29 Patient sample collection and use of the laboratory

Lec. 1

Most clinical laboratories take stringent precautions to control the analytical accuracy and precision of results. Indeed, many laboratories undergo carefully regulated accreditation and inspection procedures to ensure that they are fit for practice. Generally, laboratory users need only limited knowledge of the technical details of the laboratory tests. However, they should understand that the appropriate collection of patient specimens can affect results (Table 29.1), and they should therefore work with the laboratory in its attempt to produce answers rapidly and accurately, with appropriate interpretation when required and identifiable with the relevant patient. To this end, one should understand the importance of:

- accurately completed request forms,
- the collection of specimens by the correct technique at the appropriate time,
- correctly labelled specimens,
- appropriate laboratory liaison,
- speedy delivery to the laboratory.

Remember that treatment based on technically correct results from a wrongly labelled or collected specimen may be as dangerous as a faulty surgical procedure. 'Unlikely' results are checked in most laboratories to make sure that they have not been transposed with the results for another patient.

All patient samples are potentially infection risks. Informed consent may be needed for acquired immunodeficiency syndrome (AIDS) testing and also certain genetic tests. All blood specimens should be sent in leak-proof, sealed plastic bags, with the request form in a different pocket in the bag. Failure to comply with these guidelines may put many people, including porters and laboratory staff, at unnecessary risk.

Table 29.1 Some extra-laboratory factors leading to erroneous results

Cause of error	Some possible consequences
Patient not fasting	High plasma triglyceride and glucose
Keeping blood overnight before sending it to the laboratory or refrigerating blood sample	High plasma potassium, phosphate, LDH, AST
Haemolysis of blood	As above, lower plasma ALP
Prolonged venous stasis during venesection	High plasma protein, total calcium and cholesterol
Taking blood from an arm with an infusion running into it	Dilution of blood constituents such as electrolytes and glucose
Putting blood into wrong vial or tipping it from one vial into another	e.g. EDTA or oxalate cause low plasma calcium or ALP
Blood for glucose not put into fluoride	Low blood or plasma glucose
Delay in analysing blood gases	Low bicarbonate concentration
Failure to keep sample cool or delay separating and freezing plasma	Low PTH, ACTH, insulin
Incorrect anticoagulant	e.g. gut peptide hormones falsely low if no protease inhibitor used
Palpation of prostate by rectal examination, passage of catheter, enema in last few days	High tartrate-labile acid phospatase and PSA
Inaccurately timed urine collection	Poorly timed 24-h urinary excretion values Abnormal renal clearance values
Urine collections without preservative	Falsely low result, e.g. urea or calcium
Loss of stools during faecal fat collection	Falsely low faecal fat results (test now rarely done)

ACTH, adrenocorticotrophic hormone; AST, aspartate transaminase; ALP, alkaline phosphatase; EDTA, ethylenediamine tetra-acetic acid; LDH, lactate dehydrogenase; PTH, parathyroid hormone; PSA, prostate-specific antigen.

REQUESTING PATIENT SAMPLES

Patient identification

Accurate and legibly written information about the patient is essential, although electronic requesting systems are now widespread in some areas. This information includes the patient's:

- hospital case number, and/or healthcare number,
- surname and first name(s), correctly and consistently spelt,
- date of birth, rather than age.

These would usually be considered the minimum acceptable dataset for patient identification details.

Any of these may be recorded inaccurately on the form and, unless there is complete agreement with previous details, results may be entered into the wrong patient's record either on a computer or in the patient's case notes, causing confusion and possible danger to the patient. The National Health Service (NHS) number is being used as a unique individual identifier in the UK.

It is important also to include relevant clinical details so as to facilitate correct interpretation of the results.

Location of the patient and identification of the clinician

It should be obvious that, if the ward or department is not stated, it may take time and effort to determine where the results should be sent. The requesting doctor must sign the form legibly, and also state how he or she can be notified rapidly, for example by 'bleep number', in case abnormal results requiring urgent action are found or advice needs to be sought about treatment. The doctor must check the completed request form to be sure that the information given is correct; it is also important to include his or her name and contact details.

Request forms designed by pathology and other departments ask only for information that is essential to ensure the most efficient possible service to the clinician and therefore to the patient. Now quite widespread is the use of electronic test requesting, which should improve patient identification and speed up the process and may replace 'paper' requests.

COLLECTION OF PATIENT SPECIMENS

Collection of blood

If a clinically improbable result has been checked analytically and the second result is in close agreement with the first, a fresh specimen should be analysed. Although contamination at some stage of blood collection is an obvious possibility, this is relatively rare and should not be accepted until other, more common, causes have been excluded. Of course, it should also be remembered that the sample could be incorrectly labelled with wrong patient details.

Effect on results of procedures before venepuncture

- *Some tests may require the patient to fast,* for example plasma glucose or triglyceride (see Chapters 12 and 13).
- *Oral medication*: some assays may be affected by oral medication.
  - Blood should be taken for drug assays at a standard time after the dose; misleadingly high plasma concentrations may occur at the time of peak absorption (see Chapter 25).

CASE 1

A 22-year-old man had the following post-operative biochemical results after an appendectomy:

Plasma
Sodium 165 mmol/L (135–145)
Potassium 1.9 mmol/L (3.5–5.0)
Urea 1.1 mmol/L (2.5–7.0)
Creatinine 38 µmol/L (70–110)
Glucose 43 mmol/L (3.5–6.0)

The clinical biochemistry laboratory suggested an immediate repeat blood sample, which gave the following results:

Plasma
Sodium 136 mmol/L (135–145)
Potassium 3.9 mmol/L (3.5–5.0)
Urea 5.4 mmol/L (2.5–7.0)
Creatinine 89 µmol/L (70–110)
Glucose 4.5 mmol/L (3.5–6.0)

DISCUSSION

The first sample seems to display profound hypernatraemia, hyperglycaemia and hypokalaemia. In addition, the plasma urea and creatinine concentrations are both low. The repeat sample values are completely different. It later transpired that the first sample had been taken out of the patient's drip arm, which had a dextrose–saline infusion going into it. This had resulted in dilution of the analytes and elevated sodium and glucose concentrations.

  - There may be significant hypokalaemia for a few hours after taking potassium-losing diuretics due to rapid clearance of potassium from the extracellular fluid (ECF). The plasma concentration returns to its 'true' level as equilibration occurs between cells and ECF (see Chapter 5).
  - Interfering substances: previous administration of a substance may affect a plasma analyte concentration for some time; for example certain antibiotics may interfere with chemical reactions used in creatinine assays (see Chapter 3).
- *Effect of posture:* for example the concentrations of plasma proteins and of substances bound to them are lower when the patient is supine than when standing up (see Chapter 19).
- *Intravenous infusion:* for example a spuriously low plasma sodium concentration may result if the sample is taken from a dextrose drip arm (see Chapter 2).
- *Clinical procedures:* for example palpation of the prostate by rectal examination may possibly release large amounts of prostate-specific antigen (PSA) into the circulation; the spuriously elevated concentrations in blood may persist for several days (see Chapter 24).

Effects on results of the technique of venepuncture

Venous stasis

It is usual to apply a tourniquet proximal to the site of venepuncture to make it easier to enter the vein with the needle. If occlusion is maintained for more than a short time, the combined effect of raised intracapillary pressure and hypoxia of the vessel wall increases the rate of passage of water and small molecules from the lumen into the surrounding interstitial fluid. Large molecules, such as proteins, cannot pass through the capillary wall at the same rate; their plasma concentrations therefore rise.

Many plasma constituents are partly bound to protein. Prolonged venous stasis can raise the plasma total calcium concentration, sometimes to equivocal or slightly high levels. Therefore, ideally, blood samples for plasma calcium estimation should be taken without stasis, especially if high plasma concentrations have previously been found (see Chapter 6).

Prolonged stasis may also cause local hypoxia; consequent leakage of intracellular constituents, such as potassium and phosphate, may cause falsely high plasma concentrations. It is sometimes difficult to enter 'bad veins' without applying stasis. A tourniquet may be used and released as soon as the needle is in the vein; a suitable specimen may be obtained after waiting at least a further 15 s before withdrawing blood.

Site of venepuncture

If the patient is receiving an intravenous infusion, the administered fluid in the veins of the same limb, whether proximal or distal to the infusion site, has not mixed with the total plasma volume; local concentrations will therefore be unrepresentative of those circulating through the rest of the body. Blood taken from the opposite arm will give valid results. Note that glucose infusion may cause systemic hyperglycaemia and glycosuria. Only if the hyperglycaemia persists after infusion has stopped should the diagnosis of diabetes mellitus be considered (see Chapter 12).

Containers for blood

Most hospital laboratories issue a list of the types of container suitable for different assays (Fig 29.1); this list may vary from hospital to hospital. For example, most laboratories specify that:

- Blood for glucose estimation should be put into a tube containing an inhibitor of erythrocyte glycolysis, such as fluoride.
- Potassium should ideally be estimated on plasma from heparinized blood rather than serum, although many laboratories accept serum tubes for potassium on the pragmatic grounds that the differences are

Figure 29.1 Blood collection tubes: if in doubt about what tubes to use be sure to contact the laboratory for advice. Reproduced with kind permission of Greiner Bio-One.

usually non-significant. Potassium is released from cells, especially platelets, during clotting. Serum potassium concentrations are usually higher than those of plasma by a variable amount; this difference can be clinically misleading. Marked differences may be found in patients with leukaemia, in whom the number of white blood cells is usually significantly increased.

Laboratories should accept blood only in the correct containers. Even with this precaution, serious errors can arise if blood is decanted from one container to another, although this is less likely to occur if a closed vacuum system is used for obtaining blood. The anticoagulant actions of oxalate and of sequestrene (ethylenediamine tetra-acetic acid, EDTA) depend on the precipitation or chelation of calcium, respectively, thus invalidating the results of calcium estimation. Ethylenediamine tetra-acetic acid is usually in the form of its potassium salt; therefore, it renders the sample unsuitable for potassium analysis.

The use of sodium (instead of lithium) heparin or trisodium citrate may give a falsely high plasma sodium result. This anticoagulant is often used in specimens taken for 'blood gases'; apparent plasma sodium concentrations of 160–170 mmol/L can result from transferring an aliquot of sodium heparinized blood into a lithium heparin vial. The use of lithium heparin leads to a falsely high plasma lithium concentration and it should therefore not be used as an anticoagulant for lithium determination.

CASE 2

A blood sample had been taken from a 44-year-old woman on the medical ward and the results were as follows:

Plasma
Sodium 140 mmol/L (135–145)
Potassium > 10 mmol/L (3.5–5.0)
Urea 4.1 mmol/L (2.5–7.0)
Creatinine 78 µmol/L (70–110)
Albumin-adjusted calcium < 0.5 mmol/L (2.15–2.55)
Phosphate 0.92 mmol/L (0.80–1.35)

Repeat blood sampling on the same day gave the following results:

Plasma
Sodium 139 mmol/L (135–145)
Potassium 3.6 mmol/L (3.5–5.0)
Urea 4.2 mmol/L (2.5–7.0)
Creatinine 78 µmol/L (70–110)
Albumin-adjusted calcium 2.43 mmol/L (2.15–2.55)
Phosphate 0.90 mmol/L (0.80–1.35)

DISCUSSION

The first blood sample had been collected in a potassium ethylenediamine tetra-acetic acid (EDTA) tube (this is normally used for certain haematology tests such as full blood count) by mistake and then the blood had been decanted by the doctor into the correct chemistry lithium heparin tube. Note in the first sample the raised plasma potassium and low calcium concentrations due to chelation by potassium EDTA.

Effects of haemolysis and delayed separation of blood

Haemolysis

The concentration of many substances is very different in erythrocytes from that in the surrounding plasma. Haemolysis releases the cell contents into plasma and, consequently, if this occurs in vitro, the concentrations of some plasma constituents, such as potassium, phosphate and aspartate transaminase, may be falsely increased. The increase is variable and is not related to the intensity of the red colour of plasma due to haemoglobin. It is uncommon for haemolysis to occur in vivo because these constituents are distributed throughout the total extracellular, not just plasma, volume.

Haemoglobin may interfere with some chemical reactions, falsely increasing the apparent plasma bilirubin concentration and lowering alkaline phosphatase activity. The chance of haemolysis is minimized if the blood is treated gently and if a closed vacuum system is used.

Delayed separation of blood

It is important to check the sample date. The differential concentrations of some analytes across cell membranes are maintained by energy, derived from glycolysis. In vitro, erythrocytes soon use up the available glucose and therefore the energy source; concentrations of these analytes in plasma will then tend to equalize with those in erythrocytes by passive diffusion across cell membranes. If plasma is not separated from blood cells within a few hours, the effect on plasma concentrations will be similar to that resulting from haemolysis. However, there are a few important differences:

- Because haemoglobin is not released, the plasma colour looks normal; the error is therefore easily overlooked.

CASE 3

A blood sample had been taken in the morning from a 43-year-old man and was then sent to the laboratory from the local health centre. The sample was analysed in the evening of the same day, as there had been a transport delay, and the following results were obtained:

Plasma
Sodium 143 mmol/L (135–145)
Potassium 6.0 mmol/L (3.5–5.0)
Urea 5.1 mmol/L (2.5–7.0)
Creatinine 88 µmol/L (70–110)

The laboratory contacted the patient's general practitioner and an urgent repeat sample showed the following results.

Plasma
Sodium 145 mmol/L (135–145)
Potassium 4.2 mmol/L (3.5–5.0)
Urea 5.2 mmol/L (2.5–7.0)
Creatinine 88 µmol/L (70–110)

DISCUSSION

The first patient sample shows hyperkalaemia, but repeat analysis on a 'fresh' sample shows a 'normal' potassium concentration. The spuriously raised potassium result in the first sample was due to the delay in sample assay and leakage of intracellular potassium ions out of cells, resulting in pseudohyperkalaemia. It is important to transport samples to the laboratory as quickly as possible to avoid storage artefacts.

- The plasma potassium concentration rises as the plasma glucose falls; initially, there may be a slight fall in the plasma potassium concentration as it moves into the erythrocytes.

Many plasma constituents, such as bilirubin, deteriorate even if the plasma is correctly separated and stored. If the plasma sample is haemolysed or separation has been delayed, the plasma potassium concentration may still be clinically significant. For example, if the sample is visibly haemolysed and the plasma potassium concentration is only 2.8 mmol/L, this may indicate profound hypokalaemia; the laboratory staff should contact the requesting doctor directly to discuss the significance of the comment 'haemolysed specimen' on the report form.

Refrigeration

The refrigeration of whole blood has the effect of raising the plasma potassium concentration probably by reducing the activity of the adenosine triphosphatase pump. Hence, blood samples for urea and electrolytes should not be refrigerated. Transport of samples in cold weather may have a similar effect. Freezing will result in haemolysis. Therefore blood specimens must be centrifuged and the plasma separated from the cells before storing, for example overnight.

Collection of urine

Urine estimations performed on timed collections are expressed as units/time (for example mmol/24 h); this figure is calculated by multiplying the concentration by the volume collected during the timed period. The accuracy of the final result depends largely on that of the urine collection. Sometimes, if the patient is a child or incontinent, accurate urine collection is particularly difficult.

For example, a 24-h specimen may be collected between 09.00 h on Sunday and 09.00 h on Monday. The volume excreted by the kidneys during this period is the crucial one: urine already in the bladder at 09.00 h on Sunday was excreted earlier and should not be included. Therefore the procedure is as follows.

- *09.00 h on Sunday*: the bladder is emptied completely, whether or not the patient feels the need, and the specimen is discarded.
- Collect all urine passed until *09.00 h on Monday* at which point the bladder is completely emptied, whether or not the patient feels the need, and the urine specimen is added to the total collection.

The shorter the period of collection, the greater the error if this procedure is not followed.

Before collection, a urine container should be obtained from the laboratory; this may contain a preservative to inhibit bacterial growth (which might destroy the substance being estimated) or acid preservative to prevent precipitation or sample degradation, but that does not interfere with the relevant assay. The patient must be told not to discard the preservative in the container and that it might be toxic and harmful if spilt.

Collection of faeces

Rectal emptying is usually erratic and, unlike that of the bladder, can rarely be performed to order. Results

of faecal estimations of, for example, fat may vary by several hundred per cent in consecutive 24-h collections. If the collection period lasted for weeks, the *mean* 24-h output would be very close to the true daily loss from the body into the intestinal tract.

To render collection more accurate, orally administered 'markers' are used in certain circumstances. Faecal collections and estimations are time consuming and unpleasant for all concerned and are now rarely required.

Labelling patient specimens

All specimens must be accurately labelled and the information should correspond with that on the accompanying request form in every detail, as error may cause a medicolegal disaster. The date, and preferably the time, of specimen collection should be included, and should be written at the time of collection.

Sending the specimen to the laboratory

Many estimations can be performed rapidly if the result is needed urgently. Non-urgent late blood samples can be separated from cells and stored overnight, although some laboratories work 24/7. In cases of true clinical emergency, the requesting clinician should notify the laboratory, preferably before the specimen is taken, indicating the reason for the urgency, so that the laboratory can be ready to deal with the specimen as soon as it arrives. It is the clinician's responsibility to indicate the degree of urgency. The misuse of emergency services may delay truly urgent results, and some urgent samples may need to be separated immediately and kept on ice.

SUMMARY

- It is essential to liaise closely with the laboratory when collecting patient samples to help ensure correct sampling times and collection conditions.
- In vitro haemolysis can lead to a spurious increase of predominantly intracellular ions in the plasma, such as potassium (pseudohyperkalaemia).
- Particular attention should be paid to avoiding blood samples being collected from the same arm as an intravenous infusion, leading to 'drip arm' results.
- It is also essential to ensure correct sample labelling and patient identification to avoid potentially life-threatening errors and medicolegal disasters.