REQUESTING LABORATORY TESTS

There are many laboratory tests available to the clinician. Correctly used, these may provide useful information, but, if used inappropriately, they are at best useless and at worst misleading and dangerous.

In general, laboratory investigations are used:

- to help diagnosis or, when indicated, to screen for metabolic disease,
- to monitor treatment or detect complications,
- occasionally for medicolegal reasons or, with due permission from the patient, for research.

Overinvestigation of the patient may be harmful, causing unnecessary discomfort or inconvenience, delaying treatment or using resources that might be more usefully spent on other aspects of patient care. Before requesting an investigation, clinicians should consider whether its result would influence their clinical management of the patient.

Close liaison with laboratory staff is essential; they may be able to help determine the best and quickest procedure for investigation, interpret results and discover reasons for anomalous findings.

HOW OFTEN SHOULD I INVESTIGATE THE PATIENT?

This depends on the following:

- How quickly numerically significant changes are likely to occur: for example, concentrations of the main plasma protein fractions are unlikely to change significantly in less than a week (see Chapter 19), similarly for plasma thyroid-stimulating hormone (TSH; see Chapter 11). See also Chapter 3.
- Whether a change, even if numerically significant, will alter treatment: for example, plasma transaminase activities may alter within 24 h in the course of acute hepatitis, but, once the diagnosis has been made, this is unlikely to affect treatment (see Chapter 17). By contrast, plasma potassium concentrations may alter rapidly in patients given large doses of diuretics and these alterations may indicate the need to instigate or change treatment (see Chapter 5).

Laboratory investigations are very rarely needed more than once daily, except in some patients receiving intensive therapy. If they are, only those that are essential should be repeated.

WHEN IS A LABORATORY INVESTIGATION ‘URGENT’?

The main reason for asking for an investigation to be performed ‘urgently’ is that an early answer will alter the patient’s clinical management. This is rarely the case and laboratory staff should be consulted and the sample ‘flagged’ as clearly urgent if the test is required immediately. Laboratories often use large analysers capable of assaying hundreds of samples per day (Fig. 1.1). Point-of-care testing can shorten result turnaround time and is discussed in Chapter 30.

Figure 1.1 A laboratory analyser used to assay hundreds of blood samples in a day. Reproduced with kind permission of Radiometer Limited.
Laboratories usually have ‘panic limits’, when highly abnormal test results indicate a potentially life-threatening condition that necessitates contacting the relevant medical staff immediately. To do so, laboratory staff must have accurate information about the location of the patient and the person to notify.

**INTERPRETING RESULTS**

When interpreting laboratory results, the clinician should ask the following questions:

- Is the result the correct one for the patient?
- Does the result fit with the clinical findings? Remember to treat the patient and not the ‘laboratory numbers’.
- If it is the first time the test has been performed on this patient, is the result normal when the appropriate reference range is taken into account?
- If the result is abnormal, is the abnormality of diagnostic significance or is it a non-specific finding?
- If it is one of a series of results, has there been a change and, if so, is this change clinically significant?

Abnormal results, particularly if unexpected and indicating the need for clinical intervention, are best repeated.

**CASE 1**

A blood sample from a 4-year-old boy with abdominal pain was sent to the laboratory from an accident and emergency department. Some of the results were as follows:

**Plasma**

- Bilirubin 14 µmol/L (< 20)
- Alanine transaminase 14 U/L (< 42)
- Alkaline phosphatase 326 U/L (< 250)
- Albumin 40 g/L (35–45)
- γ-Glutamyl transferase 14 U/L (< 55)
- Albumin-adjusted calcium 2.34 mmol/L (2.15–2.55)

**DISCUSSION**

The patient’s age was not given on the request form and the laboratory computer system ‘automatically’ used the reference ranges for adults. The plasma alkaline phosphatase activity is raised if compared with the adult reference range, but in fact is within ‘normal limits’ for a child of 4 years (60–425). See also Chapters 6 and 18.

Test reference ranges

By convention, a reference (‘normal’) range (or interval) usually includes 95 per cent of the test results obtained from a healthy and sometimes age- and sex-defined population. For the majority of tests, the individual’s results for any constituent are distributed around this mean in a ‘normal’ (Gaussian) distribution, the 95 per cent limits being about two standard deviations from the mean. For other tests, the reference distribution may be skewed, either to the right or to the left, around the population median. Remember that 2.5 per cent of the results at either end will be outside the reference range; such results are not necessarily abnormal for that individual. All that can be said with certainty is that the probability that a result is abnormal increases the further it is from the mean or median until, eventually, this probability approaches 100 per cent. Furthermore, a normal result does not necessarily exclude the disease that is being sought; a test result within the population reference range may be abnormal for that individual.

Very few biochemical tests clearly separate a ‘normal’ population from an ‘abnormal’ population. For most there is a range of values in which ‘normal’ and ‘abnormal’ overlap (Fig. 1.2), the extent of the overlap differing for individual tests. There is a 5 per cent chance that one result will fall outside the reference range, and with 20 tests a 64 per cent chance, i.e. the more tests done, the more likely it is that one will be statistically abnormal.

No result of any investigation should be interpreted without consulting the reference range issued by the
laboratory carrying out the assay. Some analytes have risk limits for treatment, such as plasma glucose (see Chapter 12), or target or therapeutic limits, such as plasma cholesterol (see Chapter 13).

Various non-pathological factors may affect the results of investigations, the following being some of the more important ones.

**Between-individual differences**
Physiological factors such as the following affect the interpretation of results.

**Age-related differences**
These include, for example, bilirubin in the neonate (see Chapter 26) and plasma alkaline phosphatase activity, which is higher in children and the elderly (see Chapter 18).

**Sex-related differences**
Examples of sex-related differences include plasma urate, which is higher in males, and high-density lipoprotein cholesterol, which is higher in pre-menopausal women than in men (see Chapters 13 and 20). Obviously, sex hormone concentrations also differ between the sexes (see Chapter 9).

**Ethnic differences**
These may occur because of either racial or environmental factors, for example plasma creatine kinase may be higher in black than in white people (see Chapter 18).

**Within-individual variations**
There are biological variations of both plasma concentrations and urinary excretion rates of many constituents, and test results may be incorrectly interpreted if this is not taken into consideration. Biological variations may be regular or random.

**Regular**
Such changes occur throughout the 24-h period (circadian or diurnal rhythms, like those of body temperature) or throughout the month. The daily (circadian) variation of plasma cortisol is of diagnostic value, but, superimposed on this regular variation, ‘stress’ will cause an acute rise (see Chapter 8). Plasma iron concentrations may fall by 50 per cent between morning and evening (see Chapter 21). To eliminate the unwanted effect of circadian variations, blood should ideally always be taken at the same time of day, preferably in the early morning and, if indicated, with the patient fasting. This is not usually possible, and these variations should be taken into account when serial results are interpreted.

Some constituents vary monthly, especially in women during the menstrual cycle. These variations can be very marked, as in the results of sex hormone assays, for example plasma oestriadiol, which can only be interpreted if the stage of the menstrual cycle is known; plasma iron may fall to very low concentrations just before the onset of menstruation. Other constituents may also vary seasonally. For example, vitamin D concentrations may be highest in the summer months. Some of these changes, such as the relation between plasma glucose and meals, have obvious causes.

**Random**
Day-to-day variations, for example in plasma iron concentrations, can be very large and may swamp regular cycles. The causes of these are not clear, but they should be allowed for when serial results are interpreted—for example the effect of ‘stress’ on plasma cortisol concentrations.

The time of meals affects plasma glucose concentrations, and therefore correct interpretation is often only possible if the blood is taken when the patient is fasting or at a set time after a standard dose of glucose (see Chapter 12).

**Methodological differences between laboratories**
It has been pointed out that, even if the same method is used throughout a particular laboratory, it is difficult to define normality clearly. Interpretation may sometimes be even more difficult if the results obtained in different laboratories, using different analytical methods, are compared. Agreement between laboratories is close for many constituents partly due to improved standardization procedures and because many laboratories belong to external quality control schemes. However, for others, such as plasma enzymes, different methods may give different results. For various technical reasons, the results would still vary unless the substrate, pH and all the other variables were the same.

**IS THE ABNORMALITY OF DIAGNOSTIC VALUE?**

**Relation between plasma and cellular concentrations**
Intracellular constituents are not easily sampled, and plasma concentrations do not always reflect the situation in the cells; this is particularly true for those...
constituents, such as potassium and phosphate, that are at much higher concentrations intracellularly than extracellularly. A normal, or even high, plasma potassium concentration may be associated with cellular depletion if equilibrium across cell membranes is abnormal, such as in diabetic ketoacidosis. Analyte concentrations may differ between plasma (the aqueous phase of anticoagulated blood) and serum (the aqueous phase of clotted blood). The concentration of potassium, for example, is higher in serum than in plasma samples because of leakage from cells during clotting, and the total protein concentration is lower in serum than in plasma because the protein fibrinogen is removed during the clotting process.

Non-specific abnormalities

The concentrations of all protein fractions, including immunoglobulins, and of protein-bound substances may fall by as much as 15 per cent after as little as 30 min recumbency, owing to fluid redistribution in the body. This may account, at least in part, for the low plasma albumin concentrations found in even quite minor illnesses. In-patients often have blood taken early in the morning, while recumbent, and plasma concentrations of protein and protein-bound substances tend to be lower than in out-patients (see Chapter 19).

**CASE 2**

A 54-year-old Nigerian man was seen in an accident and emergency department because of chest pain. His electrocardiogram (ECG) was normal. The following results were returned from the laboratory, 6 h after his chest pain started:

<table>
<thead>
<tr>
<th>Plasma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase</td>
<td>498 U/L (&lt; 250)</td>
</tr>
<tr>
<td>Troponin T</td>
<td>10 pg/L (&lt; 20)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The raised plasma creatine kinase activity suggested an acute myocardial infarction (see also Chapters 18 and 22). The patient was, however, subsequently found not to have had a myocardial infarction (confirmed by a normal troponin T result) and the raised plasma creatine kinase activity was thought to be due to his racial origin. (The reference range of <250 U/L was based on that of the predominantly white UK population; normal plasma creatine kinase activity may be two to three times higher in black than in white people.)

**DIAGNOSTIC PERFORMANCE**

Before one can interpret day-to-day changes in results and decide whether the patient's biochemical state has altered, one must know the degree of variation to be expected in the results derived from a normal population. We have already discussed intraindividual (same person) analyte variation. However, there is also unavoidable analytical variation.

**CASE 3**

One hundred patients with chest pain were screened with a new biochemical test that showed 80 to be positive for chest pain. What is the test's sensitivity?

**Answer:** $\frac{80}{100} \times 100\% = 80\%$

The same test was used on 100 patients without chest pain, and 95 had a negative screening result. What is the test's specificity?

**Answer:** $\frac{95}{100} \times 100\% = 95\%$

**DISCUSSION**

Sensitivity is true-positive rate per total affected.

Specificity is true-negative rate per total unaffected.

**Reproducibility of laboratory estimations**

Most laboratory estimations should give results that are reproducible to well within 5 per cent; some, such as those for sodium and calcium, should be even more precise, but the variability of some hormone assays, for example, may be greater. Small changes in results produced by relatively imprecise methods are not likely to be clinically significant.

Imprecision is the term used to describe the random changes that reduce the agreement between replicate assay measurements. This can be considered in terms of the within-assay precision, which is the assay variability when the same material is assayed repeatedly within the same assay batch, or day-to-day precision, which is the variability when the same material is assayed on different days.

The assay coefficient of variation (CV) is used to express imprecision and can be calculated by the following equation:

$$CV\% = \frac{\text{standard deviation of the assay}}{\text{mean of the assay results}} \times 100\%$$  \hspace{1cm} (1.1)

This should be as small as possible for each assay, and can be expressed as the intra-assay CV when describing the imprecision within a single run or batch.
Diagnostic performance

Test sensitivity and specificity

Diagnostic sensitivity is a measure of the frequency of a test being positive when a particular disease is present, that is, the percentage of true-positive (TP) results. Diagnostic specificity is a measure of the frequency of a test being negative when a certain disease is absent, that is, the percentage of true-negative (TN) results. Ideally, a test would have 100 per cent specificity and 100 per cent sensitivity.

The usefulness of tests can be expressed visually as receiver operating characteristic (ROC) curves (Fig. 1.3).

Unfortunately, in population screening, some subjects with a disorder may have a negative test (false-negative, FN); conversely, some subjects without the condition in question will show an abnormal or positive result (false-positive, FP).

The predictive value of a negative result is the percentage of all negative results that are true negatives, that is, the frequency of subjects without the disorder in all subjects with negative test results. A high negative predictive value is important in screening programmes if affected individuals are not to be missed. This can be expressed as:

$$\frac{TN}{TN + FN} \times 100\%$$ (1.2)

The predictive value of a positive result is the percentage of all positive results that are true positives: in other words, the proportion of screening tests that are correct. A high positive predictive value is important to minimize the number of false-positive individuals being treated unnecessarily. This can be expressed as:

$$\frac{TP}{TP + FP} \times 100\%$$ (1.3)

The overall efficiency of a test is the percentage of patients correctly classified by the test. This should be as high as possible and can be expressed as:

$$\frac{TP + TN}{TP + FP + TN + FN} \times 100\%$$ (1.4)

If the cut-off, or action, limit of a diagnostic test is set too low, more falsely positive individuals will be included, and its sensitivity will increase and its specificity decline. Conversely, if a diagnostic test has its cut-off or action limit set too high, fewer falsely positive individuals will be encompassed, but more individuals will be falsely defined as negative, that is, its sensitivity will decrease and its specificity will increase.

Likelihood ratios of laboratory tests

Some may find predictive values confusing, and the likelihood ratio (LR) may be preferable. This can be defined as the statistical odds of a factor occurring in one individual with a disorder compared with it occurring in an individual without that disorder.

The LR for a negative test is expressed as:

$$\frac{1 - \text{sensitivity}}{\text{specificity}}$$ (1.5)

The LR for a positive test is expressed as:

$$\frac{\text{sensitivity}}{1 - \text{specificity}}$$ (1.6)

The greater the LR, the more clinically useful is the test in question.

SUMMARY

- Careful thought is required when it comes to requesting and interpreting clinical biochemistry tests.
- Communication with the laboratory is essential to ensure optimal interpretation of results and patient management.
- The laboratory reference range should be consulted when interpreting biochemical results, and results should be interpreted in the light of the clinical findings.
- Just because a result is ‘abnormal’ does not mean that the patient has an illness; conversely, a ‘normal’ result does not exclude a disease process.