Praise for previous editions

“...This is a really useful book for midwives... I would recommend this for any team or ward reference library.”

The Practising Midwife, January 2009

“If you have not come across this book before then I would certainly recommend this new improved edition.”

Accident and Emergency Nursing, 2007

Understanding Laboratory Investigations helps nurses, midwives and healthcare professionals to better understand how the work of clinical laboratories contributes to patient care. It answers the following questions:

- Why is this test being ordered on my patient?
- What sort of sample is required?
- How is that sample obtained?

And most importantly:

- What is the significance of the test result for my patient?

Retaining its accessible and user-friendly style, this new and updated edition remains a key resource to provide nurses with as much relevant information as possible about the most commonly requested laboratory tests. This is not a book about laboratory technique—its focus is on the clinical significance of test results, and therefore the patient. This edition discusses a more comprehensive range of tests, and includes new content on paediatrics and colour to aid accessibility.

Key features

- A glossary of terms commonly used in laboratory medicine
- Discussion of tests most commonly requested and likely to be encountered by nurses
- Relevant to nurses, other healthcare workers and students of biomedical sciences interested in pursuing a career in laboratory medicine
- A brand new chapter on neonatal screening as well as discussion of tests not previously covered in earlier editions
- More case studies designed to illustrate the practical clinical use of the test being discussed
Understanding Laboratory Investigations

A Guide for Nurses, Midwives and Healthcare Professionals
For Mary, Tom and Jon again
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PREFACE

The purpose of this book is to help nurses to understand better how the work of clinical laboratories contributes to patient care. It is intended to answer the following questions:

- Why is this test being ordered on my patient?
- What sort of sample is required?
- How is that sample obtained?

And most importantly:

- What is the significance of the test result for my patient?

Answers to these questions must be based on an understanding of basic science. Care has been taken to introduce this science, which includes some basic biochemistry, physiology and anatomy, in a way which is accessible to all those with an interest in how the body works. Much will be familiar to nurses.

The format of the book is simple. After two introductory chapters (one of which emphasises the role of nursing staff in the process of laboratory testing), each chapter is devoted to consideration of a single test or group of related tests. Each of these chapters begins with some relevant biochemistry, physiology or anatomy to put the substance being measured (i.e. the test) in some physiological context. A consideration of the sample requirements follows, and finally interpretation of the test results. Wherever possible, patient pathology, symptoms and test results are related. Mock case histories are included at the end of each chapter to illustrate the practical clinical use of the test being discussed and give a human face to the science.

It is not possible in a book of this size to discuss all of the tests performed in clinical laboratories in this degree of detail, so it has been necessary to be selective. The tests discussed are the most commonly requested, and those which nurses are most likely to encounter. Taken together the tests discussed in this book account for around 70–80% of the total workload of clinical laboratories in the average district general hospital.
Although the primary audience for this book is nursing staff, it should be of interest to other healthcare workers and also students of biomedical sciences interested in pursuing a career in laboratory medicine.

The general format and philosophy of this book remains unchanged for this third edition but some revision has been necessary in the light of changing practice and new research. Significant additional features include a chapter on neonatal screening for disease, and discussion of some tests not considered in previous editions. These include the D-dimer test and the B-natriuretic peptide (BNP) test; inclusion of both is justified on the basis of their increasing clinical use.

In response to the welcome advice of reviewers, the number of mock case studies has been significantly increased and an attempt has been made to render the text more relevant to midwives and neonatal nurses.

Acknowledgements

Very special thanks to my son Jon who conceived and prepared the artwork for previous editions and willingly agreed to update it once again for this third edition. Also to my wife Mary for her continued support and patience.

Thanks too of course to everyone at Wiley-Blackwell involved in the production of the book, especially Magenta Styles for commissioning this edition and Catriona Cooper for assistance during preparation of the manuscript.
PART 1

Introduction
InIntroduction To Clinical Laboratories

Patients may be subjected to many kinds of investigative procedure. These range in complexity from ward or clinic based measurements familiar to all nurses, such as determining body temperature, pulse and blood pressure, through monitoring of heart function by electrocardiographic (ECG) machines to body imaging techniques, such as X-ray, computed tomography (CT) and magnetic resonance imaging (MRI) scan. All of these require the presence of the patient; they are performed on the patient, if not by nurses, at least often in their presence.

In contrast, all the investigations described in this book are performed on samples removed from the patient. The remoteness of patients from the site of laboratory testing might engender the understandable though misguided perception that laboratory testing has little to do with nursing care. In fact an understanding by nursing staff of the work of clinical laboratories is important for several reasons.

Nurses are in a unique position to satisfy the need expressed by many patients for information about the tests that they are subjected to. Recent research confirms the intuitive notion that patients want to understand the purpose of tests and significance of their test results. This may be to allay fears and anxieties among those who have never undergone such a test before, or it may simply reflect a right to know. Most laboratory tests are only minimally invasive but can only be done with a patient’s implied informed consent. Of course many patients will express no interest, but some have questions that must be addressed.

Key learning topics
• The role of the nurse in patient testing
• The five sub-disciplines of clinical pathology
• Clinical laboratory staffing and costs

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Nurses sometimes have responsibility for the collection and timely, safe transport of patient samples. It is vital that anyone collecting samples is aware of the importance of good practice during this pre-testing phase.

Nurses are frequently involved in the reception of laboratory test results. It is important that they are familiar with the terminology and format of laboratory reports and are able to identify abnormal results, particularly those that warrant immediate clinical intervention.

For many years nurses have performed limited testing of blood and urine samples (e.g. blood glucose and urine dipstick testing) in wards and clinics. With advances in technology, an ever increasing repertoire of tests can now be performed, within minutes, outside the laboratory in clinics and wards by nursing staff. This so called ‘point of care testing’ is particularly well established in intensive care, coronary care and emergency room settings where speed of analysis has proven benefit for patient care. It is important that nurses involved in the analysis of patient samples understand the pitfalls, limitations and clinical significance of this aspect of their work.

Traditionally, doctors have had sole responsibility for both requesting and interpreting laboratory test results, but the developing role of the clinical nurse specialist has required that some nurses become involved in both of these processes. In any case all...
qualified nursing staff members have to make judgements about how the results of laboratory tests might impact on the formulation of nursing care plans for their patients.

Finally, there are those nurses whose professional role requires especially detailed knowledge of the work of the laboratory as well as close co-operation with laboratory staff. These include haematology nurse specialists, blood transfusion nurse specialists, infection control nurses and diabetic nurse specialists. Figure 1.1 summarises the role of nursing staff in laboratory testing.

All the tests described in this book are performed – although, as has been made clear not exclusively so – in clinical pathology laboratories. The second part of this introductory chapter serves to describe in outline the work of the five sub-disciplines of clinical pathology. The range of samples tested is listed in Table 1.1.

Table 1.1 Range of samples used for laboratory investigation.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical analysis</td>
<td>Usually blood or urine&lt;br&gt;Less common: &lt;br&gt;• Faeces &lt;br&gt;• Cerebrospinal fluid (CSF): this is the fluid that surrounds the brain and spinal cord &lt;br&gt;• Pleural fluid: this is the fluid that surrounds the lungs in the pleural cavity and is only obtainable when there is abnormal accumulation, a condition called pleural effusion &lt;br&gt;• Ascitic fluid: this is the fluid that surrounds the abdominal organs in the peritoneal cavity and is only obtainable when there is abnormal accumulation, a condition called ascites</td>
</tr>
<tr>
<td>Haematological analysis</td>
<td>Usually blood only&lt;br&gt;Less common: &lt;br&gt;• Bone marrow aspirate &lt;br&gt;• Bone marrow biopsy</td>
</tr>
<tr>
<td>Microbiological analysis</td>
<td>Common: &lt;br&gt;• Urine &lt;br&gt;• Faeces &lt;br&gt;• Sputum &lt;br&gt;• Swabs of any (potentially infected) accessible site including: throat, nose, eye, wound, vagina&lt;br&gt;Less common: &lt;br&gt;• Cerebrospinal fluid &lt;br&gt;• Pleural fluid &lt;br&gt;• Vomit &lt;br&gt;• Skin scraping</td>
</tr>
<tr>
<td>Histopathological analysis</td>
<td>Tissue samples (biopsy) only</td>
</tr>
<tr>
<td>Cytopathological analysis</td>
<td>Urine &lt;br&gt;Sputum &lt;br&gt;Cellular material obtained by scraping the surface of organs or aspirating abnormal fluids (e.g. cysts)</td>
</tr>
<tr>
<td>Immunological analysis</td>
<td>Usually blood only</td>
</tr>
</tbody>
</table>
The clinical chemistry laboratory

(also known as chemical pathology, clinical biochemistry)
Clinical chemistry is concerned with the diagnosis and monitoring of disease by measuring the concentration of chemicals, principally in blood plasma (the non-cellular, fluid portion of blood) and urine. Occasionally, chemical analysis of faeces and other body fluids, for example cerebrospinal and pleural fluid, is useful.

Blood plasma is a chemically complex fluid containing many inorganic ions, proteins, carbohydrates, lipids, hormones and enzymes, along with two dissolved gases, oxygen and carbon dioxide. In health the concentration in blood of each chemical substance is maintained within limits that reflect normal cellular and whole body metabolism. Disease is often associated with one or more disturbances in this delicate balance of blood chemistry; it is this general principle that underlies the importance of chemical testing of blood for the diagnostic process. The range of pathologies in which chemical testing of blood and urine has proven diagnostically useful is diverse and includes disease of the kidney, liver, heart, lungs and endocrine system. Diseases that result from nutritional deficiency can be identified by chemical analysis of blood. The cells of some malignant tumours release specific chemicals into blood. Measurement of these so called tumour markers allows a limited role for the clinical chemistry laboratory in the diagnosis and monitoring of some types of cancer.

The safe and effective delivery of some drug therapies depends on measuring the blood concentration of those drugs. This is just one aspect of the broader monitoring of treatment role that the clinical chemistry laboratory serves.

Most chemical testing of blood and urine is performed on highly sophisticated, automated machinery. Modern clinical chemistry analysers can perform up to 1000 tests per hour; 20 or more different chemical substances can be measured simultaneously on these analysers using a single blood sample. Results of the most commonly requested tests, which include nearly all those discussed in this book, are usually available within 12–24 hours of receipt of the specimen. More rarely, requested tests may be performed just once or twice a week, and a small minority are only performed in specialist centres. Nearly all clinical chemistry laboratories provide an urgent 24 hour per day service for a limited and defined list of tests; results of such urgently requested tests can usually be made available within an hour.

Critically ill patients being cared for in intensive care units and emergency rooms often require frequent and urgent monitoring of some aspects of blood chemistry. In these circumstances blood testing is performed by nursing staff, using dedicated analysers sited close to the patient; this represents one aspect of point of care testing.

The haematology laboratory

Haematology is concerned principally with the diagnosis and monitoring of diseases that affect the number, size and appearance of the cellular or formed elements of blood. These are: the red blood cells (erythrocytes), the white blood
cells (leucocytes) and platelets (thrombocytes). The full blood count (FBC) is probably the most frequently requested laboratory test and certainly the most frequently requested haematology test, reflecting the range of common and less common disorders which affect both the numbers and appearance of blood cells. It is in fact not one but a battery of tests.

Modern haematology analysers are able to process FBC tests at the rate of up to 400 samples per hour. The detailed information about the blood cells which these analysers provide has dramatically reduced the number of specimens that need to be examined under the microscope, but the microscope remains an essential tool to the haematologist for examination of bone marrow biopsy specimens and, in some circumstances, blood.

Apart from the cells in blood, haematology is also concerned with measurement of the concentration of some of the proteins present in blood plasma that are involved in the complex process of blood coagulation.

Disorders of blood in which haematology testing is vital include haematological malignancy (e.g. the leukaemias, Hodgkin’s disease, myeloma), anaemia and diseases in which disturbances of blood coagulation result in an increased tendency to bleed (e.g. haemophilia) or an increased tendency for blood to coagulate within blood vessels (e.g. deep vein thrombosis). Some haematology tests including the FBC are useful in the diagnosis or clinical management of common, non-haematological diseases. For example, infectious disease is usually associated with an increase in the number of white blood cells. Anaemia is often a feature of kidney disease, chronic inflammatory disorders such as rheumatoid arthritis and some diseases that result from nutritional deficiency.

Many patients at risk of heart and blood vessel (cardiovascular) disease are prescribed medication that inhibits the process of blood clotting (coagulation). This anticoagulation therapy must be monitored by regular blood testing to prevent bleeding, a potentially dangerous side effect of such therapy.

Most haematology test results are routinely available within 12–24 hours. However if the need is clinically justified, results of some haematology tests, including FBC, can be made available within an hour, at any time of the day or night.

**The clinical microbiology laboratory**

Clinical microbiology is concerned with the diagnosis and monitoring of disease caused by infective agents, mostly bacteria but also viruses, fungi and parasitic worms. Much of the work involves isolation and identification of bacteria from many sorts of sample, including urine, sputum, faeces, blood, cerebrospinal fluid and swabs taken from a variety of infected sites. Bacteria can sometimes be seen by examining these specimens under the microscope, but more precise identification can only be made after culture, or ‘growth’ of bacteria on nutrient enriched media. One of the problems encountered by the microbiologist when dealing with clinical specimens is that many bacterial species are normally present in many sites around the body; indeed in some cases they are essential for normal health. The
microbiologist must isolate those that are pathogenic (i.e. cause disease) from those that are normally present, and from any environmental bacterial contaminant introduced during sample collection. Some body fluids are normally sterile; these include blood, cerebrospinal fluid, and fluid aspirated from joints and the pleural cavity. Bacteria isolated from these sites are always pathogenic.

Having isolated and identified a species of pathogenic bacteria, the next step is to test the sensitivity of the organism to a range of antibiotics. This information helps in deciding which antibiotic therapy is likely to be most effective in eradicating the infection.

Blood testing plays an important and evolving role in detecting infections that are caused by organisms difficult to isolate by culture. During any infection the immune system produces specific antibodies directed at specific antigens present on the surface of the invading organism. A rising amount of the antibody in blood provides evidence of current infection.

Specific antigens present on the surface of microorganisms also provide a means of identifying infective agents. Testing blood for the presence of viral antigens is an important means of diagnosing viral infections such as those which cause hepatitis and acquired immune deficiency syndrome (AIDS).

Some microbiological investigation may take from several days to several weeks to complete; this delay is governed largely by the speed of bacterial growth in culture. Initial microscopical examination can be performed immediately on receipt of the specimen if clinically necessary, and results can usually be made available on the day the sample is received in an interim report.

Clinical microbiology laboratories operate a 24 hour service for the rare cases when urgent culture and microscopical examination of samples is necessary. These include suspected cases of immediate life threatening infections of blood (septicaemia) and central nervous system (meningitis).

Quite apart from their diagnostic role, hospital microbiology laboratories play an important role with infection control nurses in the monitoring and prevention of nosocomial infectious disease, that is infectious disease acquired by patients whilst in hospital – an ever present problem, which impacts on the working life of all nursing staff.

**The blood transfusion laboratory**

Blood transfusion is concerned with the provision of a safe supply of blood and blood products. In contrast to other pathology departments, blood transfusion has limited diagnostic function. In some senses its function more resembles a pharmacy, in that its main purpose is to supply therapeutic products. Transfusion of whole blood is very rarely practiced nowadays; rather specific components of whole blood are transfused. The most frequently transfused blood product is red cells, to correct anaemia and to replace blood lost during surgery or as a result of trauma or complication during childbirth. Much less commonly, the white cells of blood, platelets and the proteins present in blood plasma are therapeutically useful.
The National Blood Service (NBS) is responsible for the collection and supply of safe (disease free) donated blood products to hospital blood transfusion laboratories. Here, each donated unit of red cells must be tested for compatibility with the patient's blood before it can be transfused. The transfusion of incompatible blood products can have very serious health consequences and is potentially fatal. Advances in compatibility testing have ensured that compatible red cells can be made available for transfusion usually well within an hour of a patient's blood sample arriving in the laboratory; this service is available 24 hours a day.

The blood transfusion department also has an important specific diagnostic role for some forms of haemolytic anaemia, in which the body produces antibodies against its own red cells. One important aspect of this work is haemolytic disease of the newborn, a potentially fatal condition in which the red cells of the developing foetus are destroyed by antibodies present in the mother’s blood. All pregnant women are routinely tested for the presence of such antibodies.

The histopathology laboratory

(also known as morbid anatomy, cellular pathology)

Histopathology, the oldest of all pathology disciplines, is concerned with the diagnosis of disease by microscopical examination of tissue samples (biopsies). The rationale for this approach is that disease processes, for example, malignancy, inflammation, infection etc., are characterised by specific changes at the tissue and cellular level, which are evident when tissue is viewed under the microscope. There are many ways of recovering tissue samples from the body. Tissue from the gastrointestinal tract, lungs and urinary tract are commonly sampled at the time of endoscopic examination. An endoscope is an instrument used to visually examine internal organs directly by fibre optics. The instrument includes small forceps which can be used to remove small pieces of tissue during the examination. Tissue may be taken during surgery by incision or excision biopsy. Incision biopsy is the removal of a sample cut from an area of diseased tissue, whereas excision biopsy involves removal of the whole area of diseased tissue.

Before transport to the laboratory, biopsy samples must be ‘fixed’ in a chemical fixative, usually formalin, to preserve structure. This process can take from a few hours to a whole day depending on the size of the specimen. In the laboratory ‘fixed’ specimens are impregnated with paraffin wax, allowed to harden and then cut into very thin sections just 3–5 µm thick. These wafer thin sections are then mounted on glass microscope slides and stained with chemicals, before examination under the microscope. The whole process from reception of specimen to issue of a histopathological report can take from one to four days depending on the size of the biopsy sample. Sometimes it is important to make a diagnosis very quickly, and in these circumstances a frozen section is performed. Tissue is ‘fixed’ immediately by freezing. This process allows tissue sections to be cut almost immediately the sample is removed from the patient. The sections are stained and examined under the microscope. This rapid technique allows a diagnosis of, for example,
breast cancer to be made rapidly whilst the patient remains anaesthetized on the operating table. Armed with a laboratory report that confirms malignant disease, the surgeon can proceed immediately to surgical treatment.

Microscopical examination of tissue removed from the patient is probably most widely used in the diagnosis and staging of malignant disease in organs throughout the body. It is also used in the differential diagnosis of non-malignant disease of all body organs. It has a role in the diagnosis of connective tissue and skin disorders and in the early diagnosis of tissue rejection among patients who have received transplanted organs.

Clearly all histopathological tests are invasive, often requiring surgical intervention to recover samples. Both financial and patient safety consideration ensure that, unlike other laboratory investigations, histopathological investigations are reserved for those patients in whom there is a strong suspicion of serious disease. In many cases this suspicion will have been raised by the abnormal results of blood and urine tests performed in other pathology laboratories, so that histopathological examination of tissues can represent the final stage in laboratory diagnosis.

Finally, post mortem examinations to determine cause of patient death are conducted in the hospital mortuary, which is administratively part of the histopathology department.

**Cytopathology**

Cytopathology is a sub-discipline of histopathology. Whereas histopathology is concerned with microscopical examination of tissue samples, the focus of the cytopathologist is the cells that are normally exfoliated from the epithelial surface of organs. Sample recovery is less invasive than that required for histopathological investigation. Typically cells are scraped from the surface of organs such as the cervix, the mucosal surface of the duodenum and stomach and lungs. Cells can also be recovered by aspiration using a fine needle and syringe, from the pleural and peritoneal cavities, or from solid tumours, for example, in the breast. The cells are spread onto a glass microscope slide, fixed and stained and then examined under the microscope. Cytopathology is almost exclusively concerned with diagnosis of pre-malignant and malignant disease. The cervical smear test, used to screen all women for risk of cervical cancer, accounts for a large proportion of the workload of the cytopathology laboratory.

**The immunology laboratory**

Clinical immunology laboratories are concerned principally with blood testing for the diagnosis of autoimmune diseases, in which the body’s normally protective immune system produces antibodies against its own tissue antigens. These self-reacting, destructive antibodies are called autoantibodies. The detection in blood of organ specific autoantibodies is helpful in the diagnosis of many diseases with an autoimmune component including coeliac disease, thyroid disorders, pernicious anaemia, systemic lupus erythematosus (SLE) and autoimmune disease of kidney and liver disease.
Clinical laboratories are staffed by graduate trained biomedical scientists (BMS), who are responsible for the analysis of samples and the overall day to day management of the laboratory departments. They are helped in the analytical task by medical laboratory assistants (MLAs) who may have the additional responsibility of blood sample collection (phlebotomy) from patients. Cytoscreeners are a specially trained group whose work is confined largely to the examination of cervical smears.

Each laboratory department is headed by a medically qualified doctor of consultant status who has specialised in one area of laboratory medicine (in some cases a non-medically qualified clinical scientist fulfils this role). They provide consultancy for doctors and nurses on all aspects of laboratory medicine, so that they might advise both on the most appropriate laboratory investigation in particular cases, and the clinical significance of test results. Haematology consultants also have clinical responsibility for the care of patients suffering haematological disease (e.g. leukaemia). Medical consultants attached to clinical chemistry departments are often responsible for the clinical care of patients suffering diabetes and other metabolic and endocrine disorders, whilst a microbiology consultant advises doctors on the most effective use of antibiotic therapy and is responsible, with the control of infection nurse, for the formulation and implementation of the hospital control of infection policy.

Medically qualified histopathology consultants examine tissues prepared by biomedical scientists and make a histopathological diagnosis based on this examination. They provide diagnostic and prognostic advice to the medical team caring for cancer patients, although their clinical input is by no means confined to this patient group. They also perform all post-mortem (autopsy) examinations, with the assistance of an anatomical pathology technician.

The influential, government initiated Carter Review of Pathology Services\(^1\)\(^2\)\(^3\) determined that 70–80% of all healthcare decisions regarding diagnosis and treatment are influenced by the results of laboratory tests. The Review provides the most reliable national data on pathology workload and costs. An estimated 500 million clinical biochemical tests and 130 million haematology tests are conducted each year in National Health Service (NHS) clinical laboratories across England. Additionally, 50 million microbiology requests are processed and 13 million histopathology slides along with 4 million cytology slides are examined. Demand for pathology testing rises at the rate of 8–10% per year. Primary care (GP) test requests account for around 40% of total laboratory workload, the rest is generated within hospitals (both inpatient and outpatient departments). Carter estimates the annual cost of NHS pathology services in England to be close to £2.5 billion, equivalent to 3.5% of the total NHS budget. The median (average) cost of a routine high-volume laboratory test – which describes nearly all of those discussed in this book – varies between hospitals and pathology discipline: clinical biochemistry (average: £1.00
per test, range: £0.50–2.80); haematology (average: £2.40 per test, range: £1.50–3.70); microbiology (average: £6.10 per test, range: £4.00–9.40) and histopathology (average: £48.10 per test, range: £21.40–73.40).

The modernisation of pathology services$^4$ – begun a decade ago and reflected in recommendations of the Carter Review – continues. The principal aim is to identify novel ways of delivering high quality pathology services that are responsive to patient needs and are cost effective. This has included an expansion of point of care testing, with nurses and other non-laboratory healthcare professionals becoming more involved in patient testing. The potential for economy of scale with centralisation of routine (non-urgent) pathology services in regional centres is receiving active consideration and in some hospitals pathology services have been rationalised so that haematology, blood transfusion and clinical biochemistry laboratories are combined to form one ‘blood science laboratory’.

As in all other areas of patient care, successful clinical laboratory investigation depends on teamwork; nurses are important members of that team. Good communication between nursing and laboratory staff can help to ensure that resources consumed in delivery of pathology services are used to best effect for the patient.

References


Useful Websites

www.ibms.org – website of the Institute of Biomedical Sciences – the professional organisation that represents biomedical scientists, the largest group of pathology laboratory staff.
www.rcpath.org – website of the Royal College of Pathologists – the professional organisation that represents pathologists, medically qualified laboratory staff.
www.acb.org.uk – website of the Association of Clinical Biochemistry – the professional organisation that represents non-clinical scientists working in clinical chemistry laboratories.
www.labtestsonline.org.uk – website for patients about clinical laboratory tests – includes a wealth of information about the work of pathology laboratories.
Some General Principles of Laboratory Testing

Lab testing results are the sum of three distinct phases:

1. Pre-testing phase, which includes collection and transport of samples to the laboratory.
2. Analytical phase within the laboratory.
3. Post-testing phase, which includes communication and interpretation of test results as well as clinical response to test result in terms of patient management.

In this chapter some general principles relating to pre- and post-testing procedures are discussed.

Pre-testing procedures

It is difficult to over emphasize the importance of good practice during the pre-testing phase of laboratory investigation. The production of high quality, accurate results which are clinically useful depends as much on practice before the sample...
reaches the laboratory as it does on the analytical process within the laboratory. Aspects of the pre-testing phase that need to be considered are:

- The pathology request form.
- The timing of sample collection.
- Sampling technique.
- Collecting the right amount of sample.
- Sample containers and labelling.
- Safety during collection and transport of samples.

This chapter is concerned with principles only. The detail of pre-testing will be considered again under each test heading. However, it must be remembered that practice, although based on the principles in this book, does vary between laboratories. There is no real substitute for consultation with your local laboratory. Clinical pathology departments are now mandated to publish a manual – usually available online – for users (doctors and nurses) of local pathology services. Such user manuals provide the detail of local pre-testing procedures that should guide practice in this area.

**Pathology request form**

Each patient sample must be accompanied by the appropriate fully completed pathology request form, signed by the doctor or in some instances, where that responsibility has been delegated, the specialist nurse practitioner making the request. Attention to detail is particularly vital for blood transfusion requests. Most cases of incompatible blood transfusion are the result of documentation errors. All pathology request forms should include the following information set:

- Patient details, including: full name, date of birth and hospital number.
- Hospital ward/clinic or GP surgery.
- Nature of specimen (e.g. venous blood, urine, biopsy etc.).
- Date and time of sample collection.
- Name of test requested (e.g. blood glucose, full blood count).
- Clinical details (these should very briefly explain why the test is being requested and may include a suspected or provisional diagnosis or symptoms).
- Details of any drug therapy that might affect test analysis or interpretation.
- An indication, where relevant, of the urgency of the request.
- Some health authorities request details about budget cost centres.

**Timing of sample collection**

Whenever possible, samples should be taken to coincide with routine transport to the laboratory, so that they can be processed by the laboratory without undue delay. It is not good practice to leave samples for more than a few hours or overnight before sending them to the laboratory; in many cases the samples will be unsuitable for analysis. For a few biochemical tests, for example, blood hormone
levels, it is vital that blood is sampled at a particular time of the day. For others (e.g. blood glucose) it is simply important to know what time the sample is collected. Some tests (e.g. blood gases) require that samples be processed immediately they are taken. Collection of these must be timed by prior agreement with the laboratory. Samples for microbiological investigation are best taken before antibiotic therapy is started, since antibiotics will inhibit the growth of bacteria in culture.

**Sampling technique**

**Venous blood collection**

Most blood tests are performed on venous blood collected by a technique known as venepuncture, using either a needle and syringe or, more commonly, an evacuated tube system (Figure 2.1).

- Patients may be anxious at the prospect of a venepuncture. A calm confident manner is important. Explain in simple terms what is involved and that mild discomfort or pain is usually felt as the needle is inserted.
- If there is a history of fainting during blood collection, take the sample with the patient lying down.
- When performing venepuncture on a patient receiving IV fluids, do not take blood from the arm used for IV administration. This avoids the risk of IV fluid contamination of the sample.
- Haemolysis (the rupture of red cells) during blood collection may render the sample unsuitable for analysis. This can occur if blood is forced at speed through narrow gauge needles or if the sample is shaken vigorously. When using syringe and needle technique, remove the needle before expelling blood into the sample container.
- Prolonged use of a tourniquet can affect laboratory results. Avoid the use of a tourniquet if possible and do not collect blood if tourniquet has been in place for more than one minute or two. Release the tourniquet and try the opposite arm.
- Although the cephalic or basilic vein in the forearm at the elbow is the usual preferred site, veins in the back of the hand or the foot provide alternative sites, for those with ‘difficult’ veins.

**Capillary blood collection**

Capillary blood can be recovered by a simple skin puncture with a sterile lancet, usually on the finger tip or, in the case of neonates and babies, the heel of the foot. It is useful if only very small sample volumes (less than 1 ml) are required. The technique can be performed by patients themselves and is routinely used, after training, by diabetic patients to obtain samples for self-monitoring of blood glucose concentration.

- The finger tip or heel is wiped with alcohol. A sterile lancet or autolet device is used to puncture the cleansed skin on the side of the finger tip or heel. Puncturing the ball of the finger tip is more painful.
Vacutainer® system comprises:
- Sterile, double ended needle
- Needle holder
- Blood collection tube containing a pre-set vacuum

Additional equipment required:
- Disposable gloves
- Tourniquet
- Sterile alcohol-soaked swab
- Cotton wool

- Hold the coloured section of the needle and break the white paper seal.
- Remove and discard the white plastic needle shield. **DO NOT USE** if paper seal already broken

- Screw needle into needle holder and leave coloured shield on needle.
- Apply tourniquet about 10 cm above elbow to make veins visible and locate a suitable site for venepuncture.
- Clean site with alcohol-soaked swab. Allow to dry.

- Remove needle shield.
- Ensure patients arm is supported and straight at the elbow.
- Insert needle bevelled side uppermost into the vein.

- Insert blood collection tube into the needle-holder.
- Ensuring needle does not move within vein, push tube to end of needle-holder, gently but firmly.
- Release tourniquet as blood flows into tube to fill vacuum.

- Withdraw blood collection tube when blood flow ceases.
- Continue to hold needle and needle holder in position.
- For further samples, insert next blood collection tube as before.

- Withdraw tube from holder.
- Invert tube 8–10 times to ensure mixing of blood with any additives in tube.

- Withdraw needle-holder with needle attached.
- Cover injection site with cotton wool and apply gentle pressure for a minute or two.

- Dispose of needle and needle holder (if disposable) in accordance with manufacturer’s instruction/local safety policy.
- Label all tubes fully in accordance with local laboratory policy.

Figure 2.1 Collection of venous blood with Vacutainer® system.
• Undue pressure to ‘squeeze’ blood out, can cause inaccurate results; blood must flow freely.
• Blood should be collected immediately into an appropriate container, designated for capillary samples and mixed gently by inversion.
• Pressure must be applied to the puncture site with a sterile gauze until blood flow ceases.

**Arterial blood collection**
The only test that requires sampling of blood from arteries is blood gases. The technique, which is more hazardous and painful than venepuncture, is described in Chapter 7.

**Urine collection**
Four kinds of urine collection are commonly made:

- A mid stream urine (MSU).
- A catheter specimen urine (CSU).
- An early morning urine (EMU).
- A 24 hour urine (i.e. all the urine passed during a 24 hour period).

The test requested determines which of these is appropriate. For most non-quantitative purposes, such as dipstick testing and microbiological testing, an MSU is necessary. This is a small 10–15 ml sample of urine collected part way through micturition, which can be collected at any time of the day. A CSU is the urine sample collected from a patient who has an indwelling urinary catheter. The detail of collecting an MSU and CSU for microbiological examination is provided in Chapter 21.

The first urine passed in the day, the so called EMU is the most concentrated and an EMU provides the best method of detecting substances in the urine which are present only in low concentration. An example of its use is pregnancy testing. The urine pregnancy test is based on detection of a hormone, human chorionic gonadotrophin (HCG), which is not normally detectable in urine, but is excreted in increasing concentration during the first few months of pregnancy. Early in pregnancy, the concentration is so low that unless a concentrated urine (i.e. an EMU) is used, the result may be falsely negative.

Sometimes it is useful to know exactly how much of a particular substance (e.g. sodium, potassium) is being lost from the body in urine, on a daily basis. Quantitation of this urinary loss can only be made by collecting all the urine passed during a 24 hour period. The detail of collecting a 24 hour urine is provided in Table 2.1.

**Sputum, swab collection**
All these specimens are destined for microbiological examination and the object is to sample only from infected sites, whilst avoiding bacterial contamination from other sites on the body or from the environment. For example, a sputum specimen is intended to reflect the environment of the respiratory tract, not the mouth. Saliva is not sputum. Sputum is best collected first thing in the morning and must be coughed
up from the lungs. Washing out the mouth before sampling reduces the risk of salivary contamination. When collecting throat swabs it is important to ensure that the swab does not come into contact with the tongue or sides of the mouth. This can be avoided by use of a tongue depressor. The swab should be gently rubbed only over the area at the back of the mouth (pharynx) and tonsils, especially inflamed areas.

Wound swabs are obtained by sampling the affected site only, avoiding contact with surrounding normal skin or tissue. When collecting any microbiological specimen, it is important to minimise environmental contamination by using aseptic technique and replacing swabs back in sterile containers or transport medium immediately.

### Tissue (biopsy) collection

A very brief reference to tissue sampling techniques necessary for histopathological examination has already been made in Chapter 1. Such sampling is always the responsibility of doctors and is beyond the scope of this book. Nurses are however involved in sampling of cervical cells for the cervical smear test (Chapter 24).

### Collecting the right amount of sample

The amount of blood required for laboratory testing is governed largely by local laboratory equipment and is therefore a matter of local laboratory policy. In general, continuing technological advance serves to significantly reduce the amount of blood required for tests. The local laboratory user guide includes a list of tests

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**Table 2.1 Protocol for collection of 24-hour urine.**

Clinical diagnosis or monitoring is occasionally aided by measurement of the rate of urinary excretion of a substance normally present in urine.

This requires collection of a timed (usually 24 hour) urine sample.

The validity of results of these tests depends crucially on an accurately timed sample; the object is to collect **ALL** urine passed during the 24 hour period.

- Obtain a 24 hour urine container for the test requested from the laboratory. Some tests require a container with an acid preservative. This may be a corrosive acid e.g. concentrated hydrochloric acid, so care must be taken.
- Label the bottle with patient details and date and time of start of urine collection.
- Explain to the patient that **ALL** urine passed during the 24 hour period must be saved.
- At a convenient time (usually 09.00) any urine in the bladder is voided and discarded.
- **ALL** the urine passed after 09.00 must be collected into the container.
- At 09.00 on the following day, the bladder is again emptied. This last sample must be added to the container. No urine passed after 09.00 on the second day should be added to the container.
- The urine collection, along with relevant test request form should be transported to the laboratory as soon as possible.

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*Notes: Sometimes a patient’s 24-hour urine volume exceeds the 2 l capacity of the collection container. If this is the case a second container must be obtained to complete the collection. **ALL** the urine passed **MUST** be collected.

If the patient inadvertently discards some urine during the collection period, all the urine collected to that point must be discarded, a new container obtained and collection restarted.

The urine container should be stored in the ward sample fridge during the collection period.*
with the minimum blood volume requirements. Anyone responsible for blood collection must be familiar with this local list. Some blood specimen bottles contain pre-weighed amounts of chemical preservatives and/or anticoagulants that determine the optimum volume of blood that the bottle should contain; this volume is stated on the side of the bottle. Erroneous results may occur if these blood volume instructions are not observed.

Whilst the volume of urine collected for MSU and CSU is not critical, it is vital, when collecting 24 hour urines, that all the urine passed in the collection period is collected, even if a second collection bottle is required.

In general, the size or amount of sample is important for successful isolation of bacteria. For example, it is more likely that bacteria will be isolated from a large specimen of sputum than a small specimen. Aspiration of pus with a needle and syringe is more likely to result in isolation of the causative organism than a swab of the pus. Falsely negative blood culture results can occur if insufficient blood is added to culture bottles.

**Sample containers**

Pathology laboratories supply a bewildering array of sample bottles and containers. Each container has specific uses; it is vital for accurate results that the correct container is used for the test requested. Guidance on this is contained within the local laboratory user manual.

The colour coded tops of blood sample containers indicate the chemicals, either in liquid or powder form, that they contain (Table 2.2). These chemicals serve two main purposes: prevention or acceleration of blood clotting; and preservation of blood cell structure or the concentration of some blood constituent. It is important that these chemicals are well mixed with the blood sample by repeated gentle inversion of the sample bottle.

A preservative may be necessary to preserve urine during collection of a 24 hour urine. The need for a preservative is determined by the substance in urine to be measured.

All sample containers for microbiological examination, for example, urine, swabs, blood culture bottles etc., are sterile and should not be used if seals are broken. Some bacteria will only survive outside the body if preserved in special transport media.

The structure of tissue samples is preserved by ‘fixing’ the tissue in formalin. Biopsy sample containers contain this preservative.

All sample containers must be fully labelled including patient’s full name, date of birth and location (ward, clinic or GP). Laboratories receive many hundreds of specimens every day, which may include specimens from two or more patients with the same name. It is vital, if results are to find their way back to the correct patient records, that specimen labels accurately and fully identify the patient. Inadequately labelled specimens or labelled specimens that cannot be incontrovertibly linked to the accompanying pathology request card may be rejected by the laboratory, resulting in the need for the patient to be re-tested; an entirely avoidable waste of time and resources for both patient and staff.
Safety during sample collection and transport

All laboratories have a locally written safety policy relating to the safe collection and transport of patient specimens, based on the premise that all patient specimens are potentially hazardous. Anyone involved in sample collection should be familiar with this policy. Among the many hazards that may be present in pathological specimens are viruses that cause AIDS and hepatitis, both of which can be transmitted by contact with infected blood. Tuberculosis can be transmitted by contact with infected sputum and gastrointestinal infections by contact with infected faeces. Good practice can have a major impact in reducing the risk to all staff and patients. The detail of good practice is included in the local safety policy. Some general points are included here.

Table 2.2 Some common additives present in blood collection tubes.

<table>
<thead>
<tr>
<th>Additive</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylenediaminetetraacetate (EDTA) – present as the potassium salt of this acid, i.e. K⁺-EDTA</td>
<td>An anticoagulant that prevents blood from clotting by binding to and effectively removing the calcium present in blood (calcium is required for clotting to occur). EDTA also preserves the structure of blood cells. Principle use of EDTA tubes: full blood count (FBC) and some other haematology tests.</td>
</tr>
<tr>
<td>Colour code of tube top: lavender/purple</td>
<td></td>
</tr>
<tr>
<td>Heparin – present as the sodium or potassium salt of this acid, i.e. sodium or potassium heparin</td>
<td>An anticoagulant that prevents blood from clotting by inhibiting the formation of thrombin from prothrombin. Principle use of heparin tubes: chemistry tests that require blood plasma.</td>
</tr>
<tr>
<td>Colour code of tube top: dark green or orange</td>
<td></td>
</tr>
<tr>
<td>Citrate – present as the sodium salt of this acid, i.e. sodium citrate</td>
<td>An anticoagulant that prevents blood from clotting by precipitating calcium, similar in action to EDTA. Principle use of citrate tube: coagulation study tests.</td>
</tr>
<tr>
<td>Colour code of tube top: light blue</td>
<td></td>
</tr>
<tr>
<td>Oxalate – present as either the sodium or ammonium salt of this acid, i.e. sodium or ammonium oxalate</td>
<td>An anticoagulant that prevents blood from clotting by precipitating calcium, similar in action to EDTA. Principle use of oxalate: used with sodium fluoride (see sodium fluoride entry) in tubes specifically for blood glucose measurement.</td>
</tr>
<tr>
<td>Colour code of tube top: yellow or grey</td>
<td></td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>This is an enzyme poison that prevents continued metabolism of glucose by blood cells and thereby preserves blood glucose concentration. Principle use of sodium fluoride: used with oxalate in tubes specifically for blood glucose measurement.</td>
</tr>
<tr>
<td>Colour code of tube top: yellow or grey</td>
<td></td>
</tr>
<tr>
<td>Clot activator and gel</td>
<td>This speeds up the blood clotting process and aids the separation of blood serum from blood cells. Principle use of clot activator/gel tubes: chemistry tests that require blood serum.</td>
</tr>
<tr>
<td>Colour code of tube top: red or gold</td>
<td></td>
</tr>
</tbody>
</table>