

# *best tests*

February 2013

General principles of  
laboratory investigations  
Reproductive hormones



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better medicine

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# Best tests?

The general principles of laboratory investigations in primary care

Laboratory investigations are essential for the diagnosis and management of many conditions. However, laboratory tests do not provide clinical value in every scenario, and in some cases, they may even cause harm. Before a laboratory test is requested, clinicians should consider the aim of the test and have a clear understanding of how the result will be interpreted and how the patient's management will be affected by the result. Understanding the clinical situations where laboratory testing may be problematic can help to improve the overall approach to testing.

### **“Testing, testing: one, two, three”**

- 1. Think twice before you test**
- 2.. Select the right test, at the right time, for the right patient**
- 3.. Ask yourself: can I improve my testing?**

### **Think twice before you test**

Laboratory tests are generally requested in primary care for one of the following reasons:

1. **Diagnosis:** to either include or exclude a disease, e.g. thyroid stimulating hormone (TSH) levels in a patient with suspected thyroid dysfunction
2. **Establishing a baseline prior to treatment initiation:** e.g. liver function test (LFT) before commencing methotrexate
3. **Monitoring:**
  - a. To ensure a medicine is within a therapeutic range, e.g. patients taking lithium where the serum lithium concentration relates to clinical effect
  - b. To detect early signs of an adverse effect to treatment, e.g. full blood count in patients taking clozapine
  - c. To monitor or predict the response to treatment, e.g. INR assessment in patients taking warfarin, serum urate monitoring in patients taking allopurinol, or antimicrobial susceptibility of a pathogen
  - d. To monitor long-term conditions for disease control and associated complications, e.g. the monitoring of HbA<sub>1c</sub> and albumin creatinine ratio (ACR) in people with diabetes
4. **Targeted testing,** e.g. antenatal screening for rubella status, lipid levels as part of a cardiovascular assessment

In each of these situations the test result will benefit the patient and the clinician by allowing better decisions to be made about future management.

When considering laboratory investigation it is important to acknowledge that testing is not always beneficial, and that in certain situations the balance may shift from benefit to harm. Understanding the clinical situations which may lead to a poor outcome can provide insight into when to be more cautious in deciding if laboratory investigation is needed, or if a request for tests should be deferred or delayed.

## Considerations before testing

Before requesting a laboratory test it may be helpful for clinicians to consider their answers to the following questions:

- What is my reason for requesting this test?
- 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?
- How will the test result be interpreted?
- How will the test result influence patient management?
- Are there potential harms of doing this test?

The following examples demonstrate potentially problematic scenarios when considering laboratory investigations.

### Laboratory tests may reveal incidental findings

The early discovery of dormant conditions or incidental findings that have little or no long-term consequences to the patient's health can be unveiled by laboratory investigations. Once a condition is identified, it can sometimes be difficult for the patient to understand and accept that treatment is not necessary.

Over-diagnosis and over-treatment are the most important adverse effects of screening programmes. Estimates of over-diagnosis of indolent cancers (slow growing and low-grade) in PSA screening populations are 27% at age 55 years and 56% at age 75 years.<sup>1</sup> The risk of patients receiving a diagnosis and treatment for a cancer that would not have affected their long-term outcome is one of the reasons why PSA screening is controversial, especially in older patients with co-morbidities.

A discussion with patients about the potential risks of testing, and consideration of what a positive or negative result will mean for the patient's management, can help when making an informed decision about whether to test or not.

### Some symptoms are medically unexplained

In some cases, patients with underlying emotional distress or psychological illness present with a complex pattern of

medically unexplained symptoms, leading to a degree of diagnostic uncertainty. An increasing level of uncertainty about the patient's presenting symptoms and signs, leads to an increasing number of laboratory tests requested.<sup>2</sup> Rather than clarifying the situation, sometimes this can lead to "digging an even deeper hole", and emphasises the importance of thoughtful test requesting and interpretation.

Normal laboratory results are often not helpful in reassuring such patients. In addition, multiple test requests are also likely to eventually result in a value being identified outside the normal reference range, regardless of whether it is clinically significant (Page 9). This ultimately leads to dissatisfaction for both patients and clinicians, and increased health costs.

This situation provides the "perfect storm" of clinical uncertainty. Instead of requesting a battery of tests, it may be more appropriate to identify any psychological or environmental stressors, or administer a formal depression screening tool. In this scenario, providing an explanation for the patient's symptoms in relation to the psychological problems is likely to have more benefit than a series of laboratory tests.<sup>3</sup>

### Patients ask for tests themselves

Patients often ask for laboratory tests based on their own research, or following consultation with friends or family. A common scenario is for a patient to be concerned about possible dietary deficiencies. Trace element testing, e.g. zinc, copper and selenium, has been increasing in New Zealand in recent years.<sup>4</sup> However, in most cases, patients are unlikely to have a deficiency, and borderline low levels are a non-specific finding, with low predictive value of organic disease (see Pages 8 and 9 for further discussion of these terms).<sup>4</sup> For example, transient inflammation is a common cause of low levels of iron and zinc, but can also result in raised copper levels (due to an increase in its binding protein ceruloplasmin).

Education and evidence-based discussions can be helpful in explaining to patients why testing is not always appropriate. Patients need to be aware that they may need to pay for some tests themselves, if they are not clinically justified.



## Selecting the right test, at the right time, for the right patient

There are approximately 200 standard laboratory tests in New Zealand, with many more available on request. It has been estimated that if one patient undergoes 12 biochemical tests there is almost a 50% chance that one or more of the results will be outside the normal reference range, even in a healthy person.<sup>5</sup>

Once the decision is made to request laboratory investigation, selecting the right test at the right time for the right patient can sometimes be a challenge. This decision may be influenced by many factors including patient and family/whānau expectations, emerging evidence, changing guidelines, clinical experience and individual clinical, social and cultural factors. All of which are combined with the need to identify the problem within the consultation time, and the natural concern of the clinician not to get it wrong and miss a diagnosis.

In the search for a diagnosis, some patients may undergo numerous investigations, some of which, only in hindsight, will be unnecessary. This does not mean that a carefully chosen test with a normal result was wasted, rather it may have redirected the investigation to another likely explanation. However, in the worst case scenario, a “shotgun” approach to laboratory testing can lead to misdiagnosis and patient harm. By having a clear purpose when selecting a test and selecting the right test, in the right circumstances, with a clear understanding of how results will be interpreted, clinicians can improve patient outcomes while making the best use of tests.

### Selecting the most appropriate test

It is estimated that over half of all errors that occur in the process of laboratory investigation, take place during the test selection process.<sup>6</sup> To reduce the likelihood of errors clinicians should be careful not to request tests that are likely to cause confusion or false reassurance. For example, a faecal occult blood test (FOBT) is inappropriate in an older patient with anaemia, where there is a high suspicion of bowel cancer, as a negative result is not sufficiently reassuring to avoid definitive investigation and a positive result could be due to other causes.

Sometimes it is clear that an investigation is required, but there may be uncertainty as to what test to use. For

example, the routine use of laboratory microscopy and culture is inappropriate for testing for microscopic (non-visible) haematuria. Dipstick analysis alone is sensitive enough to determine the presence of haematuria in patients with suspected renal disease.

The usefulness of some tests depends on the clinical setting. For example, tumour markers are useful tests when used in the appropriate clinical context, e.g. patients receiving cancer treatments, but as a first-line rule in/rule out test for cancer, they have a limited diagnostic value in the large majority of clinical circumstances. In a United Kingdom-based study, requests for tumour marker tests from General Practitioners were studied retrospectively over a 34 month period, and the appropriateness of each test reviewed. Comparison with best-practice guidelines suggested that 84% of the tests requested were inappropriate.<sup>7</sup>

### The timing of laboratory tests is an important consideration

Even if a test is appropriate, it needs to be requested at the right time for the patient, and with the right preparation, where necessary.

Some tests require certain factors to be present (or not present) in order to produce a meaningful result. For example, the measurement of antibodies to tissue transglutaminase (TTG) in a patient with suspected coeliac disease may be falsely-negative if the patient has already removed gluten from their diet. A patient undergoing skin prick testing for allergies needs to avoid antihistamine medicines for at least 72 hours prior to undertaking the procedure, in order not to mask any response.

Other tests must be undertaken at specific times. For example, a patient undergoing therapeutic drug monitoring must have samples taken at certain intervals to measure the drug concentration relative to dosing, in order to maximise clinical effect, while avoiding toxicity. Similarly, tests such as cortisol and iron should be measured in the morning, as diurnal variation leads to a fall in levels later in the day.

Some tests must be timed to coincide with a certain stage of the disease cycle. For example, if an HIV serology test is requested too early, seroconversion may not have occurred, and therefore a false-negative result is possible.

In another example, raised serum urate levels are the most important risk factor for gout, however, ideally levels should not be measured during an acute episode of gout, as they may be misleadingly normal during this time in 11–49% of people.<sup>8</sup>

## Can I improve my testing?


There are several examples of ways in which clinicians can use laboratory investigations in a more effective way.

### Use serial testing rather than parallel testing

Serial testing is when subsequent tests are requested, based on the results of initial tests, rather than testing all at once (i.e. parallel testing). For example if a patient presents with feeling “tired all the time” a clinician may consider a full blood count, ferritin and TSH as first-line tests. Based on these results, the clinician can then instruct the laboratory to further analyse the sample for other tests, such as B12/folate and electrophoresis, if there is unexplained anaemia.

### Manage test ordering forms

Electronic laboratory test ordering forms can be customised to reduce the temptation for “checkboxitis”, i.e. routinely selecting certain tests with every laboratory request. Consider moving the position of tests that are frequently ticked, such as antinuclear antibody (ANA) and serum magnesium, to another tab. In a large population of 3000 physicians, reformatting a computerised ordering form resulted in a 36% – 53% decrease in requests for vitamin B12, folic acid and ferritin tests after two months.<sup>9</sup>

 You can find out the number of tests ordered by your practice from your “2012 Annual Report: Pharmaceutical & laboratory test utilisation”.

### Be aware of standing orders for tests

Repetition of unnecessary tests can occur when regular tests are automatically repeated, without checking that the clinical justification for testing is still present. For example, continuing to test INR levels in patients who are no longer receiving warfarin or testing lipids in patients no longer receiving lipid-lowering medicines.

### Consider if treatment can commence without testing

Vitamin D testing is an example of a laboratory test that is frequently unjustified in New Zealand. Due to seasonal variation in sunlight, most people’s vitamin D levels fluctuate through the year, making interpretation of vitamin D test results difficult. It is therefore recommended that instead of testing people for vitamin D deficiency, clinicians should focus on treating individuals who have a high likelihood of deficiency, e.g. older people in residential care, dark-skinned people or people with evidence of osteoporosis.<sup>10</sup>

Despite this guidance, the rate of vitamin D testing in New Zealand has been increasing. Between 2000 and 2010, the number of vitamin D tests in the Auckland region increased almost four-fold from 8 500 to 32 800.<sup>10</sup> At a cost of over \$31 per test, more than \$1 million was spent on vitamin D testing in the Auckland region in 2010.<sup>10</sup> The vast majority of this testing did not reveal a vitamin D deficiency, and in some individuals multiple testing was required before a deficiency was detected - one individual was tested 13 times before a deficiency was found, at a total cost of \$404.<sup>10</sup> This compares to the treatment cost of \$10 per year of vitamin D supplementation.<sup>10</sup>

### Consult with the laboratory

When in doubt about what test to order, or how to interpret the results, phone the laboratory. Laboratory staff, including pathologists, are available to provide expert assistance, and this resource should be utilised.





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## Deciding when a test is useful: how to interpret the jargon

The usefulness of any laboratory test is determined by the clinical context. For example, a study of diagnostic tests ordered by 87 General Practitioners for over 1200 patients found that when a test was ordered purely for patient reassurance, approximately 66% of results outside the reference range were interpreted as normal, however, when a test was ordered to confirm a suspected diagnosis, only 28% of results outside the reference range were interpreted as normal.<sup>11</sup>

To determine the likelihood that a patient has a specific condition, based on a test result, the clinician must first consider:

1. How likely is it that the patient has this condition? This is termed the pre-test probability, and is based on the clinical characteristics of the patient, the local prevalence of the diseases being considered, and the clinician's personal experience.
2. How accurate is this diagnostic test? This is determined by the sensitivity and specificity of the test.

**Pre-test probability** is defined as the probability that the condition being tested for is the cause of the symptoms, before a diagnostic test result is known. The pre-test probability helps clinicians to decide whether it is worthwhile requesting a diagnostic test. This probability may be altered during the consultation as symptoms and signs are weighted as being "somewhat more suggestive" or "somewhat less suggestive" of the suspected medical condition.

**The sensitivity of a test** is defined as the proportion of people with the disease who have a "positive" result (above or below the diagnostic threshold used), i.e. the ability of the test to correctly identify patients with the condition. Because the number of false-negatives decreases as the sensitivity of the test increases, a highly sensitive test is useful for "ruling out" a disease if the patient tests negative. Highly sensitive tests, with deliberate use of an appropriate diagnostic threshold for follow-up, are used when the consequences of missing a particular disease are

potentially very serious, such as for an acute myocardial infarction.

**The specificity of a test** is defined as the proportion of people without the disease who have a "negative" result, i.e. the ability of the test to correctly identify patients without the condition. Because the number of false-positives decreases as the specificity of the test increases, a test with a high specificity is useful in "ruling in" a disease if a person tests positive. As with sensitivity, the specificity of a test will vary somewhat depending on the diagnostic threshold chosen.

Unfortunately, almost no test is perfect with complete (100%) sensitivity and specificity. The choice of what threshold is used depends on the parameters of the test and what the purpose is when using it. Deliberately setting the threshold for optimum sensitivity can result in increased numbers of false positives (above or below the threshold) as well, resulting in reduced specificity. Conversely, in other circumstances optimising specificity may be more relevant, at the cost of reduced sensitivity.

Performing several tests serially increases the overall specificity for detecting a particular disease, with each test being sequentially more specific than the previous one.

### Positive predictive value

The positive predictive value is defined as the probability that a patient with a positive test result really does have the condition for which the test was requested. Unlike sensitivity and specificity which are independent of the population being tested, the positive predictive value of a test changes depending on the prevalence of the disease in the population being tested.

For example, a theoretical ELISA test for HIV may have a sensitivity and specificity of 99.9%. Among 1000 intravenous drug users with an HIV prevalence of 10%, the test will correctly detect approximately 100 (99.9) people with the disease, but incorrectly label one person (0.9)

without the disease as being HIV-positive. This is a positive predictive value of 99%.<sup>13</sup> However, in a population of blood-donors (already screened for HIV) the prevalence of HIV would be much lower, closer to 0.1%.<sup>13</sup> For every 1000 blood-donors screened for HIV the test would correctly detect one person (0.9) with HIV, but incorrectly label one person (0.9) as being falsely-positive for HIV. In this second population the positive predictive value of the test falls to 50%.<sup>13</sup>

**The negative predictive value** is defined as the probability that a patient with a negative test result really is free of the condition for which the test was conducted.

**The probability of an abnormal result increases when the number of tests increases**

The risk of a healthy individual having a result outside the reference interval increases as the number of tests selected increases. This is because the normal reference interval for most biochemical tests is defined as being two standard deviations from the mean of a healthy population.<sup>5</sup> Therefore, an average of 5% of all test results from healthy patients will fall outside the normal range and be recorded as abnormal (Table 1).<sup>5</sup>

False-positive results are more likely when people with a low probability of a condition undergo testing. Although

false positive results can cause significant anxiety to the patient, false-negative results can often have more serious health consequences. Test results should always be interpreted in the context of other information gained from the clinical history and physical examination. Results which are borderline need to be interpreted with caution as the inter-test variability could mean the result is either normal or abnormal, so may need to be repeated after a period of time. If there is doubt, consultation with a pathologist about the test results can be helpful.

**Table 1:** Probability of a healthy person returning an abnormal biochemical test result, adapted from Deyo (2002)<sup>5</sup>

Number of tests	Probability of at least one abnormal test (%)*
1	5
6	26
12	46
20	64
100	99.4

\*Assuming each test outcome is independent

**An example of pre-test probability, sensitivity and specificity**

A D-dimer test can be used in conjunction with the Wells Rule or Primary Care Rule to determine the probability of a patient having a deep vein thrombosis (DVT). The sensitivity of the D-dimer test is 88% and the specificity is 72%.<sup>12</sup> Because of the low specificity, D-dimer is most useful as a “rule-out” test for DVT, i.e. a patient with a low or normal D-dimer level, whose symptoms and signs suggests a low pre-test probability of DVT, is unlikely to have a DVT. A patient with a high pre-test probability of DVT should be referred for ultrasound irrespective of the results of the D-dimer test.

 For further information see: “The role of thrombophilia testing in general practice”, Best Tests (Mar, 2011).





# REPRODUCTIVE HORMONES:

The right test at the right time, for the right patient

Understanding the physiology of reproductive hormones, recognising pathology and knowing what tests to order, when to order them and how to interpret the results can be daunting. Hormone tests provide important information when applied appropriately, but often they are used without a clearly thought out diagnostic pathway, or in response to patient demand, rather than being tailored to the right patient in the right situation. In such situations, hormone tests will, at best, be of no clinical use, and at worst, lead to anxiety and uncertainty.

## What are the main reproductive hormones?

### Follicle stimulating hormone (FSH) and luteinising hormone (LH)

Luteinising hormone (LH) and follicle stimulating hormone (FSH) are important pituitary hormones, required for reproductive processes in both males and females. LH and FSH are released by the anterior pituitary in response to pulsatile gonadotropin-releasing hormone (GnRH) stimulation by the hypothalamus, and the negative feedback of oestrogen or testosterone.

In females, the combined action of FSH and LH stimulates growth of ovarian follicles and steroidogenesis, with the production of androgens, which are then converted to oestrogens by the action of the enzyme aromatase. A mid-cycle surge in LH also triggers ovulation. FSH levels usually increase during menopause, because the ovaries become less responsive to FSH, which causes the pituitary gland to increase FSH production. However, fluctuating ovarian activity, especially early in perimenopause, means that FSH and oestradiol levels are not reliable predictors of menopause, as they are sometimes at pre-menopausal levels.

In males, FSH stimulates the Sertoli cells resulting in spermatogenesis and LH causes the interstitial Leydig cells of the testes to produce testosterone.

#### Reference range

The reference range for FSH and LH in adult females is:<sup>1</sup>

Phase	FSH (IU/L)	LH (IU/L)
Early follicular	3 – 10	2 - 8
Mid-cycle peak	4 – 25	10 - 75
Post-menopausal	>20	>15
Pregnancy	<1	2 – 9

The reference range for FSH in adult males is 2 – 12 IU/L and for LH is 2 – 9 IU/L.<sup>1</sup>

### Oestradiol

Oestradiol is the principal oestrogen in females who are ovulating and the dominant ovarian hormone during the follicular (first) phase of the menstrual cycle. The concentration of oestradiol varies throughout the menstrual cycle. Oestradiol is released in parallel to follicular growth and is highest when the follicle matures (prior to ovulation). Oestradiol production gradually reduces if the oocyte released by the follicle is unfertilised. Laboratory testing routinely measures E2 forms of oestradiol, most of which is bound to sex hormone-binding globulin (SHBG). Oestradiol levels decrease significantly during menopause.



In males, oestrogen is an essential part of the reproductive system, and is required for maturation of sperm. Primary hypogonadism (impaired response to gonadotropins including LH and FSH) can result in increased testicular secretion of oestradiol and increased conversion of testosterone to oestradiol. Obesity may also increase oestrogen levels in males.<sup>3</sup> An increase in the ratio of oestrogen to androgens in males is associated with gynaecomastia (the development of breast tissue).

### Reference range

The adult female reference range for oestradiol is:<sup>1</sup>

Phase	Oestradiol (pmol/L)
Early follicular	< 300
Ovulatory surge	< 500 – 3000
Luteal surge	150 – 1400
Post-menopausal	< 200

N.B. Oestradiol levels are usually undetectable in females using oestrogen-containing oral contraception as this suppresses oestradiol production from the ovary. Oestradiol levels in females taking some forms of HRT (e.g. oestrogen valerate) will be high.

The adult male reference range for oestradiol is assay dependent, so it is recommended to consult the local laboratory. An example of an adult male reference range for oestradiol is 0 – 200 pmol/L.<sup>1</sup>

### Progesterone

Progesterone is the dominant ovarian hormone secreted during the luteal (second) phase of the menstrual cycle. Its main function is to prepare the uterus for implantation of an embryo, in the event that fertilisation occurs during that cycle. If pregnancy occurs, human chorionic gonadotropin (hCG) is released which maintains the corpus luteum, which in turn allows progesterone levels to remain raised. At approximately twelve weeks gestation, the placenta begins to produce progesterone in place of the corpus luteum. Progesterone levels decrease after delivery and during breastfeeding. Progesterone levels are low in women after menopause.

In males almost all progesterone is converted to testosterone in the testes.

There are no indications, other than fertility investigation in females (in some circumstances), which requires progesterone measurement in a general practice setting.

### Reference range

Detecting ovulation – measured on day 20 – 23 of a normal 28 day cycle:<sup>1</sup>

0–6 nmol/L	ovulation unlikely
7–25 nmol/L	ovulation possible
>25 nmol/L	ovulation likely

The reference range for progesterone in adult males is < 1 nmol/L.<sup>1</sup>

### Prolactin

In females, prolactin stimulates the breasts to produce milk, after oestrogen priming. During pregnancy, prolactin concentrations begin to increase at approximately six weeks gestation, peaking during late pregnancy.

In males and non-pregnant females, the secretion of prolactin from the pituitary gland is inhibited by the hypothalamic release of dopamine. Tumours or masses that result in compression of the pituitary stalk or drugs that block dopamine receptors, e.g. psychotropics, opiates and dopamine agonists, can cause hyperprolactinemia by reducing dopamine delivery to the pituitary. Hypothyroidism can also be associated with hyperprolactinaemia if levels of thyrotropin-releasing hormone (TRH) are raised, which stimulates prolactin production.

Hyperprolactinaemia is the most common endocrine disorder of the hypothalamic-pituitary axis and causes infertility in both sexes. Prolactin-secreting tumours (prolactinomas) are the most common type of pituitary tumour. These are usually small tumours (microprolactinomas) and are characterised by anovulation or other menstrual disturbances, galactorrhoea (milk secretion from the breast) and sexual dysfunction. Rarely, tumours may be large (macroprolactinomas) and present with symptoms such



as headaches and bitemporal hemianopia (missing vision in the outer halves of the visual field).

N.B. Galactorrhoea can occur in males, but is a much less common symptom of high prolactin in males.

### Reference range

There is a diurnal variation in prolactin levels and serum levels are lowest approximately three hours after waking. Samples are best collected in the afternoon.<sup>1</sup> Stress or illness can also elevate prolactin levels, so ideally patients should be well and not taking medicines that can interfere with prolactin levels such as psychotropics, opiates or dopamine agonists.

Reference ranges are assay-specific so it is recommended to consult the local laboratory for their reference range. An example of a reference range for prolactin is 50 – 650 mU/L for adult females and 50 – 450 mU/L for adult males.<sup>1</sup>

In approximately 10% of patients, raised total prolactin can be due to prolactin binding to another serum protein (macroprolactin).<sup>1</sup> In these patients if the small biologically active prolactin is within normal limits, the raised prolactin can generally be regarded as a laboratory artefact. Laboratories usually test for this possibility in new patients presenting with unexplained hyperprolactinaemia.

Increased prolactin levels are usually associated with decreased oestrogen or testosterone levels.

## Testosterone

Testosterone is the primary androgen responsible for the development and maintenance of male sexual characteristics. It also stimulates anabolic processes in non-sexual tissues. In males, LH stimulates the Leydig cells in the testes to produce testosterone. A small amount of testosterone in males is produced by the adrenal glands.

In females, the majority of testosterone is produced by peripheral conversion of androgen precursor steroids to testosterone, with the remainder produced in the ovaries and adrenal glands. Circulating levels of testosterone fluctuate with the menstrual cycle, and increase during pregnancy. Serum levels of testosterone remain relatively stable during and after menopause.<sup>4</sup> Polycystic ovary syndrome is the most common cause of hyperandrogenism (increased testosterone levels) in females. Rarer causes

include Cushing's syndrome, congenital adrenal hyperplasia and androgen-secreting tumours.

### Reference range

The reference range for total testosterone in adult males differs between laboratories. An approximate range is 8 – 35 nmol/L. If a single early morning testosterone level is clearly within the reference range (e.g. >15 nmol/L) then no further testing is required. If a low or borderline result is obtained, a confirmatory early morning test (when the patient is well) should be conducted.

Testosterone reference ranges for females are also assay-specific. An example of an adult female reference range for total testosterone is 0.5 – 2.5 nmol/L.<sup>1</sup> Modern second generation testosterone assays generally have lower ranges in females, due to less interference from other steroids such as DHEAS.

Free testosterone can be calculated from total testosterone and sex hormone-binding globulin (SHBG). However, SHBG testing is only ever rarely required, such as when abnormalities of sex hormone binding (e.g. hyperthyroidism, anticonvulsant use, severe obesity) can cause total testosterone levels to be misleading. Discussion with an endocrinologist or chemical pathologist is recommended before requesting SHBG.

## Human chorionic gonadotrophin (hCG)

hCG is structurally and functionally identical to LH, apart from its beta chain, therefore it is often referred to as beta-hCG (or  $\beta$ -hCG).

hCG is released by trophoblast cells during pregnancy. These cells form the outer layer of the developing blastocyst following conception and embryonic implantation. hCG stimulates progesterone production by the corpus luteum and increases vascularity between the trophoblast and the uterus wall. It is detectable approximately three days after implantation of the embryo, which occurs approximately six to twelve days following ovulation and fertilisation. During a normal pregnancy hCG levels usually double approximately every two days, then plateau and begin to decrease at eight to ten weeks, but will remain elevated throughout pregnancy.<sup>5</sup> Women pregnant with twins generally produce higher levels of hCG than those with single embryos, but hCG levels cannot be reliably used to predict this.<sup>6</sup>

Urine or serum hCG measurement can be used to confirm early pregnancy (urine hCG is adequate in most cases). Serum hCG can also be useful as an initial investigation in women who have symptoms that may suggest ectopic pregnancy, miscarriage or trophoblastic disease. Transvaginal ultrasound can be used after approximately five weeks gestation, or hCG levels > 1000 – 2000 IU/L, to detect signs of pregnancy.<sup>7</sup>

A non-viable pregnancy may be indicated by a decrease or plateau in hCG levels in early pregnancy (remembering that hCG decreases in normal pregnancies after approximately nine to ten weeks gestation). However hCG alone is not a reliable predictor of ectopic pregnancy as there is no particular pattern of decrease or increase.<sup>8</sup> Following miscarriage it may take three or four weeks for hCG levels to return to non-pregnant levels (< 5 IU/L).<sup>7</sup> In incomplete miscarriage, hCG levels can remain raised and surgical intervention may be required.

In males, hCG is produced by some testicular tumours, and it is therefore used as a serum tumour marker for some forms of testicular cancer.

#### Reference range

There is a wide range of variability of hCG levels during early pregnancy. The rate of increase, i.e. doubling time, gives more useful information than the actual levels. Most urine tests turn positive with hCG levels > 20 – 25 IU/L. Serum hCG < 5 IU/L is considered negative for pregnancy.<sup>1</sup>

Excessively high hCG levels, e.g. > 100 000 IU/L may be suggestive of gestational trophoblastic disease, e.g. molar pregnancy.

## When should reproductive hormones be investigated?

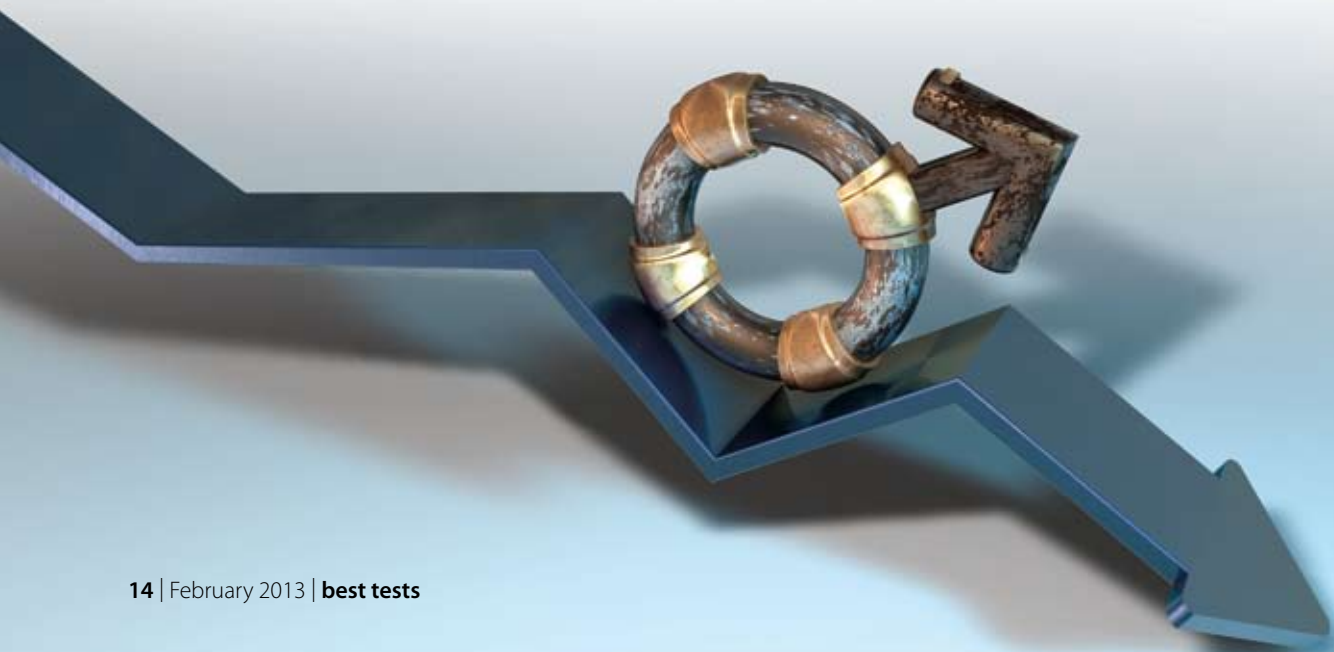
There are multiple indications for measuring reproductive hormone levels, however, in a general practice setting, the most common reasons are for investigating primary or secondary amenorrhoea or oligomenorrhoea in females, investigating hypogonadism in males, confirming pregnancy and some aspects of investigating fertility. Measuring hormone levels in women with typical symptoms of menopause is usually not necessary. Table 1 (Page 20) summarises the recommended hormone tests for some of the more common endocrine-related clinical scenarios seen in general practice.

General Practitioners are not expected to investigate and diagnose every endocrine dysfunction. The role of the General Practitioner is often to identify the patients who require referral for further assessment and diagnosis in secondary care.

### Investigating primary amenorrhoea (delayed puberty)

A possible scenario is for mothers to bring their daughters in for a consultation as they are concerned that their “periods” have not begun, and other girls in their peer group are already menstruating.

In most cases, reassurance and watchful waiting is all that is required. However, if there is no sign of breast development (the first demonstrable sign of puberty in



girls) by age 12 – 14 years, or menstruation has not begun by age 16 years in a female with otherwise normal pubertal development, investigation needs to be started.<sup>9</sup>

A common cause of primary amenorrhoea is weight loss, dieting or excessive exercise (known as hypothalamic amenorrhoea). Rarer causes include pituitary or thyroid disease, anatomical abnormalities (e.g. Mullerian agenesis) and congenital abnormalities (e.g. Turner's syndrome, Kallmann syndrome).

Laboratory investigations may be considered if concerns persist, despite a period of watchful waiting. Appropriate tests include: FSH, LH, oestradiol, prolactin, testosterone, TSH and FT4. It can be difficult to interpret the significance of abnormal results, so consultation with, or referral to an endocrinologist or gynaecologist for further investigation and diagnosis is recommended.

Oestradiol levels can indicate whether there is absolutely no evidence of ovarian oestrogen activity, or whether levels have started to rise from pre-pubertal levels, indicating that gonadal activity may be starting. Low oestradiol in association with low LH is suggestive of hypothalamic amenorrhoea.

Low levels of FSH and LH ( $< 5$  IU/L) suggests hypogonadotropic hypogonadism, of which causes include Kallmann syndrome and space-occupying pituitary tumours. High levels of FSH ( $> 20$  IU/L) and LH ( $> 40$  IU/L) suggests hypergonadotropic hypogonadism, which may be suggestive of Turner's syndrome.<sup>10</sup>

Raised prolactin levels and/or TSH and FT4 abnormalities may suggest a pituitary cause.

Although more commonly associated with secondary amenorrhoea, polycystic ovary syndrome can sometimes be a cause of primary amenorrhoea. A raised testosterone level may be suggestive of this.

Normal hormone levels in a female with primary amenorrhoea, but otherwise normal development, may suggest an anatomical abnormality such as an imperforate hymen or Mullerian agenesis (a congenital malformation that results in an absent uterus and fallopian tubes). Further investigation is required if this is suspected.

## Precocious puberty

Precocious (early) puberty is generally defined as the appearance of secondary sexual characteristics in girls aged under eight years or in boys aged under nine years. It is a much rarer presentation than delayed puberty and investigation of precocious puberty in children is complex. Any child with early signs of secondary sexual characteristics should be referred to a paediatric endocrinologist or paediatrician. Prompt management is important, as precocious puberty results in accelerated skeletal development and impaired final adult height.





## Investigating secondary amenorrhoea and oligomenorrhoea

Secondary amenorrhoea (cessation of menstruation in a female who was previously menstruating) or oligomenorrhoea (menstruation consistently > 35 days) is most commonly caused by hypothalamic amenorrhoea, polycystic ovary syndrome or premature ovarian failure (after first excluding pregnancy).

Initial investigations include FSH, LH and oestradiol. Other tests will depend on the suspected cause. Add prolactin and TSH if hyperprolactinaemia is suspected – this may be associated with galactorrhoea or symptoms of thyroid disease.

A serum FSH level > 20 IU/L and low oestradiol in a female aged < 40 years suggests that premature ovarian failure has occurred (or early menopause in a female aged < 45 years).

Low LH and oestradiol suggests a hypothalamic cause for amenorrhoea (e.g. weight loss, excessive exercise or stress).

### Hyperprolactinaemia

Stress, medicine use and hypothyroidism need to be considered as causes of hyperprolactinaemia. Macroprolactin, an inactive form of prolactin, can be a benign cause of raised prolactin levels – this can be detected by laboratory analysis. Once other possible causes of prolactin elevation have been excluded, imaging of the pituitary (MRI or CT) for prolactinoma may be considered in secondary care.

### Polycystic ovary syndrome (PCOS)


PCOS can be diagnosed based on two of the three following criteria: clinical signs or biochemical evidence of hyperandrogenism, oligomenorrhoea and/or anovulation and polycystic ovaries on ultrasound.<sup>11</sup> In most areas, publically funded ultrasound for investigating PCOS is not prioritised.

Testosterone testing is not necessarily required for a diagnosis of PCOS, and levels are not raised in all women with PCOS, especially those with minimal clinical features. Testosterone testing, along with FSH and LH, may be considered in patients with moderate hirsutism, because

significantly raised levels may suggest that other causes need to be considered. If total testosterone levels are > 5 nmol/L, further investigation is necessary to rule out other causes such as late-onset congenital adrenal hyperplasia, Cushing's syndrome, adrenal or ovarian tumour.<sup>1</sup> If pituitary disease is suspected, add prolactin, TSH and FT4 to exclude the possibility of secondary hypothyroidism.

Free testosterone levels (calculated from total testosterone + SHBG) are also sometimes measured in females with PCOS, but this is usually not necessary in the General Practice setting. It is recommended to consult with a chemical pathologist or endocrinologist before requesting SHBG.

LH levels are usually raised, while FSH levels are normal or decreased in females with PCOS.

 For further information see: "Understanding polycystic ovary syndrome", BPJ 12 (Apr, 2008).

## Investigating menopause


Hormone testing is usually not necessary for diagnosing menopause or monitoring treatment.

In women aged over 45 years with typical symptoms of menopause, hormone testing is not routinely recommended, as levels tend to fluctuate significantly over this period, and the likelihood of menopause is higher in this age group. Age and a one year history of amenorrhoea are usually sufficient for a diagnosis.

FSH testing may be beneficial in specific circumstances, such as to determine the cause of oligomenorrhoea and fertility potential in a younger woman (age < 45 years), or when oligomenorrhoea is not a relevant symptom of menopause, such as in a woman who has recently stopped taking oral contraceptives, or who has had a hysterectomy.<sup>12</sup> FSH should usually be repeated at least once (e.g. in six weeks) to confirm the result.<sup>2</sup>

N.B. FSH does not reliably predict menopause in women using combined oral contraceptives.<sup>2</sup>

There is no benefit in measuring oestradiol levels to assess doses of hormone replacement treatment.<sup>12</sup> Oestradiol measurement is also not useful in assessing post-menopausal fracture risk.

 For further information see: "Hormone replacement therapy: latest evidence and treatment recommendations", *BPJ* 12 (Apr 2008).


## Investigating hypogonadism in males

### Delayed puberty

The first sign of puberty in males is an increase in the size of the testes, which normally occurs around age 12 years. The most common cause of delayed puberty in males is constitutional delay in growth and puberty. This is more common in boys with a family history of delayed puberty. Catch-up growth, onset of puberty and the pubertal growth spurt occur later than average, but eventually result in normal adult stature, sexual development and fertility.

If clinical signs of puberty are not present by approximately age 16 years, clinical examination and investigation may be considered. Initial laboratory investigations include FSH, LH, testosterone, prolactin, TSH and FT4. It is recommended that results are discussed with an endocrinologist, and the patient is referred for further investigation and diagnosis if necessary.

Raised FSH and LH suggest primary hypogonadism. Low or normal FSH and LH levels suggest secondary hypogonadism, which in rare cases can be due to hypothalamic dysfunction, hypopituitarism, hypothyroidism or hyperprolactinaemia. Constitutional delay in puberty is associated with low FSH and LH.


 For further information see: "Selected topics in men's health", *Best Tests* (Sep, 2010).

### Gynaecomastia

Gynecomastia is a benign enlargement of the breast tissue in males, which indicates an imbalance between free oestrogen and androgens. It is important to distinguish true gynaecomastia, which is felt as a concentric, rubbery or firm mound of tissue around the nipple, from an accumulation of adipose tissue.

Gynaecomastia is quite common during mid to late puberty, when relatively higher levels of oestrogen are produced by the testes and peripheral tissues, before testosterone reaches adult levels. In almost all cases, this resolves within one to two years. The incidence of gynaecomastia rises again in older males, possibly related to a decrease in free testosterone levels.

In adult males presenting with gynaecomastia, after eliminating causes such as medicines (e.g. anti-androgens, tricyclic antidepressants, metronidazole, spironolactone, calcium channel blockers, cimetidine) or concurrent illness (e.g. cirrhosis), consider testing testosterone levels (followed by LH if low), oestradiol and hCG. hCG is measured because in rare cases, hCG production by a testicular tumour (or other ectopic hCG-secreting tumour), can lead to excessive oestrogen levels, manifesting as breast tissue enlargement.

 For further information see: "Selected topics in men's health", *Best Tests* (Sep, 2010).


### Late-onset hypogonadism

In an adult male with clinically significant signs and symptoms of hypogonadism (e.g. reduced libido, absent early morning erection), consider testing testosterone levels. The sample should be collected during the early morning, e.g. 8 am, as afternoon and evening levels may be significantly lower.

If a single early morning testosterone level is clearly within the reference range (e.g. > 15 nmol/L), then no further testing is required. If a low or borderline testosterone level is detected, a confirmatory early morning test should be conducted (when the patient is well) and a concurrent LH measurement made to differentiate possible primary from secondary hypogonadism. If LH levels are low, prolactin may be added to investigate hyperprolactinaemia.<sup>13</sup> FSH only needs to be added if investigating fertility.

High serum LH (and FSH if measured) and a low or borderline testosterone are consistent with primary hypogonadism. Low or inappropriately normal LH levels in combination with low testosterone are consistent with secondary hypogonadism.

SHBG, for measurement of free testosterone, is only rarely required, such as where abnormalities of sex hormone binding (e.g. hyperthyroidism, anticonvulsant use, severe obesity) can cause total testosterone levels to be misleading. Discussion with an endocrinologist or chemical pathologist may be helpful in such cases.

 For further information see: "Age-related testosterone decline in males", *Best Tests* (Jun, 2012).

## Investigating sub-fertility

There is a 20 – 25% chance of a healthy couple of reproductive age becoming pregnant each reproductive cycle.<sup>14</sup> This rises to 60% within six months, 84% in the first year and 92% within the second year.<sup>14</sup>

If a couple presents with fertility concerns, first give reassurance and advice about the fertile phase of the menstrual cycle and optimal frequency of intercourse, i.e. every two to three days. Temperature charting is not helpful and should not be recommended.<sup>15</sup> Lifestyle factors that affect fertility, such as BMI < 18 or > 30 and smoking, should be addressed.

Investigations may be considered if pregnancy is not achieved after 12 months in a woman aged < 35 years or after six months in a woman aged > 35 years.<sup>15</sup> Both partners should be assessed and examined. Initial investigations in primary care include establishing whether the female is ovulating, and whether the male has a normal semen analysis.<sup>15</sup>

In a female with regular menstrual cycles, it can be presumed that ovulation is occurring. However, if there is doubt (i.e. pregnancy has not occurred within an expected time frame), progesterone can be measured seven days before the expected date of menstruation, e.g. on day 21 of a regular 28 day cycle, to test if ovulation has occurred – ovulation is likely if progesterone is >25 nmol/L. In women with prolonged cycles, progesterone may be tested on day 21 of the cycle, then repeated every seven days until menstruation occurs (or just seven days prior to expected menstruation if cycles are prolonged but regular).

FSH, LH and oestradiol should be checked early in the menstrual cycle (day two – six, where day one is the first day of menstruation).<sup>15</sup> Elevated FSH suggests reduced ovarian reserve and the possibility of impending premature ovarian failure.<sup>15</sup>

Females with absent or irregular cycles should be investigated as for secondary amenorrhoea (Page 16).

If the male partner has an initial abnormal semen analysis, repeat six weeks later for confirmation. Further investigation of underlying causes of an abnormal semen analysis is usually carried out in secondary care. However, testosterone, FSH and LH may be investigated if there is suspicion of hypogonadism (Page 17).

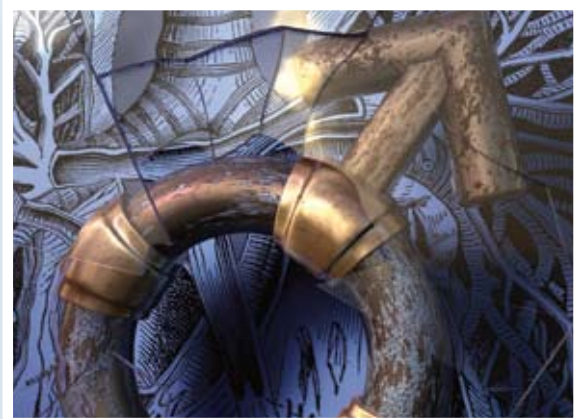
The criteria for publicly funded specialist assessment of sub-fertility in females are:

- BMI 18 – 32
  - Non-smoker or ex-smoker for > three months
  - Age < 40 years
  - Less than two children from current relationship
  - At least two years sub-fertility,
- or

one year if age <35 or six months if age > 35, with anovulation, azoospermia, oligospermia, bilateral salpingectomy, tubal obstruction, oophrectomy or premature ovarian failure

## Androgen resistance


Oestradiol measurement is recommended in males with a finding of high serum testosterone and LH levels, along with features of undermasculinisation (e.g. sparse pubic, facial or body hair, underdeveloped scrotum, penis and testes) as this is suggestive of androgen resistance.<sup>13</sup>





Early referral to a fertility specialist or relevant specialist should be offered if:<sup>16</sup>

- The female partner is aged over 35 years
- The female partner has amenorrhoea/ oligomenorrhoea
- The female partner has a history of abdominal or pelvic surgery
- The female partner has an abnormal pelvic examination
- The female or male partner has a history of sexually transmitted infection (including pelvic inflammatory disease)
- The male partner has an abnormal semen analysis
- The male partner has undescended testes or other genital pathology
- The male partner has a history of urogenital surgery
- The male partner has a varicocele
- The couple are very concerned and would be reassured by a consultation

 Individual fertility clinics may have specific criteria for referral, check with your local clinic for advice.

### Investigating early pregnancy

A random urine hCG test can be used in primary care to diagnose early pregnancy. A positive urine test is possible

on the first day of a missed period, however, delaying the test decreases the likelihood of a false-negative result. A false-negative result can occur if the urine test is performed too soon after implantation of the embryo, especially if the urine sample is dilute. If pregnancy is still suspected despite a negative test, the test should be repeated after one week. Depending on the type of test kit used, hCG levels > 20 – 25 IU/L will show a positive result. Most brands of home pregnancy tests have a similar threshold for detection, and are considered reliable when used according to manufacturer's instructions.<sup>17</sup> A positive or negative result from a home pregnancy test should usually be confirmed by a clinician, to ensure that correct sampling technique was followed.

Females of reproductive age that present with lower abdominal pain should be offered a urine hCG test to exclude the possibility of pregnancy.

A serum hCG test can detect hCG at lower levels than a urine test to confirm pregnancy, however, there is no need to request this if there is a positive urine hCG test. It is not recommended that serial hCG tests are used in a general practice setting to ensure that a pregnancy is progressing normally, as this may cause unnecessary anxiety for the patient. If there are any clinical concerns about the viability of a pregnancy, the patient should be referred to an early pregnancy clinic or gynaecology department for further investigation and ultrasound. Common causes for lower abdominal pain or vaginal bleeding, such as urinary tract infection or constipation, should first be ruled out.

#### ACKNOWLEDGEMENT

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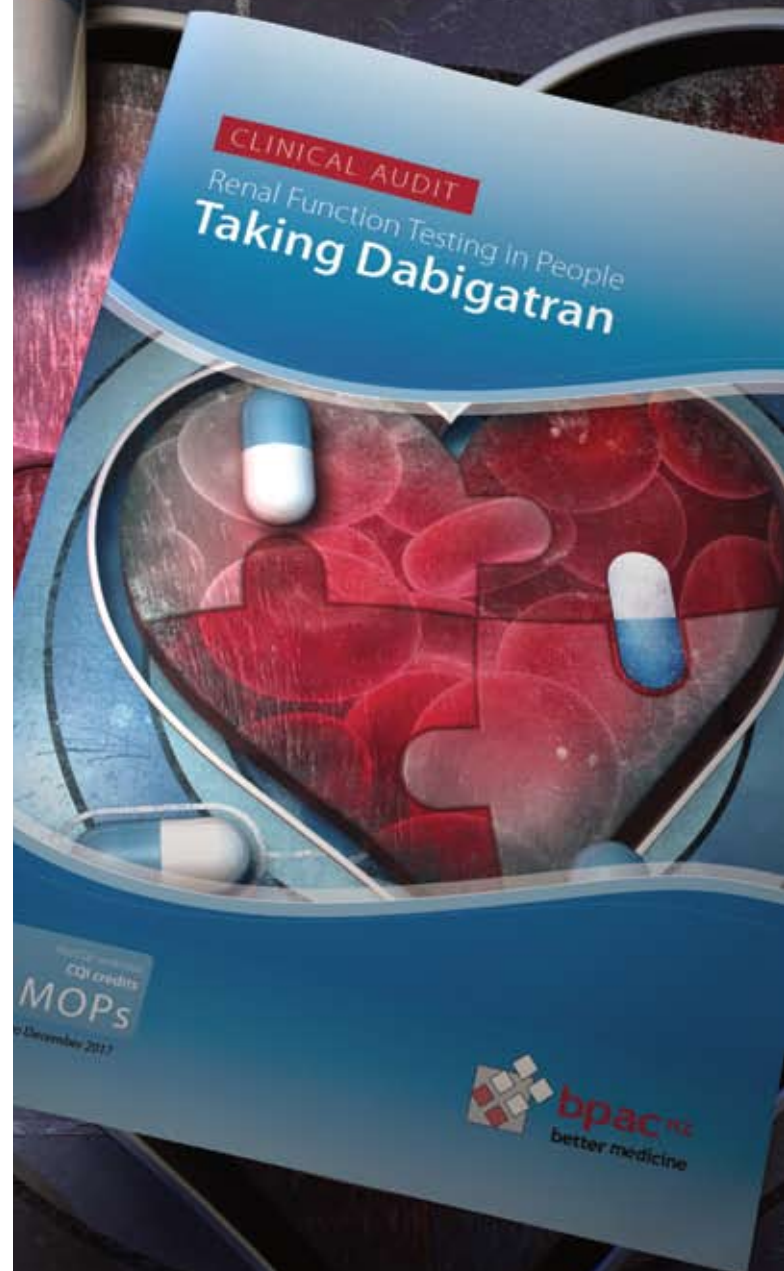
**Table 1:** Recommended hormone tests in the general practice setting

Clinical scenario	FSH	LH	Oestradiol	Prolactin	Testosterone	hCG	Notes
Primary amenorrhoea	✓	✓	✓	✓	✓	±	Also TSH and FT4. First exclude pregnancy where appropriate.
Secondary amenorrhoea or oligomenorrhoea	✓	✓	✓	±	±	✓	First exclude pregnancy. Tests dependent on suspected cause, may add TSH, FT4.
Menopause	±	-	-	-	-	-	Only consider if age < 45 years, hysterectomy or recently stopped ocp
Delayed puberty in males	✓	✓	-	✓	✓	-	Also TSH and FT4
Late-onset hypogonadism in males	±	±	±	±	✓	±	Add LH if low testosterone. Add FSH only if fertility concerns. Tests dependent on suspected cause. Add oestradiol and hCG if gynaecomastia is suspected.
Fertility in females with regular menstruation	✓	✓	✓	-	-	-	May add day 21 progesterone. If irregular menstruation, investigate as per secondary amenorrhoea.
Fertility in males	±	±	-	-	±	-	Following abnormal semen analysis. Add testosterone if hypogonadism suspected, followed by LH and FSH if low
Confirming pregnancy	-	-	-	-	-	✓	Urine test usually sufficient

Key: ✓ = Recommended ± = may be required - = not usually required

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