




Factors that can affect laboratory investigations

In 2013 we published an article entitled “Best tests?” (Best Tests 18; Feb, 2013). In this article we challenged clinicians to consider whether they could improve the method and manner in which they request laboratory investigations. To put it simply, it is about selecting the right test, at the right time, for the right patient. After making the decision that an investigation is necessary, and selecting the most appropriate test, consideration must be given to what factors are present that may affect the interpretation of results, or even the decision to proceed with the test at that time.

In many scenarios, a diagnosis is based predominantly on the patient’s history, with supporting evidence from physical examination and laboratory investigation. Diagnosis is rarely based on the results of laboratory investigations alone, and testing is not always the best clinical course of action. Consideration needs to be given to whether testing will add meaningful information to the overall clinical picture, and then to the factors which may need to be taken into account when interpreting the result. Can the test be done immediately or is the patient required to prepare in some way, e.g. fasting? Is the patient taking any medicines that may influence or invalidate the results, e.g. taking antibiotics prior to faecal antigen testing for *Helicobacter pylori*? Does the test need to be performed at a certain time, e.g. measuring testosterone in the morning? Is the test being done at the right stage of illness, e.g. testing for antibodies after seroconversion has occurred? A test result returned from the laboratory will usually include a reference range or a threshold value based on guidelines. However, this result “on paper” does not always represent the clinical significance of a test, which is only apparent once all other factors for that individual patient have been taken into account.

This article is not intended to be a comprehensive guide, but rather an overview of general concepts to guide clinicians in considering the wide array of factors that can influence laboratory investigations or interpretation of results.

 If you are uncertain about how a test may be affected by a specific factor you have identified, discuss this with the testing laboratory first, or include this detail on your investigation request form.

To test or not?

Considerations prior to requesting a laboratory investigation include:

- What is my reason for requesting this test? E.g. am I investigating symptoms and signs? Am I monitoring a disease or effect of a medicine? Is this a screening test?
- Has this test already been done? Does it need to be repeated? If so, when?
- Will the test improve patient (or in some cases, family or partner) care?
- Is this the right test or combination of tests for the clinical situation?
- Is it the right time to do the test?
- How should the sample be taken?
- How should the sample be stored and transported?
- How will the test result be interpreted?
- How will the test result influence patient management?
- What will be the consequences of a false positive result?
- Are there potential harms of doing this test?
- How will the patient be informed of the result?

Biological variation

There are certain variations in laboratory test results that can be expected due to non-modifiable biological factors, such as age, biological rhythms and physiological changes during pregnancy. These factors may be controlled for, e.g. by selecting the most appropriate time in the day, month or year for a test, or may be taken into consideration in the interpretation of results, e.g. different reference ranges or thresholds for clinical significance depending on age, sex or pregnancy status.

Advancing age


The physiological changes associated with ageing, along with increasing co-morbidities and polypharmacy, mean that older people are more likely to have test results that fall outside of the normal reference range. For some tests, laboratories are able to provide an age-adjusted reference range, but for other tests, a result outside of the range in an older patient, needs to be interpreted in the context of their overall clinical picture.

In many cases, assessing the rate and magnitude of change over time offers more information than interpreting the value of an individual result. Often the population range of a test shows much more variation than that for an individual patient, e.g. serum creatinine, liver enzymes. In such cases the patient's own previous results are a useful baseline.

An example of the effect of age, sex and other variables on interpretation of laboratory results is serum alkaline phosphatase (ALP), which may be requested as part of liver function tests. The upper reference limit is markedly increased during puberty as this is the time of maximum bone remodelling. After this period ALP levels fall to a new upper limit through younger adult life, and then rise again, particularly in females around the time of perimenopause, largely reflecting an increase in bone turnover at that time. Marked increases in serum ALP may also occur in women in late pregnancy (due to production of ALP by the placenta), and during other times such as in the weeks after healing of a fracture.

Another example of laboratory values which change with age is lipids. In adults, total cholesterol, LDL and triglyceride levels increase with age, until approximately age 50 to 60 years in males and age 60 to 70 years in females, when they begin to decline in most people; triglyceride levels tend to continue to increase in older females.¹ Many laboratories are


no longer reporting reference ranges for lipid levels, as this is considered less clinically useful than treatment targets based on the underlying cause of raised levels and cardiovascular risk.²

 When interpreting laboratory results in an older patient, ensure that an age-appropriate reference range is used if available. Normal age-related changes, e.g. deterioration in renal function, may explain results outside of the reference range in an older patient, but age alone should not be considered as the only cause of an abnormal result.

Biological rhythms

Many laboratory parameters vary depending on the time of day, week, month or year when they are sampled. Body temperature, hormone production (e.g. cortisol, testosterone), platelet and cardiac function and cognitive function follow a circadian (24 hour) rhythm.¹ To allow for this effect, some laboratory tests are recommended at specific times of the day, e.g. testosterone should be sampled between 7 am – 10 am. This is because peak testosterone levels usually occur in the early morning; evening levels are often substantially (up to 50%) lower, especially in younger males.² For most purposes, serum cortisol* should preferably be sampled in the early morning as there is marked diurnal variation, with early morning levels at least 50 – 100% higher than levels in the late afternoon.²

Testing vitamin D levels (25-hydroxyvitamin D) is seldom necessary, however, if levels are obtained, they need to be interpreted in the context of the time of year they are sampled. Seasonal variations occur with vitamin D, with the lowest levels usually observed at the end of winter (in countries with defined seasons, such as New Zealand). For example, if a patient has a mild vitamin D deficiency at the end of winter, this is likely to be less clinically significant than a patient with a mild deficiency at the end of summer.

 For further information, see "Age-related testosterone decline in males" Best Tests (Jun, 2012) and "Vitamin D supplementation: navigating the debate" BPJ 36 (Jun, 2011).

* Serum cortisol levels have a wide reference range, and therefore this is a relatively inaccurate measure of cortisol excess or deficiency. The dexamethasone suppression test is used to help exclude Cushing's syndrome and the Synacthen stimulation test is used to investigate for primary or secondary hypoadrenalism.²

Menstrual cycle


Females who are menstruating have predictable monthly rhythms of FSH, LH, oestrogen and progesterone. In a general practice setting, these hormone levels may be requested to investigate conditions such as oligo/amenorrhoea or sub-fertility. The interpretation and meaningfulness of the results is dependent not only on the value, but on the stage in the cycle when the hormone was measured.

In a normal menstrual cycle LH levels peak mid-cycle to trigger ovulation. FSH levels also peak mid-cycle. Oestradiol (the predominant oestrogen in women who are ovulating) is highest prior to ovulation, and then reduces if fertilisation does not occur. Unless ovulation is being investigated, levels of these hormones are best measured early in the menstrual cycle.²

N.B. Investigation of oestradiol levels is not useful in women who are taking oestrogen-containing oral contraceptives as this suppresses the pituitary ovarian axis. LH and FSH are also suppressed in women taking depot progesterone.²

Progesterone levels peak in the second phase of the menstrual cycle, after ovulation has occurred, to prepare the endometrium for implantation of an embryo. Progesterone levels are sometimes measured to establish if ovulation has occurred; usually seven days before the expected date of menstruation, i.e. approximately day 21 if the woman has a regular 28 day cycle.


Oestradiol levels decrease and FSH levels increase during menopause, but monitoring these hormone levels is not always reliable in predicting when a woman is entering menopause, as fluctuations occur with varying ovarian activity.

 For further information, see "Reproductive hormones: the right test at the right time, for the right patient" Best Tests (Feb, 2013).

Pregnancy

Physiological changes during pregnancy result in alterations in many laboratory parameters, such as blood volume, liver and renal function and hormone levels (Table 1, over page). Reference ranges for different stages of pregnancy are available for some laboratory tests, however, these ranges are often not as well defined as the general reference range. In addition, pregnancy-related changes, such as alterations

in binding proteins, can affect assays differently, e.g. free hormone levels can be assay dependent. Therefore caution is recommended in interpreting results based on reference ranges and the laboratory should be contacted if there is any doubt.

 When requesting a laboratory investigation in a woman who is pregnant, note the gestational week on the laboratory request form.

Individual variations

A patient's diet, health status and lifestyle factors can all have a pre-analytical influence on laboratory parameters.

Diet and nutritional status

Fasting, calorie restriction, food exclusion diets, malnutrition and dehydration can all affect laboratory results. The significance of some laboratory tests is dependent on controlling dietary factors, e.g. ensuring there is sufficient gluten in the diet for at least several weeks prior to serology investigations for coeliac disease, or fasting prior to assessing the effect of intervention in a patient with previously high triglyceride levels. In other scenarios, assessing dietary factors can help to interpret unexpected laboratory results, e.g. a vegetarian or vegan diet can result in decreased levels of vitamin B12, a low carbohydrate diet can cause increased ketone levels (as part of urinalysis) and a high protein diet can result in increased uric acid levels.

Fasting for 12 hours prior to laboratory testing may be helpful or even necessary, depending on the clinical scenario, to get the most accurate result for the following tests, which are affected by the ingestion of certain foods:⁴

- Glucose; only if indicated – for most patients HbA_{1c} is now recommended as the test of first choice for the diagnosis and monitoring of type 2 diabetes, and does not require fasting
- Triglycerides; for most patients fasting is not required for lipid testing, but may be useful for monitoring in people with high triglyceride levels
- Uric acid; fasting is not usually required in practice to get accurate results but the effect of recent dietary intake may help to explain unexpected results
- Creatinine; a recent meal with high meat content can have a significant influence on serum creatinine, and this should be considered when monitoring eGFR⁵


Table 1: Examples of laboratory values that change with pregnancy^{1, 2, 3}

Increases	Decreases
Alpha-fetoprotein (AFP); peaks in third trimester	Haemoglobin; due to haemodilution caused by greater blood volume
Alkaline phosphatase (ALP); up to four fold increase in third trimester	Ferritin; decreases as pregnancy progresses
Blood volume (mean plasma volume); increases by 30–50%	FT4; may decrease slowly in late pregnancy (can be assay dependent)
Lipids; up to 40% increase in cholesterol, triglyceride levels can markedly increase in some women (due to the effect of oestrogen)	Prothrombin and partial thromboplastin times
Creatinine clearance; glomerular filtration rate increases 40–60% (eGFR cannot be reliably calculated)	TSH; decreases first trimester, then returns to normal (due to the effect of hCG)
ESR; increasing to 30–60 mm/h as pregnancy progresses	Sodium; slight decrease due to changes in blood volume and fluid homeostasis
Hormones; oestrogen, testosterone, progesterone, human chorionic gonadotrophin (hCG), prolactin	
Iron binding (transferrin levels); significant increase even in a non-iron deficient woman (due to the effect of oestrogen)	
White blood count; may increase to 15–18 x10 ⁹ /L	

Sustained low caloric intake and starvation can result in numerous changes to laboratory parameters such as glucose, thyroid function, electrolytes, liver function, renal function and lipids.⁴ Uric acid levels may be increased as a result of ketonaemia (causing reduced clearance).³

Malnutrition has varying effects on laboratory results, depending on the nature of the patient’s nutritional status. Malnutrition is classically thought of as a deficiency of protein and energy, with or without micronutrient deficiencies. However, malnutrition may be defined as under-nutrition, over-nutrition or deficiency of specific nutrients. Malnutrition should be considered as a cause for results such as decreased ferritin, folate and vitamin B12 levels.

Dehydration can be considered as a cause of sodium and potassium imbalances, and can also affect numerous other indices, such as creatinine and urea, albumin, lipids and haematology indices.

 For further information see: “A primary care approach to sodium and potassium imbalance”, Best Tests (Sep, 2011) and “Strategies to improve nutrition in elderly people”, BPJ Special Edition (May, 2011).

Caffeine

The effect of caffeine on laboratory parameters has not been fully studied. It has a short half life of three to seven hours, but this varies among individuals.⁴ Caffeine intake causes transient increases in blood glucose levels and impairs glucose tolerance.^{3, 4} It can also affect other specialised investigations such as interpretation of metanephrines when investigating hypertension.

Alcohol

The effect of alcohol consumption on laboratory investigations depends on the duration and extent of use. Acute (transient) effects of alcohol consumption (within two to four hours) include decreased serum glucose and increased plasma

Reference range

A reference range for a laboratory test is a statistically-derived numerical range of results that is obtained by testing a sample of “healthy” individuals. Defining “healthy”, however, is not straightforward, and depends on a wide range of factors and assumptions; in many cases to define a range simply using “perfectly healthy” patients would make it unrealistic and unusable.

The range is also commonly assumed to have a Gaussian distribution, in that 68% of values lie within one standard deviation (SD) of the mean value, 95% within two SDs and 99.7% of values within three SDs.¹ However, many ranges do not have a Gaussian distribution, but rather the upper end of the distribution is skewed. In these cases the range can be derived either by log transformation of the data, or simply by identifying the relevant 2.5th and 97.5th percentiles in the population being studied (with attempts to exclude those patients likely to have an underlying pathology by clinical, laboratory and statistical means).

Reference ranges for laboratory results usually include two SDs from the mean value meaning that one in 20

“healthy” individuals will have a test result outside the reference range.¹ Reference ranges vary between laboratories, and can change if new evidence becomes available. The upper and lower limits of the range are not absolute and do not define “normal” and “abnormal”, but are points at which the probability of clinical significance tends to increase.

Some “reference ranges” are based on recommendations from international bodies for optimising patient outcomes, rather than on a population statistical distribution. For example, the upper reference limit for TSH in early pregnancy is based on guidance statements from the Endocrine Society and the American Thyroid Association. The recommended limit for serum uric acid in patients taking uric acid lowering treatment (0.36 mmol/L) is based on the European League against Rheumatism (EULAR) guidelines.

Interpretation of a result outside the stated reference range is therefore very dependent on the clinical background of the patient, the pattern of other abnormalities, and the clinical question(s) being asked.



Timing of investigation in relation to stage of illness

The significance of an investigation can be dependent on when the sample was taken in relation to the stage of the disease process. The stage of illness can also influence the selection of the most appropriate investigation. For example, a serology test for syphilis may be falsely negative if the sample is taken too early after exposure, and therefore seroconversion has not yet occurred. Different types of serology test will provide information about active or past infection.

Acute illness can also affect the result of some investigations, e.g. ferritin is an acute phase protein and levels can be increased by inflammation and infection, as well as chronic disease and malignancy.

lactate with a reduction in urinary uric acid excretion due to the inhibition of hepatic gluconeogenesis.³

Chronic effects of alcohol consumption on laboratory investigations include:²

- Elevated gamma glutamyl transferase (GGT) and mean cell volume (MCV) which are commonly used to test for excessive alcohol consumption
- Elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT) and AST/ALT ratio
- Elevated triglyceride levels
- Elevated uric acid and ferritin levels due to fatty liver and alcoholic hepatitis
- Elevated creatine kinase due to alcoholic myopathy
- Other haematological abnormalities, e.g. anaemia and thrombocytopenia

Alcohol consumption can also contribute to vitamin and mineral deficiencies due to replacement of food with alcohol or as a result of interference of absorption of vitamin and minerals, e.g. decreased folate, vitamin A, vitamin B and calcium levels.

Tobacco smoking

Regular smoking and exposure to nicotine can have both acute and chronic effects on laboratory investigations, although the mechanisms behind these changes are not fully understood. Within one hour of smoking one to five cigarettes, plasma/serum concentrations of fatty acids, adrenaline, glycerol, aldosterone and cortisol are increased.³ People who are chronic smokers may have persistent increases in leukocyte counts, heavy metals, lipoproteins, tumour markers and haematocrit (PCV), and decreases in the activity of some enzymes (e.g. angiotensin-converting enzyme).³

Exercise

The effect of exercise on laboratory parameters is dependent on the health status of the patient, air temperature during exercise and intake of food and water during or following exercise.⁴ Extreme exercise or vigorous exercise in a person unaccustomed to this level of activity can result in changes to some laboratory parameters. For example, the most common cause of elevated creatine kinase (CK) levels is



exercise. Intense exercise can cause an elevation in CK levels for several days to a week. Well-muscled people often have CK levels persistently above normal.²

Thyroid function is also known to be altered in people undergoing high-intensity exercise. For example, anaerobic exercise increases TSH and FT4 levels, but decreases FT3.⁴ Liver function (AST and to a lesser extent ALT) tests can increase after exercise. Transient proteinuria and haematuria are also common after exercise, but usually resolve after a few days. Other analytes that can be increased by exercise include urea, creatinine, lactate dehydrogenase, prothrombin time, and D-dimer levels. Fibrinogen and the activated partial thromboplastin time (APTT) can be reduced.⁴ Most of these effects are likely to be transitory (e.g. persistent for a few hours to a few days after exercise), but this depends on individual patient factors.


Medicines

The medicines that a patient is taking can significantly affect some laboratory results, therefore this needs to be taken into consideration when interpreting results. It is good practice to note the relevant medicines that a patient is taking on the laboratory request form, especially if they may potentially influence results, e.g. antihypertensives being taken when investigating secondary causes of hypertension or hormone replacement therapy being taken when requesting endocrine tests.

Medicines can have a direct effect on the sample or laboratory testing process, causing an inaccurate result. For example, when investigating for *H. pylori*, a false-negative result of a faecal antigen test may occur in patients taking a course of antibiotics or proton pump inhibitors (PPIs) as this would decrease the gastric load of *H. pylori*.

Medicines may also cause a biological effect to the patient which would account for an altered result. For example, some antibiotics (e.g. cotrimoxazole and erythromycin), cardiovascular medicines (e.g. amiodarone and propranolol), NSAIDs (e.g. piroxicam) and gastrointestinal medicines (e.g. omeprazole) may account for a raised INR result in a patient taking warfarin who normally has a stable INR.² Long term use of metformin or PPIs is a possible explanation for a low vitamin B12 level.² Many medicines have an effect on the balance of sodium and potassium in the body, e.g. diuretics may cause hypernatraemia (especially loop diuretics), hyponatraemia (especially thiazides), hyperkalaemia (especially potassium-

sparing diuretics) and hypokalaemia (loop and thiazide diuretics).²

 For further information, see: "A primary care approach to sodium and potassium imbalance", Best Tests (Sep, 2011).

When monitoring the serum concentration or effect of a medicine, the laboratory test needs to be timed depending on the drug's metabolism, e.g. a blood sample for testing lithium levels should be collected 10–14 hours after the last dose and a sample for testing digoxin should be collected at least eight hours after the last dose.² When initiating a patient on warfarin, INR levels should be sampled daily in the morning, after an evening dose of warfarin, to calculate necessary dose adjustments.²

Analytical variation

Analytical variation occurs due to imperfections in testing methods and equipment, which may cause analyte values to be slightly different each time they are measured. Modern testing methods and laboratory equipment mean that analytical variation is usually less of a factor in differing test results than biological variation. Ideally the variation in measurement (expressed as analytical coefficient of variation, or CVa) should be less than half the individual patient biological variation of the analyte in question (CVi).

 For further information on variation with specific analytes, see: www.westgard.com/biodatabase1.htm


Collection, storage and transport of samples

If a sample is being collected at the practice, it is important to be familiar with the type of collection container and sample medium that is required by the laboratory for the specific test, as this can affect results, sometimes markedly. For example, a swab for PCR testing for pertussis should be transported in a dry tube or a tube with universal viral transport medium, but not in a tube with charcoal transport medium (which is acceptable for swab culture).



Other examples of collection or transport requirements for optimal test results include:

- Blood samples for coagulation studies, including platelet count, D-dimer, prothrombin time, APTT and fibrinogen, should be transported to laboratory within four hours of collection⁶
- Urine specimens for culture should be stored in a fridge prior to transportation to reduce the rate of multiplication of microorganisms
- Samples for glucose analysis should be separated as soon as possible after collection; this applies even with samples collected in fluoride or oxalate collection tubes, as reduction in glucose concentration still occurs for 60–90 minutes
- Samples for potassium or phosphate should not be left overnight, especially in the fridge, as results can be markedly altered, e.g. late evening collection with delayed transport to the laboratory
- Faecal samples for culture and microscopy should preferably be transported to the laboratory within four hours
- Semen samples for fertility testing should be kept by the patient at body temperature, e.g. by storing in a clothing pocket, and transported to the laboratory within one hour of collection.² The same level of urgency is not required for post-vasectomy semen analysis.

 Refer to your local laboratory for specimen collection requirements. Some providers have online resources, e.g. www.labtests.co.nz/referrers/tests/collection-guide

Haemolysis

Haemolysis is the destruction of red blood cells, resulting in release of haemoglobin and cellular constituents, e.g. potassium, into the plasma. It is a cause of inaccurate blood test results and can occur either *in vitro* during collection, storage or transportation of a blood sample or *in vivo* resulting in haemolytic anaemia if severe.

In vivo and *in vitro* haemolysis have different analytical features, e.g. haptoglobin levels are normal with *in vitro* haemolysis, therefore analysis can reveal if haemolysis occurred during the collection of the blood sample or if it was already present.² The possibility of haemolysis should usually be noted on results by the laboratory staff, including

the likely degree of interference and reliability of the result. If the haemolysis is severe, a recollection of the sample may be advised.

Some of the analytes that can be affected when *in vitro* haemolysis has occurred include:²

- Elevations in potassium, AST (ALT is less affected), lactate dehydrogenase, phosphate
- Reductions in bilirubin, troponin T, insulin

Standardised sample collection and transport processes can help to prevent *in vitro* haemolysis, including:³

- Allowing alcohol to dry completely when it is used for skin sterilisation prior to venepuncture
- Not leaving a tourniquet on for longer than two minutes
- Using an appropriately sized needle for collection (20 – 22 gauge needles can be used for most routine collections)
- Not removing the needle from the vein if the vacuum tube is attached
- Not exposing the specimen to extremes in temperature
- Avoiding vigorous mixing or shaking of tubes
- Avoiding delay in sending samples to the laboratory

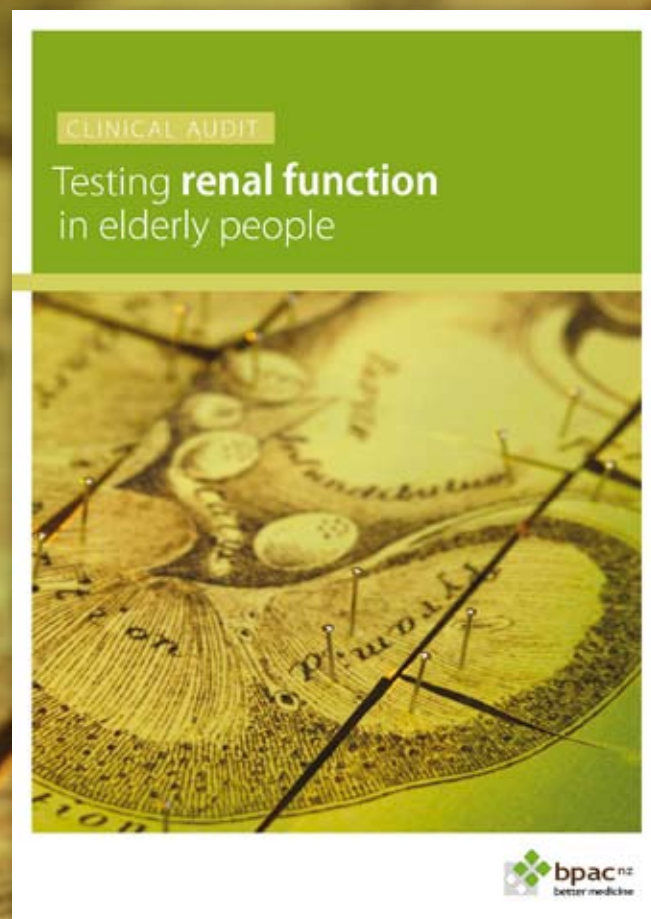


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