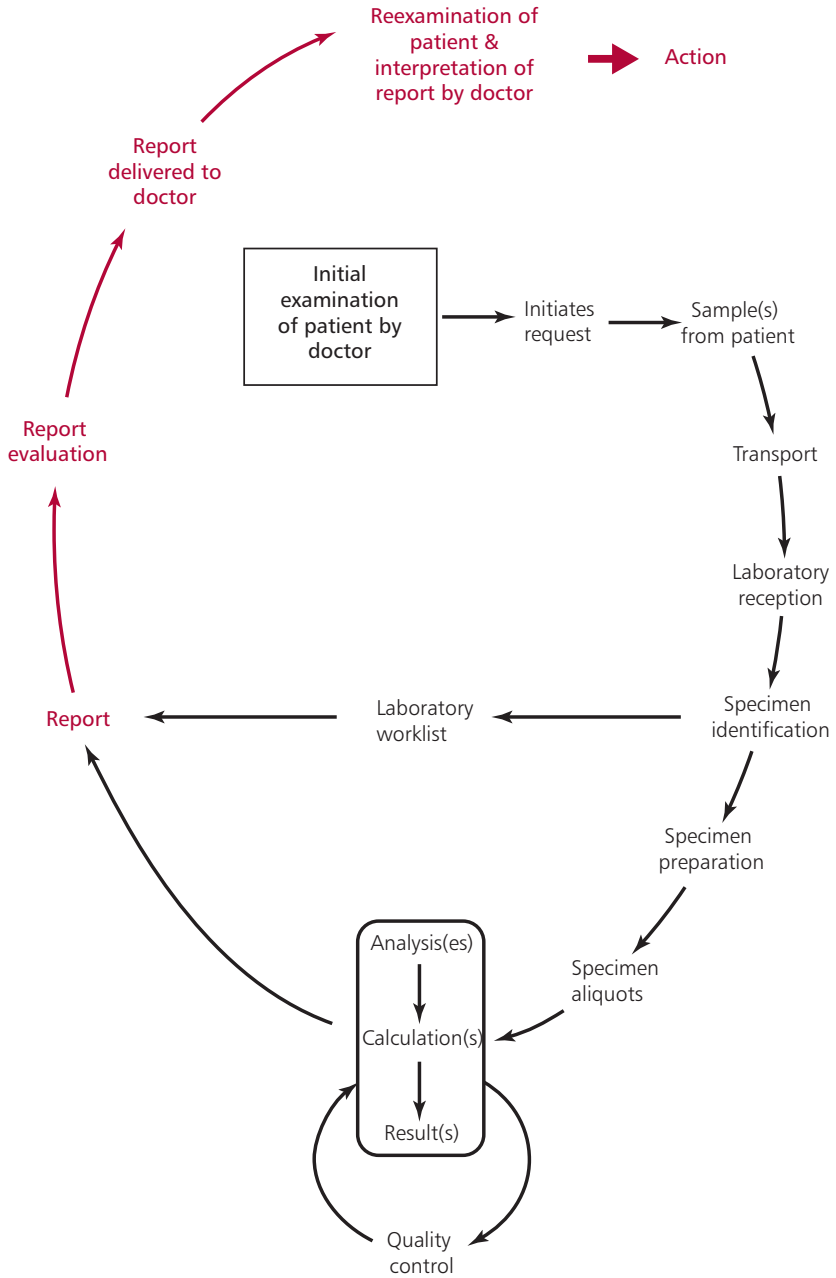

Figure 1.18 Sequence of events involved in obtaining a clinical test result.

Figure 1.19 Photograph of some tubes used in the collection of clinical specimens. Courtesy of BUPA Hospital, Manchester, UK.

Margin Note 1.2 Mean, standard deviation and coefficient of variance

The mean ($\bar{x}$) is the arithmetic average value of a particular group of measurements. It can be calculated from:

$$\bar{x} = \Sigma x / n$$

where n is the number of individual measurements and Σ is the total of individual values (x).

The standard deviation (SD) is a measure of the dispersion of the data. This is defined as the square root of $\Sigma (x-\bar{x})^2 / n-1$. Hence the smaller the value of the SD relative to that of the mean, the less dispersed the data. It can be seen from *Figure 1.20* that the $\bar{x} \pm$ SD include about 68% of the samples, the $\bar{x} \pm$ 2SD will include approximately 95% of the samples.

The coefficient of variance (cv) is also a measure of dispersion. It is related to both the $\bar{x}$ and SD:

$$cv = 100.SD / \bar{x}$$

Since both the mean and standard deviation have the same units, the coefficient of variance is a percentage; the lower its value the lower the dispersion.

to preserve the pathogens. Blood specimens that have been stored overnight may show erroneously high concentrations of serum K^+, phosphate and activities of erythrocyte enzymes because these all leak from the cells during storage. To prevent this happening, the serum should be separated from blood cells immediately following collection and stored separately if it is to be analyzed the following day.

Certain samples require a timed collection, for example, the collection of urine specimens over a 24-hour period for determination of creatinine clearance values (*Chapter 8*) or the collection of stools over a three-day period for fecal fat determination to assess malabsorption (*Chapter 11*). The results obtained for such tests often lack accuracy because of the practical difficulties in obtaining accurately timed specimens from the patient.

1.9 EVALUATION OF LABORATORY TESTS

The clinical tests used in hospital laboratories undergo thorough evaluation prior to usage in a laboratory. A number of factors are assessed including accuracy, precision, reliability, practicality, safety, ease of use, duration and cost.

Accuracy refers to the ability of a method to give results that are close to the true value of the substance (analyte) being measured. The results obtained from laboratory tests may be based on subjective assessment as, for example, following the microscopic examination of a tissue section obtained after biopsy during the investigation of a possible malignancy. These types of assessments rely heavily on the experience of the practitioner in recognizing and identifying key changes. Many tests, however, provide quantitative data such as blood glucose concentrations in diabetics, or the concentration of thyroid stimulating hormone (TSH) in the serum of a patient with suspected hypothyroidism. The interpretation of many clinical tests for analytes requires referral to its reference range (*see Section 1.9*).

The **precision** of a method refers to its ability to provide the same result every time it is used. Precision is assessed by repeatedly measuring samples taken from a single specimen and from batches of samples. The variation in the results may be assessed by calculating statistical parameters, such as standard deviation (SD) or coefficient of variance (cv).

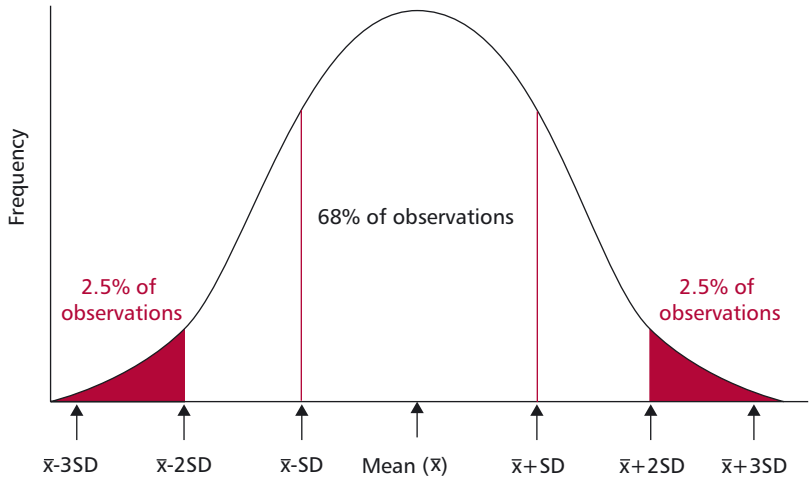

Figure 1.20 Gaussian distribution for results of a test in a healthy population. The reference range encompasses 95% of these results within –2 and +2 SD of the mean.

Often substances in biological materials are present in extremely low concentrations and it may be necessary for the clinical test to detect such low concentrations of analyte and, indeed, monitor changes in its concentration. The **analytical sensitivity** of a method is its ability to detect small amounts of the analyte under investigation. A related term is the limit of detection, which is the smallest amount of a substance that can be distinguished from the zero value. Biological material contains many components and some of these may interfere with the test being used, giving rise to unreliable results. For this reason, the **analytical specificity** of a test, that is, the ability of the method to detect only the test substance, may be determined.

In addition to analytical variation, test results are subject to biological variation. The discrimination between normal and abnormal results can be influenced by a number of biological factors. These include the sex and age of the patient, his or her diet, the time of collecting the sample, the posture adopted, whether the patient is stressed or had been exercising, the menstrual state of a female patient and whether she is pregnant, whether the person is taking drugs (legal or illegal). All these influence the results of the test. Thus, for example, plasma iron and urate values are higher in male than female patients; the activity of serum alkaline phosphatase is greater in growing children than in adults. Variations in diet may affect concentration of certain analytes, such as cholesterol (*Figure 1.21*). The values of some analytes, such as the concentration of cortisol in the plasma, show a diurnal variation. Blood should be collected from a seated patient since differences in posture at the time of blood collection can influence the concentrations of a number of analytes including the concentration of plasma proteins. Stress influences the release of a number of hormones, such as adrenaline and cortisol, while the concentrations of serum analytes, like creatine kinase and lactate, increase following exercise. The concentrations of ovarian hormones are strongly influenced by the menstrual cycle and corticosterone is known to vary by as much as 50% during different stages of the cycle. The nature and concentrations of many hormones change during pregnancy. Lastly, some drugs can influence results. For example, patients on estrogen-containing oral contraceptives often have an increased concentration of total plasma protein.

Analytical methods need to satisfy certain criteria to ensure they are practical and suitable for use in the laboratory. New methods are introduced into the laboratory only if they offer significant advantages over existing methods. New methods are assessed for their speed, that is, how many specimens can be processed in a given time and how long it takes to produce a result. The time a test takes may, of course, be vitally important in the care and treatment of a patient when urgent intervention is necessary.

Hospital laboratories have witnessed an increasing workload in recent years, and although they are helped by automation (*Figure 1.10*), they process very large numbers of specimens on a daily basis. Like all organizations, they have a finite budget and it is vital that the cost of tests is kept to a minimum. As well as direct costs, such as those for reagents, equipment and labor, there are indirect costs, such as the heating and lighting of laboratories. Safety is also of importance and biological, chemical, mechanical and electrical hazards associated with the method need to be assessed to ensure the safety of hospital laboratory staff.

1.10 REFERENCE RANGES

Reference ranges were mentioned earlier (*Section 1.7*). The reference range for any particular analyte can be obtained by measuring it in healthy individuals from a representative sample of the local population. Most laboratories use healthy blood donors. It may be important to know the normal range

Figure 1.21 Computer generated model of cholesterol.

in adults, children, males, females, particular ethnic groups, or pregnant or postmenopausal women. However, since no analytical method is 100% accurate and since individuals vary, care must be taken when establishing a reference range. To determine the reference range, the values for the measured analyte are plotted against their frequency in the selected population. In most cases, the resultant graph shows a normal or Gaussian distribution with most values clustered around the center as seen in *Figure 1.20*. The mean ($\bar{x}$) and standard deviation (SD) can be determined from these data.

In general, the reference range is taken to be between two standard deviations either side of the mean. This will cover 95% of the values obtained for the selected sample (provided the curve is Gaussian). The 95% reference range was selected as this minimizes any overlap between the results for a healthy population and those for a population with the disease. However, choosing a 95% range is one of the major limitations of reference ranges, since 5% of healthy individuals will, by definition, give results that are outside these values. Thus a test result outside the reference range does not necessarily imply that the individual is ill although it does indicate that there is a greater likelihood of the presence of disease.

The profile of test results for some substances, for example serum bilirubin, does not give a Gaussian curve but shows a skewed distribution. This skewed distribution can be transformed mathematically to a Gaussian distribution and a normalized reference range calculated.

The values of a number of analytes, such as serum iron and alkaline phosphatase vary with the age or sex of the patient. In such cases, age- and sex-matched reference ranges are required. When interpreting results for a particular patient, the ideal reference value would be obtained from the same patient before their illness and this is sometimes possible. For example, the concentrations of electrolytes in serum can be measured in a patient before an operation for comparison with those obtained postoperatively. However, in most cases the results for the patient before they became ill are not available.

1.11 QUALITY OF TEST RESULTS AND CLINICAL AUDITING

The results of tests performed in pathology laboratories assist with diagnosis of disease or the monitoring of treatment. Thus they can greatly influence the management of patients, and it is essential to assure the quality of laboratory results. Erroneous results have the potential to cause considerable harm (both physical and psychological) to patients and must be avoided. All laboratories have practices and procedures to ensure erroneous results are minimized and that good quality results are provided. Errors can arise at the three different stages of analysis, that is, preanalytical, analytical and postanalytical**.** Preanalytical errors occur before the sample has been analyzed. Analytical errors arise during the laboratory testing procedure. Postanalytical errors arise after the specimen has been analyzed.

In general, preanalytical mistakes result from inappropriate methods of collection or incorrect labeling, handling, transport or storage of the specimen. Experimental mistakes that can give rise to analytical errors are detected by introducing systems for each clinical test to warn when errors occur. This is normally achieved by analyzing a **control** sample within each batch of tests. A control sample is one that is identical in composition to the test samples except that it contains a known concentration of the test analyte. All samples, including the control sample, must be treated identically. For example, if the concentration of glucose in serum is being determined, then the control should be serum, not water, containing a known concentration of glucose.

Margin Note 1.3 False negatives and false positives

It is clear that false negative or false positive results from a test can have serious consequences (and no test is 100% reliable). This applies even more to tests that patients can carry out at home. One can imagine the anguish or joy that a false positive in a pregnancy test might cause. The recent development of a quick home test for HIV based on a saliva test is perhaps a more severe example. This HIV test, approved in the US, has raised fears that people who find that they are infected (or obtain a positive result) may kill themselves.

The control sample is usually an aliquot from a larger sample for which the mean and standard deviation have already been determined. The results for control samples are usually recorded graphically so that changes in the quality of the method are detected as soon as they arise. A common chart used for quality control purposes is the Levey-Jennings chart (*Figure 1.22*) in which the control limits are set at the mean ± 2 SD and ± 3 SD. If the control values fall outside the ± 2 SD limit, that is, there is drift away from the accepted limits, there is only a 5% probability that the result lies in a normal distribution around the mean and is still valid. Any results that lie outside the 3 SD warning limit suggests that a problem is occurring with the method. Problems could include unstable reagents in the analyzer, problems with temperature control or contamination, all of which require investigation. Occasionally there are gross (and usually very obvious) inaccuracies in the value of a test result such that it bears no resemblance to values seen in health or disease. These are referred to as 'blunders'. Blunders usually arise because of transcriptional errors in reporting the result. To reduce the number of blunders, results should be checked thoroughly by senior staff before being sent to clinicians.

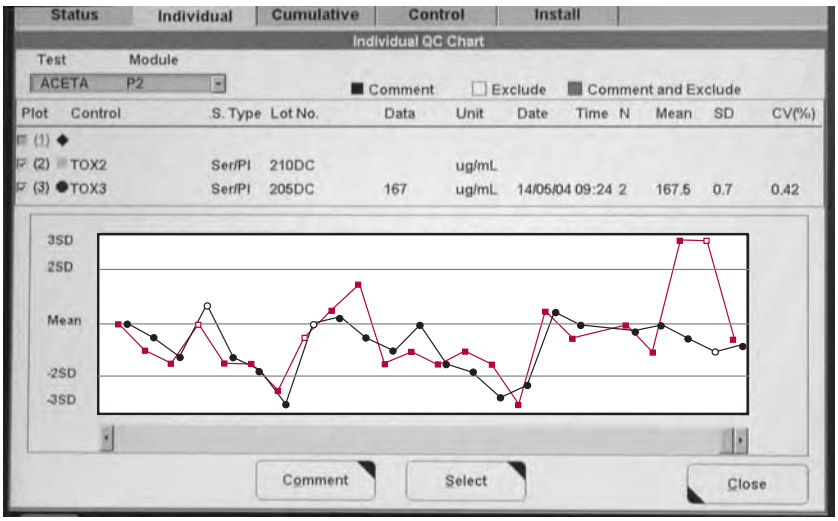

Figure 1.22 Levey-Jennings Chart used to assess quality control of quantitative results of tests. The values of controls are plotted as means ± 2 and 3 SD. When results are plotted out like this, any trend affecting quality of test can be detected, for example the values for one control (red) lie outside ± 3 SD. Courtesy of Department of Clinical Biochemistry, Manchester Royal Infirmary, UK.

Most laboratories have their own quality control samples and these are used for internal quality assurance purposes. Many countries now participate in external quality assurance, whereby quality control samples are sent to participating laboratories from a central source to assess the analytical performance of their methods for particular analytes. Furthermore, to ensure quality of service provision, many laboratories follow a set of procedures required for accreditation by external agencies. These procedures ensure good laboratory practice (GLP) and cover all aspects of the laboratory that are involved in the production of test results. These procedures ensure that all laboratory staff are adequately trained and have clearly defined responsibilities. The equipment used should be of adequate standard with a logbook showing a full record of maintenance and faults. All methods used in the laboratory are standardized, fully documented and appropriate for the analysis. Full details of each method are provided as a standard operating procedure (SOP) that includes details of specimen handling, the analytical method, equipment used and quality control procedures.

To improve the quality of the services they provide, many laboratories participate in some form of audit. **Clinical audit** (*Figure 1.23*) is a process whereby practices and procedures involved in patient care are monitored and, if necessary, revised to provide a more efficient and cost-effective service that should ultimately benefit the patient. Audit is part of the process of ensuring

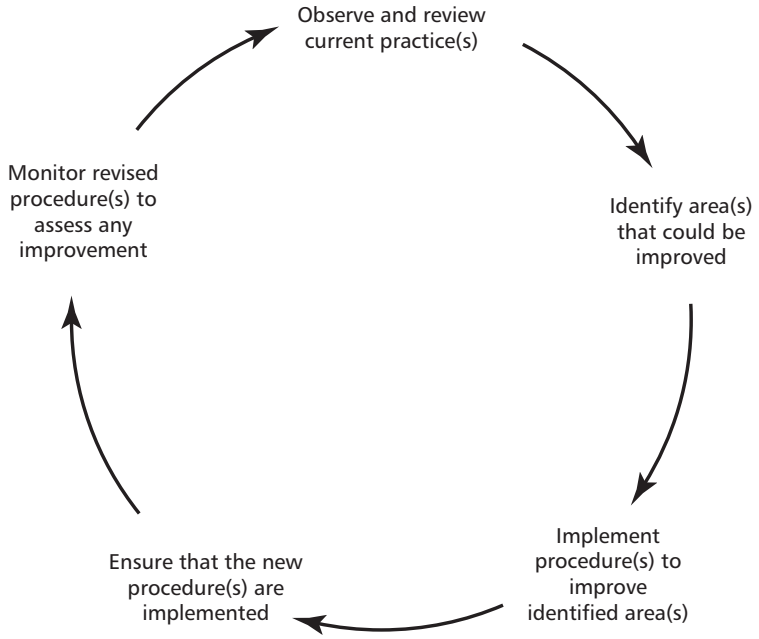

Figure 1.23 Outline of the five stages of a clinical audit.

quality and is usually divided into five stages. The first is the observation and review of current practice and procedures in the laboratory. The second stage involves the identification of areas of concern that could be improved and questions are asked as to whether the current service can be provided more economically. Third, a series of changes are devised to rectify and improve the identified area(s) of concern. Fourth, these changes are implemented and steps taken to ensure compliance and, finally, at the fifth stage, the changes are monitored and compared with previous procedures in order to assess whether there is indeed any improvement in the service provided or whether the revised procedures are actually more cost effective.

A clinical audit is usually followed by a re-audit after an appropriate period of time. The audit may include several processes such as the initial stages of test requesting, specimen collection and transport. The audit may wish to investigate whether appropriate advice is available to clinicians requesting tests, whether the test request forms are easy to use or whether appropriate containers are provided for specimen collection. Other types of audit processes may relate to the analytical service provided by the laboratory, such as whether the repertoire of tests offered is appropriate to the needs of the clinical service. A clinical audit may wish to investigate whether provision of the laboratory service out of hours is efficient and cost-effective and whether test results are being returned to the clinicians at the right place and within an appropriate time.

CASE STUDY 1.1

The serum K^+ concentration of Emma, a 22-year-old patient, about to undergo surgery was determined. The laboratory sent a value of 35 mmol dm^{-3} to the ward. The reference range for serum K^+ is 3.5 to 5.0 mmol dm^{-3}.

Question

What is the most likely explanation of this result? Discuss its implications.

CASE STUDY 1.2

A new test for the detection of prostate cancer has been developed and is undergoing clinical evaluation to determine how effective it is in the diagnosis of this condition. The study included 100 healthy men and 100 men with known prostate cancer. All were screened using the new test, which involved the measurement of a serum tumor marker. The patients were rated either positive or negative depending on whether the determined concentration of the tumor marker was above or below a certain cut-off value. The results obtained were as follows:

	Positive	Negative
Patients with prostate cancer	95	5
Healthy individuals	5	95

Question

What are the sensitivity and specificity of the test? Discuss the values.

1.12 SUMMARY

Health is a 'state of physical, mental and social well-being, not merely the absence of disease'. Disease refers to any abnormality or failure of the body to function properly. For medical treatment to commence the disease needs to be diagnosed and its etiology established. This also helps to define the prognosis, that is, the likely course of the disease and its outcome. Diseases have a number of possible causes. They may be exogenous, such as infections or trauma (accidents) or they may be endogenous such as diabetes or cancer. Some diseases may be caused by a range of factors and are called multifactorial and, for some, the cause may be unknown and these are referred to as idiopathic.

The pathogenesis describes how the etiological agent produces the clinical signs and symptoms and the pathological changes characteristic of that disease. This enables the physician to make a diagnosis and prescribe treatment. The prognosis should also emerge at this stage.

Diseases may be classified into a number of types: genetic, infectious, endocrine, traumatic, degenerative, immunological, nutritional, homeostatic, neoplastic (cancer), toxic, psychogenic and iatrogenic (caused by the treatment itself). Epidemiology is the study of how diseases spread in populations. This is of importance in the control of diseases.

Various types of laboratories specialize in investigating the pathology of diseases. A laboratory may measure the concentrations of analytes in blood and urine, identify infectious agents such as bacteria and viruses, characterize genetic diseases by looking at the patient's chromosomes, or identify problems with the blood, for example those involving defective hemoglobin or clotting factors. The laboratories report their findings to the clinician in charge of the patient to help in the process of diagnosis and in making decisions on treatment and how well any treatment is working. Pathology laboratories must work to high standards of accuracy, otherwise wrong treatments may be given and patients may be misinformed about their disease and its prognosis. Therefore pathology laboratories take steps to standardize and check their procedures on a daily basis and investigate new tests exhaustively before they are introduced.

QUESTIONS

1. Which of the following terms best describes a test used to detect disease before it presents clinically?
   a) diagnostic test;
   b) sensitive test;
   c) screening test;
   d) prognostic test;
   e) specific test.

2. The term used to describe the cause of a disease is:
   a) manifestation;
   b) etiology;
   c) pathogenesis;
   d) mortality;
   e) epidemiology.

3. A Na^+ solution of 10 mmol dm^{-3} was measured by two methods X and Y. The value obtained with method X was 11.8 mmol dm^{-3} and for method Y it was 9.7 mmol dm^{-3}. Which one of the following statements is correct?
   a) Method X is more accurate than method Y.
   b) Method X is more precise than method Y.
   c) Method Y is more accurate than method X.
   d) Method Y is more precise than method X.
   e) Method X is as accurate as method Y.

4. The concentration of Mg^{2+} in the serum of an individual is 0.5 mmol dm^{-3}. The reference range is 0.7 to 1.0 mmol dm^{-3}. Is it possible to be 100% certain that this individual has a deficiency of Mg^{2+}? Explain your answer.

FURTHER READING

Henny, J and Hyltoft Petersen, P (2004) Reference values: from philosophy to a tool for laboratory medicine. *Clin. Chem. Lab. Med.* **42:** 686–691.

Hooper, J, McCreanor, G, Marshall, W and Myers, P (1996) *Primary Care and Laboratory Medicine*. ACB Venture Publications, Cambridge.

Irjala, KM and Gronroos, PE (1998) Preanalytical and analytical factors affecting laboratory results. *Ann. Med.* **30:** 267–272.

Khan, KS, Dinnes, J and Klijnen, J (2001) Systematic reviews to evaluate diagnostic tests. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **95:** 6–11.

Libeer, J-C (2001) Role of external quality assurance schemes in assessing and improving quality in medical laboratories. *Clin. Chim. Acta* **309:** 173–177.

Moyer, VA and Kennedy, KA (2003) Understanding and using diagnostic tests. *Clin. Perinatol.* **30:** 189–204.

Stewart, A (2002) *Basic Statistics and Epidemiology*. Radcliffe Medical Press, UK.

Stolley, PD and Lasky, T (1998) *Investigating Disease Patterns*. Scientific American Library, NY.

Thagard, P (1996) The concept of disease: structure and change. *Commun. Cogn.* **29:** 445–478.

Wachel, M, Paulson, R and Plese, C (1996) Creation and verification of reference intervals. *Lab. Med.* **26:** 593–597.

Weinstein, S, Obuchowski, NA and Lieber, ML (2005) Clinical evaluation of diagnostic tests. *Am. J. Roentgenol.* **185:** 14–19.