ARTICLE

Preparation for blood tests: what can go wrong before the sample reaches the lab

Linn Lee
Chemical Pathology Registrar[1]
Endocrinologist[2]

Wayne Rankin
Chemical Pathologist[1]
Endocrinologist[3]

[1] SA Pathology, Adelaide
[2] South Australian Medical Specialists, Adelaide
[3] Royal Adelaide Hospital Adelaide

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SUMMARY

Many errors can occur in the pre-analytical phase of laboratory testing, such as during patient preparation, sample collection, handling, storage and transport. Minimisation of errors during this phase is key to optimising the usefulness of laboratory tests and may reduce the need for repeat sampling.

Various patient factors can affect laboratory test results, including posture, fasting status, circadian variation, medications and other interfering agents. Clinicians should be aware of these factors and advise patients on the necessary preparation before testing.

Patient identification, collection timing, haemolysis, contamination and sample volume are important considerations when collecting and handling a sample.

Individuals should consult their local laboratory for specific test instructions or protocols, as these can vary.

Introduction

Laboratory tests play a crucial role in screening, diagnosis and management of medical conditions, with 60 to 70% of clinical decisions based on test results.[1] The testing cycle can be divided into different phases: the pre-analytical phase, which occurs before the sample arrives in the laboratory and starts with a clinical question; the analytical phase, in the laboratory; and the post-analytical phase, consisting of reporting and interpretation of results, clinical decision-making, possible revision of the clinical question, and further testing.

The pre-analytical phase of laboratory testing involves multiple steps, including test requesting, patient preparation, sample collection, handling, storage and transport. This phase can involve multiple personnel, and there may be inconsistent adherence to standardised protocols and a lack of supervision by laboratory staff. Errors during the pre-analytical phase are common, with 46 to 68% of errors occurring in this phase,[2] and many occur before blood has even been drawn.[3] Clinical diagnostic laboratories comply strictly with international standards for quality and competence, thereby minimising errors in the analytical phase, which are estimated to account for only 7 to 13% of total errors.[2] Therefore, minimisation of pre-analytical errors is key to optimising the usefulness of laboratory testing.

This article explores the factors that influence laboratory tests at various stages of the pre-analytical phase and how to minimise errors at each stage.

Requesting the test

Rational test ordering involves requesting the correct test for the patient, and ensuring the clinical question and timing of testing and retesting are appropriate.[4] It also involves recognising that a particular test may not be appropriate in certain contexts, for example, when a tumour marker such as carcinoembryonic antigen, which is best used for monitoring recurrence of malignancy, is ordered as a screening test, leading to further unnecessary testing and patient anxiety.

Clinicians should ensure that the patient is correctly identified, the request form is signed and dated, and that all information is legible. Inclusion of relevant clinical information or a specific clinical question on the form can also help laboratory staff during analysis and reporting.

Patient preparation

Given the various patient factors that can affect laboratory test results, adhering to local or international guidelines for standardised patient preparation is essential for ensuring reliability and comparability of results over time. Clinicians should be aware of these factors and the preparation

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required so they can counsel and educate patients effectively. Additionally, clinicians should proactively discuss and address any potential barriers to testing, such as dehydration, needle phobia or logistical difficulties, to help ensure that patients complete the required tests. Some important patient factors to consider are discussed below.

Posture

Transitioning from a supine to an upright position can reduce circulating blood volume by as much as 10%, as water passes from the intravascular space into surrounding tissues. The subsequent drop in central venous pressure triggers increased secretion of catecholamines, aldosterone, renin, and arginine vasopressin (antidiuretic hormone). To minimise the risk of false positive results when collecting blood for plasma metanephrines, it is recommended that patients lie supine for 30 minutes prior to venepuncture.[5] When collecting blood for aldosterone and renin testing, it is important to indicate if the patient was in an upright or supine position, as this will influence the reference ranges reported for the tests.[6]

Fasting status

After a meal, the concentrations of several analytes in the blood can change significantly. For example, glucose will rise following food intake, and bone turnover markers will be suppressed. Fasting for 10 to 12 hours prior to testing helps to minimise variability in these analytes; however, prolonged fasting (greater than 16 hours) should be avoided, particularly when preparing for a glucose tolerance test as false positive results may be seen. Restricting water unnecessarily while fasting can lead to an increase in certain analytes, such as urea,[7] and increases the risk of orthostatic hypotension and falls in older patients.[8]

Fasting for routine lipid testing is no longer recommended as postprandial changes in cholesterol and triglycerides are clinically insignificant in most people.[9]

Circadian variation

Circadian variation refers to the natural fluctuations in physiological processes over a 24-hour cycle. Hormones often exhibit specific patterns of secretion throughout the day, including cortisol, growth hormone and testosterone, and the timing of blood collection can significantly influence the concentrations of these hormones. To illustrate, cortisol is normally at its highest in the morning and lowest at night. Therefore, testing for hypocortisolism should be done in the morning, and collection of salivary cortisol when testing for excess cortisol should be done at midnight. Renin activity reaches its peak early in the morning during sleep, with aldosterone displaying a similar rhythmic pattern. Therefore, mid-morning collection of aldosterone–renin ratio is recommended.[6]

Medications and other interfering agents

Many medications can have marked influence on laboratory results, which may be via direct analytical interference, such as biotin (see below), or changes in analyte concentration in response to the medication. Some changes are predictable and well characterised, such as the effects of antihypertensive agents on aldosterone–renin ratios, for which pathways for patient preparation are well defined.[10] Others are known to laboratories but are less well known to clinicians requesting the test, such as the effects of trimethoprim on renal tubular handling of creatinine, leading to apparent decreased estimated glomerular filtration rate and the false impression of acute kidney injury in patients with otherwise uncomplicated urinary tract infections.[11]

Herbal remedies and other supplements are of particular concern, as their contents may be poorly defined, with undeclared corticosteroids, anti-inflammatory drugs and other agents present, and patients may not always inform health professionals they are taking them.[12,13]

Biotin (vitamin B7) is a common ingredient in many supplements, either taken orally or applied topically as a hair tonic. It was first identified as an interferent in thyroid function immunoassays; however, any immunoassay that uses streptavidin as part of the measuring system may be affected.[14] Biotin supplements should be withheld for at least 1 week before testing.[15] If concerned, clinicians should contact their laboratory for advice. If time-critical tests such as troponin assays are required, informing the laboratory of the presence of biotin is important so that the appropriate steps can be taken by the laboratory to mitigate its effects.

Sample collection

Patient identification

Prior to collection, the details on the request form should be matched to patient details, which should be confirmed by the patient and cross-referenced to specimen labels. It is recommended that at least 2 permanent identifiers, such as name and date of birth, are used for confirmation.

Pre-labelling of tubes with patient details should be avoided due to the risk of the wrong patient sample being put into the pre-labelled tube.

Appropriate timing of collection

As discussed above, samples for analytes, such as hormones that show diurnal or other cyclical variation, should be collected at a time when the most useful information can be obtained. This also applies to analytes that have longer cycles, such as measuring progesterone at the middle (usually day 21) of the luteal phase to confirm ovulation.

Therapeutic drug monitoring also requires careful timing of blood collection. It is important to record the time the drug was last administered. Trough concentrations (before the next dose) are optimal for most drugs, as this represents the lowest concentration across the dosing cycle. Blood should be collected a minimum of 6 half-lives after a drug dose has been changed (assuming first-order kinetics) to ensure the result reflects the steady-state concentration.

In special circumstances, such as assessing aminoglycoside pharmacokinetics, post-dose rather than trough samples are used, and local protocols for sample collection should be followed.

Avoiding haemolysis

Haemolysis, the rupture of red cells within a sample, is a major cause of sample rejection, and thus repeat sampling. Most haemolysis identified in laboratory samples is due to in vitro rupture of cells (over 98%). Haemolysis can lead to marked changes in many analytes by multiple mechanisms, such as:

- direct release of potassium, phosphate, magnesium, aspartate aminotransferase, and lactate dehydrogenase from within the cells
- dilution of analytes such as sodium
- direct inhibition or acceleration of analytical reactions by cell contents
- spectral interference by haemoglobin, seen in assays such as bilirubin.[16]

To reduce the incidence of in vitro haemolysis, care is required when taking and handling samples. Tourniquet time should be minimal, an appropriately sized needle should be used, and the skin should not be punctured before the disinfectant alcohol has dried. Blood should never be transferred from a syringe to a sample tube through a needle. If samples are collected using a needle and syringe, minimal vacuum should be applied. Ideally, samples should not be collected from an intravenous access site (the exception to this rule is when an experienced operator accesses a peripherally inserted central catheter or infusion port). Finally, collection tubes should never be shaken once filled with blood; mixing by gentle inversion is adequate.

Avoiding contamination

The 2 main sources of contamination of blood samples are intravenous fluids and cross-contamination with anticoagulants during sample collection.

Blood should never be drawn from an intravenous line or from the same arm receiving intravenous fluids, as the results will be influenced by the components of the fluid rather than the actual concentrations in the blood. These changes may be subtle and not immediately recognised as being caused by contamination, leading to incorrect management decisions.

Following the correct order of draw prevents cross-contamination between samples. A typical order of draw is shown in Table 1; however, the table does not list all the possible tubes. Individuals involved in sample collection should refer to their local laboratory's requirements as the colour of tubes and caps can vary. Once collected, blood should never be transferred from one tube to the other, even to bring the sample 'up to the line'.

Potassium ethylenediaminetetraacetic acid (EDTA) serves as an anticoagulant by chelating calcium and magnesium – 2 divalent cations critical for clotting to proceed. Cross-contamination of samples with potassium EDTA leads to a reduction in serum calcium, magnesium and zinc concentrations, false elevation of serum potassium concentration, and reduced serum alkaline phosphatase activity. This pattern is obvious when gross contamination is seen; however, it may be subtle and not recognised immediately as the cause of abnormalities.

Table 1 Recommended order of draw for sample collection

Order	Contents
1	sterile medium (blood cultures)
2	sodium citrate
3	gel
4	lithium heparin
5	EDTA (transfusion)
6	EDTA (full blood examination)
7	EDTA + Gel
8	fluoride EDTA

EDTA = ethylenediaminetetraacetic acid

Adequate sample volume

Adequate sample volume enables correct analysis, and small-volume collection tubes are available for samples from paediatric patients and those from whom blood is difficult to obtain. Small-volume samples can be accommodated for most analytes; however, serum bicarbonate concentration is often low due to loss of carbon dioxide to the dead space within the tube. Small sample volume in an EDTA tube destined for a haematology laboratory may also lead to changes in cell morphology.

Appropriate sample volume is particularly important for coagulation studies, in which sodium citrate is the anticoagulant of choice. This is present as a liquid in the tube, and a correct ratio of blood to anticoagulant is crucial because the analysis is predicated on the reversal of the anticoagulant by adding a standardised amount of calcium. Under- or overfilling a sodium citrate tube can thus lead to incorrect measurements or sample rejection.

A list of common blood tests and factors that affect the pre-analytical phase of these tests can be found in Table 2.

Sample storage and transport

Plasma and serum samples for general biochemistry analysis should be separated from cells by centrifugation as soon as possible, ideally within 2 hours of collection. Collection centres for large pathology networks will usually have a facility to centrifuge samples; however, this is not always available, particularly with in-office collections, and these samples should be transported to the laboratory promptly after collection. Typical changes in biochemical profiles due to prolonged storage without centrifugation include falsely elevated potassium and reduced glucose concentrations.

In general, most blood samples should be stored at room temperature to maintain stability. Refrigeration of samples leads to acceleration of potassium leakage from cells, due to inactivation of the sodium–potassium ATPase that maintains potassium ions within cells. In contrast, some samples, such as those for measuring adrenocorticotrophic hormone, ammonia, and blood gases, should be stored and transported on ice or refrigerated at 4°C to maintain stability. Additionally, certain analytes must be protected from light to avoid degradation, such as bilirubin and vitamin A.

The means of transport of samples can also be critical to ensuring sample integrity. In the hospital environment, blood culture bottles and blood gas samples should never be transported by pneumatic tube because of the risk of breakage, while in the community, couriers should be able to reach testing sites in a timely manner.

Some tests may not be feasible if blood is collected at a remote location because of specific handling and transport requirements. If there is any uncertainty, it is advisable to contact the laboratory to confirm whether the test can be conducted at the current location or if the patient will need to travel to a different collection centre.

Getting help

For more information on laboratory test protocols, check with your local laboratory as procedures may vary. In situations where unexpected test results raise concerns about possible pre-analytical errors, consult the pathologists and scientists from your testing laboratory.

Conclusion

Pre-analytical errors are common and minimising their occurrence is essential in ensuring correct laboratory test results. Appropriate patient preparation is crucial and should take into consideration factors such as posture, fasting status, timing of blood collection, and the presence of interfering agents. Correct patient identification, and preventing haemolysis and cross-contamination of samples, are imperative during sample collection. Samples should be stored and transported according to specific guidelines to maintain stability. If there are concerns about potential pre-analytical errors, individuals should consult their local laboratory for guidance. <

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Conflicts of interest: Linn Lee has no conflicts of interest to declare.

Wayne Rankin is the Director of Education and Training for the Australasian Association for Clinical Biochemistry and Laboratory Medicine and has been a member of their Education Committee since 2014. Wayne received travel funding from Sanofi to attend an educational meeting in 2023 and has received consultancy fees from Amicus Therapeutics.

Table 2 Common blood tests, factors that affect the pre-analytical phase, and key recommendations

Name of test	Factors affecting the pre-analytical phase	Recommendations
Aldosterone	Posture Medications [NB1] Timing of collection	Best time to collect is mid-morning. Indicate if sample was taken with patient upright or supine.
Blood film	Sample time in tube	Red cell morphology may change with prolonged time in tube. Prepare blood films on-site if delay is anticipated.
Blood gases	Temperature	Place on ice and transport to lab immediately.
Coagulation studies	Overfilled or underfilled sample tube	Fill the sample tube to the indicated line.
Cortisol	Diurnal variation Corticosteroid use Combined oral contraceptive pill Oral estrogen Pregnancy	Serum cortisol (for hypocortisolism) should be taken between 8 and 10 am. Corticosteroids suppress endogenous cortisol and should be stopped 24 to 72 hours before testing. Oral estrogen raises cortisol-binding globulin and thus cortisol concentrations, and should be stopped for 6 weeks before testing.
Creatinine	Trimethoprim Cephalosporins (some assays only) Creatine supplements	Stop offending agents if feasible and allow washout before retesting. Contact your laboratory for advice.
CrossLaps (C-terminal telopeptide)	Fasting status	Ensure patient has fasted before collection.
Fasting glucose	Fasting status Collection tube Delayed separation	Fast for at least 8 hours. Use appropriate collection tube (check with laboratory). Process samples promptly.
Iron	Diurnal variation Fasting status	Fasting samples are preferred, as results can be falsely elevated if not fasted.
Lipid studies	-	Routine fasting is not required.
Plasma metanephrines	Posture Caffeine Medications (MAOIs, SNRIs, SSRIs, TCAs)	Allow patient to lie supine for 30 minutes prior to collection. Allow washout of interfering medications before testing.
Potassium	Haemolysis Delayed separation Leucocytosis Thrombocytosis EDTA contamination	Take measures to reduce haemolysis. Process samples promptly. Ensure correct tube use (do not pour blood from one tube to another).
Renin	Cryoactivation Medications [NB1]	Store and transport samples at room temperature.
Testosterone	Diurnal variation	Collect sample between 8 and 10 am, or as soon after waking as possible.
Thyroid function tests	Biotin	Discontinue biotin-containing supplements or products at least 1 week before testing.
Urea	Dehydration	Ensure patient is adequately hydrated before sample collection.
Vitamin A	Photolability	Protect from light (wrap in aluminium foil).
Vitamin C	Photolability Temperature	Protect from light (wrap in aluminium foil). Place on ice immediately after collection.

EDTA = ethylenediaminetetraacetic acid; MAOIs = monoamine oxidase inhibitors; SNRIs = selective noradrenaline reuptake inhibitors; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants

NB1: Medications that affect renin and aldosterone concentrations include mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, dihydropyridine calcium channel blockers, thiazide and loop diuretics, methyldopa and clonidine. Antihypertensives that do not interfere include verapamil, prazosin, moxonidine and hydralazine.

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