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The New Zealand Laboratory Schedule and Test Guidelines

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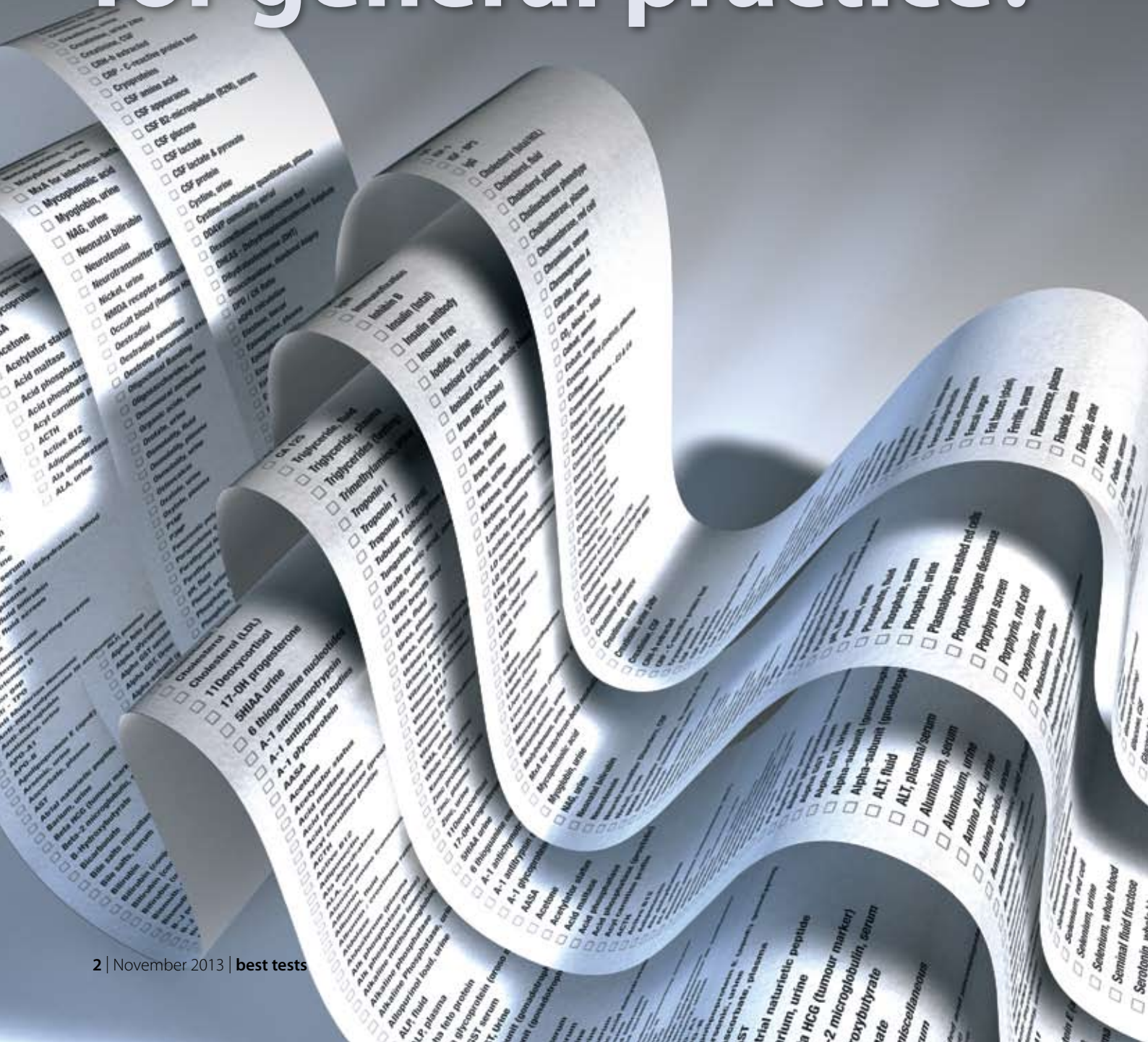
The New Zealand Laboratory Schedule and Test Guidelines: **What does it mean for general practice?**



A new schedule for laboratory testing in New Zealand

In October, 2013, a new laboratory test schedule and accompanying referral guidelines were completed and are now available online. It is anticipated that clinicians will become more aware of these guidelines over time as District Health Boards (DHBs) begin to adopt the recommendations.

The project, which involved a review of all publicly funded laboratory tests available in New Zealand, was managed by DHB Shared Services. The schedule and guidance documents were developed by the Laboratory Schedule Review group and several specialist subgroups (see opposite).

The aim of the laboratory schedule review project was to develop a consistent list of tests that are available and funded across DHBs. These tests have been categorised into two groups, termed Tier One and Tier Two (Page 4). Guidelines for the appropriate ordering of selected tests were also developed. Laboratory tests that were regarded as obsolete or clinically inappropriate have been removed, or in some cases superseded with newer tests.

The Laboratory Schedule Test List and Laboratory Test Guidelines are available from:
www.dhbsharedservices.health.nz/Site/Laboratory/Laboratory-Schedule-Review-Project.aspx

Why was the review required?

In the longer term it is expected that the Laboratory Schedule will form the basis of a national test schedule. A key future goal is the integration of the test schedule and referral guidelines into Practice Management Systems in preparation for fully supported electronic test ordering (e-requests). Until that time, the schedule and guidelines are intended to form a framework and to provide recommendations for the appropriate ordering of tests. How the recommendations are implemented at this stage will be determined by individual DHBs. The guideline document aims to provide DHBs with information on which to base local clinical care pathways and funding decisions. While the test guidelines are not intended to take precedence over established local care pathways or other guideline documents, over time they should enable clinical pathways to become more nationally consistent.

Laboratory Schedule Review Group

The Laboratory Schedule Review Group included representatives from primary and secondary care, medical laboratory scientists and clinicians, and specialists and managers from DHBs, with project sponsorship and management from DHB Shared Services.

Laboratory Subgroup Members included specialists in the following fields: microbiology, clinical biochemistry, haematology, immunology, histology, cytology, anatomic pathology and genetics.

Guidance for the ordering of laboratory tests by Midwives was also developed. A separate list of tests that can be ordered by a Midwife is included in the Laboratory Schedule Test List document.

Groups of health care professionals that were not able to be considered during the development of the documents included Nurse Practitioners and community Dietitians, and clinicians who order tests in a hospital setting as part of a specialist team, e.g. House Surgeons, Dietitians and Resident Medical Officers. Management of test ordering by these health care professionals should continue as per current guidelines within each DHB or within the clinician's specialist scope of practice.

The Laboratory Schedule Test List

The Laboratory Schedule Test List categorises tests into general areas, e.g. chemical pathology, haematology, and then further categorises the tests into Tier One and Tier Two tests.

A Tier One test can be ordered by any medical practitioner* with a current practising certificate in New Zealand. Tier One tests include the "core" tests requested frequently in primary care, e.g. full blood count, INR, creatinine and electrolytes, along with many other tests that are only ordered intermittently by General Practitioners.

* A separate list has been developed for midwives (see: "Laboratory Schedule Review Group, previous page).

A Tier Two test is regarded as a specialist test that can only be ordered by a clinician with "appropriate vocational registration or credentialing". It is intended that the ordering of some Tier Two tests is restricted to the specialists named in the schedule, e.g. a request for sex hormone binding globulin (SHBG) should be from an Endocrinologist, O&G specialist or Chemical Pathologist. In practice, however, the "rules" are not intended to be unnecessarily restrictive and any practitioner can order a Tier Two test if they have endorsement or pre-authorisation by a relevant specialist, or if the test falls within their area of expertise. The clinician requesting the test can also consult with a laboratory pathologist for advice and approval for the use of the test.

For some tests in each tier a clinical guideline has been developed to direct appropriate use (see opposite). This is indicated in the comments section of the Laboratory Schedule Test List with the word "Guideline". If there are specific requirements that apply when ordering a test, these are identified within each individual guideline with the words "Referral criteria available". The laboratory may query the test if the reason for requesting it is not within these parameters. Examples of Tier One tests for which a guideline has been developed include growth hormone, amino acids, faecal calprotectin, T3 and T4 and carcinoembryonic antigen (CEA).

Some tests are categorised as both Tier One and Tier Two and also have supporting information to guide appropriate use. In some situations a Tier One test can only be ordered if the referral form contains appropriate clinical information, otherwise the test is regarded as a Tier Two test, and it should be ordered by a specialist as indicated in the schedule. For example, serum cobalt and serum chromium can be ordered by any medical practitioner if the clinical information provided states that this test is being used in a patient with a metal-on-metal joint replacement. If this indication is not specified, then the test is regarded as a Tier Two test and the laboratory may not proceed with the request.

How is the Laboratory Schedule Test List organised?

In the Laboratory Schedule Test List, tests are listed alphabetically, e.g. in the chemical pathology and microbiology test sections, or are listed in relevant subcategories within a specialty, e.g. coagulation tests within the haematology section and allergy tests within the immunology section. Approximately 80% of the tests are in the chemical pathology section.

For each individual test:

- The Tier is indicated
- Specialists who can order the test may be listed for some of the Tier Two tests
- A note in the comments box may indicate if there is a guideline available that restricts or recommends the use of the test, if there are specific referral criteria for the use of the test or if the test is unfunded and there may be a charge to the patient

In addition, the microbiology section has an extra column indicating whether the infection being tested for is Notifiable under the Health Act or the Tuberculosis Act. A number of notes also follow giving more specific advice about notification, e.g. patients with acute hepatitis B and C (including those with neonatal hepatitis B and documented hepatitis C seroconversion within 12 months) should have their condition notified to the Medical Officer of Health. Some microbiology tests include a comment that consultation with a Public Health specialist is indicated. This consultation can fulfil the requirement for specialist advice prior to ordering of tests.

The genetics section of the schedule varies from the other sections because, due to the rapid increase in the number of tests now available, it was recognised that these could not all be itemised. The list of genetic tests therefore includes the most commonly requested tests. The majority of the genetic tests listed are classified as Tier Two tests and in most situations it is anticipated that General Practitioners will not be ordering these tests. It is recommended that advice be sought before any genetic tests are requested. Genetic tests usually require prior written consent from patients. In addition, these tests are often very costly for the laboratory to undertake.

Laboratory Test Referral Guidelines

Referral guidelines have been developed for approximately 50 individual tests on the schedule. These guidelines provide recommendations for the ordering of tests, and for certain tests, referral criteria.

Depending on the individual test, each guideline may include:

- An overview of the place of the test in a clinical setting
- Supporting information explaining why the test is subject to a guideline
- Indications for the test and any referral criteria (this should be included in the clinical information on the request form)
- Specific instructions for collection of the specimen
- Information on the frequency of testing
- Links to further information
- References for the information in the guideline

What impact will the schedule and guidelines have on primary care?

At the present time, clinicians are unlikely to notice a change to their current practice as the majority of tests ordered by primary care clinicians are either Tier One tests, or within the clinician's vocational scope of practice as a Tier Two Test. Almost all of the "day-to-day" tests used in the community, such as a full blood count, CRP and liver function tests are Tier One tests and do not have a guideline or specific referral requirements.

Tests that are Tier Two and do not fall under the scope of practice for the clinician can still be ordered, but this requires prior discussion and approval from a relevant specialist or laboratory Pathologist.

Some unfunded tests are also listed. Generally these are tests where there is a limited body of evidence to support the use of the test or the test has been replaced with either a more accurate or more cost-effective alternative. For example, salivary testosterone is no longer funded due to lack of accuracy and Chlamydia IgG is also not funded because a more appropriate test is available (Chlamydia trachomatis nucleic acid amplification test – NAAT).

Examples from the Schedule and Guideline

C-Reactive Protein (CRP)
This is a Tier One test with no restrictions, referral criteria or guideline attached.

Erythrocyte Sedimentation Rate (ESR)
This is a Tier One test with a guideline that provides recommendations for appropriate use. Many individual laboratories have already produced guidance regarding the appropriate use of ESR, but the new Schedule and Guideline aims to standardise this information.

The ESR guideline provides a brief overview of the limitations of the test in terms of the accuracy of measurement, the influence of physiological variables (other than inflammation) and the role of other factors such as the patient's haemoglobin and plasma protein levels. CRP is recommended as the preferred investigation of disorders due to inflammation or infection.

The conditions included in the guideline, where it is recommended that ESR may have a role, are:

- Systemic lupus erythematosus
- Rheumatoid arthritis
- Kawasaki disease
- Rheumatic fever
- Hodgkin lymphoma
- Temporal arteritis (giant cell arteritis)
- Inflammatory bowel disease in children (initial assessment)

If the patient is suspected to have a plasma cell dyscrasia, ESR, although not restricted, is not recommended as a "screen" - the appropriate initial test is protein electrophoresis (which may be followed by serum free light chains).

Vitamin D
Vitamin D is an example of a test that is regularly used in primary care but is not strongly supported by evidence. Under the new Laboratory Schedule, vitamin D is categorised as both a Tier One and a Tier Two test, and also has an accompanying guideline.

General Practitioners can request the test, but only when following the vitamin D guideline. The guideline outlines

the limited indications for which the test can be requested by a General Practitioner. The specific indication must be clearly specified on the request form. The requirements are that the person must be at high-risk of vitamin D or calcium abnormalities, for example:

- Patients with rickets or osteomalacia, known osteoporosis, abnormalities of calcium/phosphate metabolism or a raised ALP with a likely bone origin
- Patients with cystic fibrosis, those who require a special diet (e.g. PKU), patients with a renal transplant or those taking anticonvulsant medicines
- Children aged under 16 years, refugees, and patients prior to treatment with bisphosphonates for osteoporosis, or interferon for hepatitis C

As a Tier Two test, vitamin D may be ordered by an Endocrinologist, Hepatologist, Rheumatologist, Nephrologist, Gastroenterologist or Gastrointestinal Surgeon. However, General Practitioners or any other relevant specialist, may also order the test with pre-authorisation from any of the specialists listed or a Chemical Pathologist.

Insulin (total)

Total insulin is categorised as both a Tier One and Tier Two test and a guideline has been developed for the test. As a Tier One test, total insulin can only be requested by General Practitioners for a patient following bariatric surgery in order to investigate hypoglycaemia. This indication and the patient's relevant clinical information should be included on the request form.

As a Tier Two test, total insulin may be ordered by a Paediatrician, Endocrinologist, Hepatologist or Gastrointestinal Surgeon. General Practitioners can still request the test provided they have prior authorisation from these specialists or a Chemical Pathologist.

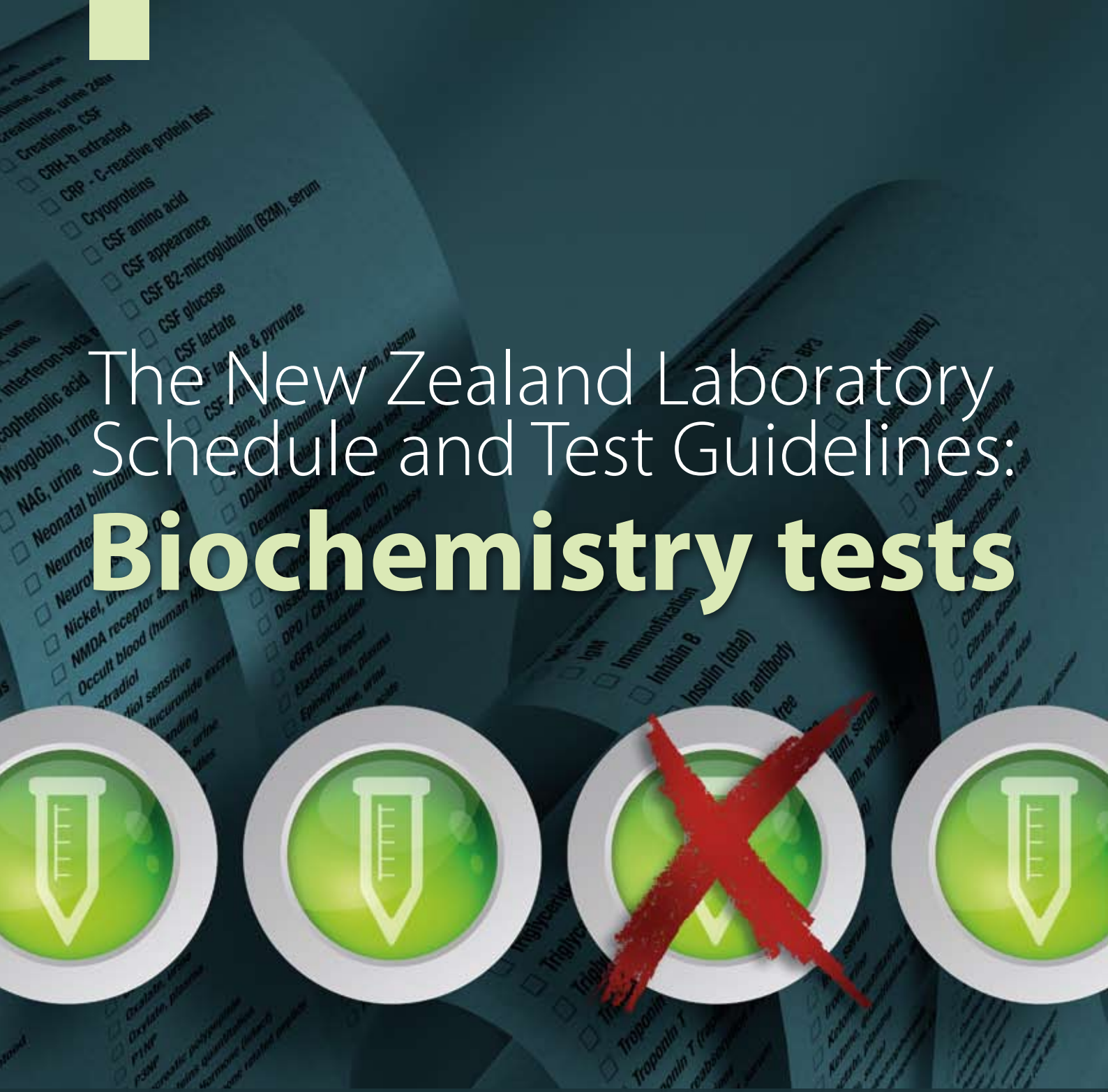
Insulin can be an important test when used in the investigation of hypoglycaemia, particularly if an insulinoma or islet cell hyperplasia is suspected, however, investigation of a patient in this clinical situation would normally be carried out in conjunction with an Endocrinologist. A plasma glucose test should also be simultaneously collected to allow correct interpretation of the results. In addition, the guideline states that fasting insulin is not recommended for assessing insulin resistance, although experts continue to debate the clinical usefulness of doing so.

Catecholamines (urine)

Testing for catecholamines in the urine is categorised as a Tier Two test. While phaeochromocytoma is a rare but important cause of secondary hypertension to consider in some patients, the most sensitive and specific first-line test is urine or plasma metanephrines, both Tier One tests. The decision on which of these tests to use will depend in part on local availability. Catecholamines should only be requested in specific and limited circumstances, such as in a patient where there is suspicion of neuroblastoma or malignant phaeochromocytoma. Investigation of these conditions would normally be done in consultation with a relevant specialist in a hospital setting.

ACKNOWLEDGEMENT Thank you to **Robyn Blue**, Laboratory Schedule Review Project Manager, DHB Shared Services, **Dr Rosemary Ikram**, Clinical Microbiologist, Chair of Microbiology Subgroup and **Dr Cam Kyle**, Clinical Biochemist, Co-Chair of Clinical Biochemistry Subgroup for expert review of this article.

The New Zealand Laboratory Schedule and Test Guidelines: **Biochemistry tests**



A new laboratory test schedule and accompanying referral guidelines have been developed for health care professionals in New Zealand. The Schedule and Guidelines were released to District Health Boards (DHBs) in October, 2013 and are also available online. The aim was to develop a consistent list of tests that are available and funded across DHBs. An article in Best Tests, Nov, 2013, introduced the new Test Schedule and explained how they have been developed. Tests have been categorised into general areas and then grouped depending on whether they are recommended as a test that can be ordered by any medical practitioner (Tier 1) or whether the test is restricted to specific clinicians (Tier 2). In this article **Dr Cam Kyle** and colleagues discuss the biochemistry tests grouping, and explain why some tests are restricted, why others are now outdated or lack evidence and some tests which are underutilised.

The Biochemistry Subgroup

As part of the wider review of the New Zealand Laboratory schedule, a biochemistry subgroup was formed to identify tests where special expertise was considered appropriate for interpretation of results, and tests where guidelines or restrictions on requesting were thought to be necessary. The group was also asked to identify tests which were outdated or of no clinical value and for which funding should be withdrawn, as well as to identify underutilised tests which should be encouraged first-line.

The key drivers for this process were:

- The desire for a national schedule that was relevant to the current evidence base and best practice
- The desire to develop more consistency of testing across DHBs
- A lack of clarity regarding appropriate and cost-effective testing, as there was no guidance on limiting testing
- The intention for the schedule to interface with an e-labs initiative, and electronic test requesting

Ultimately there was concern not only because of the increasing volume of laboratory testing in general, but also because the requesting of certain "vogue" tests had increased dramatically in a way not justified by current overall evidence. Particular attention was suggested for those tests which "create issues in terms of volume and requesting appropriateness". The background rationale was to allow appropriate, evidence-based spending on pathology testing by DHBs facing increasingly constrained laboratory budgets. The intention of the review was not to place blanket restrictions on tests, but rather to provide guidance on appropriate test requesting.

The guidelines produced are not mandatory but were developed as a resource for individual DHBs to use. They are not intended to replace well-established local protocols or clinical pathways, but rather to support them where judged appropriate by local clinicians and policy setters.

Composition and process of the biochemistry subgroup

The biochemistry subgroup was composed of six Chemical Pathologists representing different DHBs, from both public/academic and private (community) backgrounds, along with a convener from DHB Shared Services. Individual members were each allocated a range of tests to evaluate and present recommendations for wider discussion among the group. Specialists from related clinical disciplines were consulted when appropriate. In all cases where guidelines or restrictions were put in place the strength of evidence base, and the opinions of local experts were considered, and there was ultimately unanimous agreement among the group. Third party stakeholders also had the opportunity to provide feedback on an initial draft set of guidelines, and suggestions were incorporated into the final document.

In biochemistry there were a significant number of "esoteric" tests identified, which were considered to be Tier 2 tests, i.e. requiring special expertise in interpretation. Many of these tests are rarely requested and, while detailed criteria or guidelines for requesting them have not yet been recommended, requestors are encouraged to contact the laboratory or a specialist in the relevant clinical discipline to discuss appropriate requesting and interpretation.

It is intended that the Laboratory Schedule and Test Guidelines will be updated and modified as new evidence comes to light, new tests are added and others become outdated. As electronic ordering becomes standard practice there will be opportunity to guide testing based on clinical presentation and minimise inappropriate testing frequency, e.g. requesting HbA_{1c} more often than every three months without special circumstances.

Biochemistry tests (referred to as chemical pathology in the schedule) were divided into four groups:

A) Tests where it is appropriate to recommend ordering restrictions and/or criteria for funding based on clinical circumstances and/or expertise of referrer

B) Tests which are outdated and which should be funded only in very limited circumstances

C) Tests where public funding was not considered justified based on current evidence

D) Tests which were considered underutilised, but for which requesting guidelines were appropriate to optimise clinical utility

Tests with restrictions

The following tests are examples of those that have recommended guidelines or criteria for their use and should be requested only in specific clinical situations.

Androgen tests

Restricted tests include androstenedione (ASD), dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG) and free testosterone:

- In the assessment of hirsutism measurement of ASD and DHEAS is not justified unless testosterone is also elevated (except when requested by specialist Endocrinologists, or pre-authorised by a Chemical Pathologist)
- Measurement of sex hormone binding globulin (SHBG) and calculated free testosterone is not justified unless the initial total testosterone result is in a range where SHBG/free testosterone is likely to provide additional clinical value
- Measurement of dihydrotestosterone is only justified in isolated rare clinical scenarios of defective androgen action or response, e.g. partial or complete androgen insensitivity

DHEAS and **ASD** are androgens sometimes measured in addition to testosterone in the assessment of women with hirsutism and possible polycystic ovary syndrome (PCOS). Free testosterone, derived from measurement of total testosterone and SHBG, is also sometimes advocated as providing a better measure of tissue androgen exposure.

The added value of measuring these hormones is very limited in the large majority of patients being evaluated for possible PCOS. The main reason for initially performing such tests is to exclude other secondary causes, particularly virilising ovarian or adrenal tumours. However, these conditions occur very rarely and patients will virtually always have an unusual clinical presentation with relatively severe and rapidly progressive hirsutism, and/or evidence of virilisation. Even for these patients, it is extremely uncommon for there to be isolated elevation of DHEAS or ASD without testosterone elevation (which is usually marked).

N.B. Testosterone levels are not always raised in females with PCOS. Measurement of testosterone levels (total testosterone), while often carried out, is not required for diagnosing PCOS. The diagnosis is based on a constellation of findings related to clinical and/or biochemical evidence of androgen excess, menstrual irregularity and ovarian dysmorphology (usually multiple peripheral ovarian cysts).

Exclusion of other secondary causes such as Cushing's syndrome and congenital adrenal hyperplasia (mostly late onset 21 hydroxylase deficiency) involves measurement of other specific tests (urine free cortisol and/or overnight dexamethasone suppression, and 17OH-progesterone).

Measurement of DHEA or ASD has also been advocated in patients taking these as supplements. However, the biochemistry subgroup consider supplementation with DHEA or ASD ("andro") to be of unproven clinical value (and unclear long-term clinical risk), except in certain situations, such as in patients with premature ovarian failure, hypopituitarism and possibly some other limited settings, such as some female patients with SLE.[1] Even in these patients, measurement of DHEAS and ASD is of unclear and unproven value in monitoring their treatment.

Sex hormone binding globulin (SHBG), which is used to calculate free testosterone, is also of limited value in most patients. Evaluation at LabPlus shows that all female patients with a testosterone > 5 nmol/L will also have a raised free testosterone, and those with total testosterone < 1.3 nmol/L have a free testosterone within reference limits. There is little additional clinical value therefore in measuring SHBG/free testosterone for samples with total testosterone outside these limits. Even for patients with total testosterone within this range, only those with unusually high (e.g. taking oral contraceptives, hyperthyroidism) or low (e.g. obese, insulin resistant) SHBG levels are likely to have a reclassification of testosterone to within or above reference limits based on their free testosterone result. For similar reasons, in males, free testosterone adds value only if the total testosterone is between 7 – 15 nmol/L.

Dihydrotestosterone measurement is extremely expensive and adds little to the clinical management of patients with hirsutism (even those taking 5-alpha-reductase blockers, such as finasteride). This test is of established clinical utility only in patients being evaluated for very rare defects in androgen action or response (e.g. partial or complete androgen insensitivity) in specialist settings.

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

Tests of adrenal function

24h urine free cortisol (UFC) has well-established value in the initial evaluation of patients with possible Cushing's syndrome.[2] A 24 hour urinary excretion result over four times the upper reference value makes Cushing's highly likely. Lesser degrees of elevation can reflect a broad range of other factors, such as stress, illness, insomnia, depression, anorexia and alcoholism, as well as Cushing's.

The clinical utility of 24h cortisol excretion for the evaluation of possible primary or secondary hypoadrenalism is, however, very limited and the group did not consider this to be an appropriate clinical indication for this test. There are other established means with much better clinical utility to make this diagnosis, such as synacthen testing and, for primary adrenal disease, plasma adrenocorticotropic hormone (ACTH).

While there is a loose correlation between 24h urine cortisol production and cortisol output, excretion can be affected by a range of factors and can vary significantly from day-to-day, even in healthy patients exposed to temporary physical or psychological stress. Patients with primary adrenal insufficiency may also have daily excretion well within reference limits, but output is stimulated by increased ACTH stimulation (in a similar way to patients with mild hypothyroidism with free T4 maintained within reference limits by increased TSH).

Many requests for UFC are made in the belief that functional adrenal insufficiency ("adrenal fatigue") is a cause for chronic fatigue syndrome. There is no substantive evidence for "adrenal fatigue" as a real clinical entity. The use of hydrocortisone treatment in chronic fatigue syndrome is not supported by randomised controlled trial evidence,[3, 4] and both United Kingdom and Australasian guidelines specifically state that hydrocortisone should not be used in chronic fatigue syndrome.[5, 6]

Cortisol binding globulin (CBG) measurement is considered to have no clinical utiltity other than in rare situations where calculation of free cortisol adds clinical value to the patient's management, almost always in specialist settings. This would typically be where a total cortisol result (usually on stimulation testing) seemed inconsistent with the patient's clinical presentation. CBG is therefore considered a specialist test (Tier 2).

Salivary cortisol measurement is appropriate for the evaluation of patients with possible Cushing's syndrome.[2] Since saliva reflects the level of free cortisol in the tissues (salivary glands), it provides an indirect measurement of tissue cortisol exposure. Normal, unstressed patients show a marked fall in salivary cortisol in the late evening, whereas in patients with Cushing's syndrome cortisol levels, and salivary cortisol, remain elevated.[7] However, as with 24 hour urine free cortisol tests, other non-Cushing's causes of elevation can occur, such as patients with significant physical or psychological stress. A late night (10 – 11 pm) saliva sample can be collected by patients before bed and sent to the laboratory the following day.

Measuring salivary cortisol samples or profiles at other times of the day as a means of assessing tissue cortisol exposure, and thereby diagnosing cortisol excess or deficiency (organic or functional, "adrenal fatigue") is considered unproven and lacks sufficiently robust evidence at this time to justify public funding.

Tests of thyroid function

No restrictions or guidelines around thyroid stimulating hormone (TSH), Free T4 (FT4)and thyroid antibody testing have been included in the recommendations (these are all Tier 1 tests), but formal schedule guidelines on tests of thyroid function are planned.

It is important to note that:

- **FT4** is not considered an appropriate initial request for the routine assessment of thyroid status unless an unusual cause, such as pituitary disease (secondary hypo- or hyperthyroidism) is suspected. When this is not specified, reflex addition of FT4 occurs in most laboratories when TSH is abnormal.
- **The FT4/FT3** ratio may be influenced by a range of factors including drug treatment, illness and fasting status. While it may also be influenced by some trace elements such as iodine and selenium it was not considered a sufficiently reliable marker for this purpose.
- **Thyroid peroxidase (anti-TPO)** is considered the appropriate first-line antibody test for autoimmune thyroid disease. Anti-thyroglobulin may add some value when anti-TPO is raised but can cause confusion when raised in isolation. Anti-thyroglobulin testing is important, however, in the management of patients

with thyroid cancer. Repeated monitoring of anti-TPO titre has been advocated in the monitoring of iodine status, but there is little substantive evidence base for its value in this context.

FT3

Free T3 (FT3), and its precursor FT4, levels are patient-specific with an individual "set point" much narrower than the population range. This is mostly due to individual variation in tissue sensitivity to thyroid hormone, but also other factors, such as the enzymatic conversion of T4 to T3 by tissue deiodinases (mainly type 1 in the liver). This is influenced by factors such as recent calorie intake, mineral status (such as iodine and selenium), growth hormone levels and thyroid status itself.

While all routine thyroid tests (TSH, FT4, FT3) can be affected temporarily by factors such as illness and drugs, FT3 is particularly affected by illness and also by reduction in calorie intake, with both of these causing a rapid decrease in plasma level.

FT3 requests are justified in the following circumstances:

- If TSH is low and FT4 is normal (to exclude T3 toxicosis): FT3 is routinely added by most laboratories in this situation, even if not requested
- When hyperthyroidism (including secondary hyperthyroidism) is suspected or monitored based on clinical details
- If there is known or suspected pituitary/hypothalamic disease: FT3 is not considered appropriate, however, for routine monitoring of primary hypothyroidism
- In patients with thyroid cancer, where FT3 measurement is occasionally helpful to monitor the degree of replacement (which in advanced cases can be above physiological requirements)

In early hyperthyroidism or primary hypothyroidism (thyroid failure, most often Hashimoto's disease) the serum level of TSH falls, or rises, early and is a sensitive biomarker of tissue exposure. It is therefore the single most useful initial test when either primary hyper- or hypothyroidism is suspected. Serum levels of FT4 and FT3 may rise and fall compared with the patient's individual set point, but typically initially remain within population limits.

In primary hyperthyroidism FT3 may rise above population limits before FT4 (so-called "T3-toxicosis"), and it is useful to perform a FT3 assay when TSH is low (typically suppressed to unmeasurable levels in true hyperthyroidism) but FT4 is within reference limits.

In secondary hyper- or hypothyroidism (pituitary/hypothalamic disease) TSH measurement alone is unreliable, and it is very important to measure FT4 in such patients, both for initial screening/evaluation and in monitoring. FT3 measurement can also be useful, especially if there is an abnormality of growth hormone production (growth hormone insufficiency can reduce the conversion of FT4 to FT3).[8]

While theoretically the plasma level of FT3 can be of value in assessing patients with hypothyroidism, there are many factors that confound interpretation, such as the individual patient set-point (which is unknown), recent illness or calorie and iodine intake. In patients with primary hypothyroidism and in iodine deficiency FT3 levels are generally preserved within population limits until relatively late (unlike in hyperthyroidism), making it an insensitive marker.

In patients taking T3 replacement, either alone or in combination with T4 (e.g. whole thyroid extract), FT3 levels rise and fall significantly depending on time of last dose and are not considered sufficiently reliable for monitoring. As with patients taking conventional replacement treatment, TSH is considered the primary analyte by which to adjust dose.

Tests of pituitary function

Insulin-like growth factor 1 (IGF-1) is an accepted test for the initial investigation of growth hormone excess (acromegaly, gigantism), and in monitoring the treatment of such patients. Since identification of acromegaly is important and the test has well-established clinical utility (even though the diagnosis is rare), writing "*possible or known acromegaly*" on the request form is sufficient for the test to be funded.

IGF-1 may also be requested, when recommended by a Chemical Pathologist or Endocrinologist, as an initial investigation of the possibility of growth hormone deficiency. However, interpretation is much more likely to be confounded by other factors, such as nutritional status, oestrogen and thyroid hormone status. A low result is more likely to be clinically significant when prior suspicion is high, e.g. patients with other anatomical or biochemical evidence for pituitary disease. Formal diagnosis of growth hormone deficiency (i.e. to qualify for publically funded treatment) requires further testing in a specialist setting.

Measurement of IGF-1 in patients on certain weight loss diets, e.g. the intermittent fasting ("5+2") diet, is not considered sufficient reason to justify public funding.

Growth hormone measurement can be helpful in the evaluation of patients with pituitary disease, particularly when acromegaly is suspected or in children or adults when there is suspicion of hypopituitarism. The test is funded if one of these indications is specified on the request form, or when ordered by an Endocrinologist.

A major problem limiting interpretation, however, is that growth hormone is secreted in a pulsatile fashion, so unless a result is clearly high or low, a single isolated result can be impossible to interpret. Stimulation or suppression tests, or serial measurements throughout the day, provide additional information; this should only be carried out under specialist management or recommendation.

Assessment of pancreatic disease and obesity

Plasma insulin levels are a key measurement when establishing a diagnosis of insulinoma as a cause of recurrent hypoglycaemia; since insulin has a plasma half-life of minutes and insulin secretion is shut off by hypoglycaemia in normal patients, plasma insulin levels should be suppressed. As evaluation of possible insulinoma is complex, prior discussion with an Endocrinologist or Chemical Pathologist is recommended before requesting this test.

When considering possible insulinoma it is critical to:

- Measure venous plasma glucose concurrently, so that the plasma insulin level can be properly interpreted. If the plasma glucose is > 3 mmol/L, then there is no stimulus to shut off pancreatic insulin release and plasma insulin level will be unhelpful
- Document any hypoglycaemic symptoms at the time, particularly those associated with poor glucose supply to the brain (neuroglycopaenic symptoms), such as confusion, "absence" and disorientation
- Document fasting status or time since last meal

Patients who have had bariatric surgery can develop excessive inappropriate pancreatic insulin secretion. For these patients, measuring insulin and glucose together at the time they describe symptoms is considered reasonable for any referrer, as long as the clinical information details that the patient had previous bariatric surgery.

While controversial, the biochemistry subgroup felt that evidence to justify funding of plasma insulin to identify insulin resistance and the metabolic syndrome was not sufficiently robust to justify public funding, except in specialist settings and then preferably when used as part of a calculation incorporating concurrent glucose level. For example, calculation of the HOMA index of insulin resistance may be useful in assessing the probability of non-alcoholic steatohepatitis (NASH) and the need for liver biopsy to assess fibrosis.[9, 10]

Insulin levels are not useful in patients with diabetes, as they can range from very high to unmeasurably low. They should also not be used to decide whether a patient has type 1 or type 2 diabetes; other tests such as diabetes-related antibodies (anti-GAD, anti-IA2) and plasma C-peptide have greater utility.

C-peptide is stored in secretory granules with insulin and co-released in equimolar amounts. Measuring plasma C-peptide is useful in the context of evaluating possible excess endogenous insulin secretion (e.g. insulinoma) and distinguishing this from exogenous insulin administration or another cause. Fasting status or relationship to meals should be well defined and plasma glucose should be measured concurrently. Ideally the sample should be taken during a spontaneous hypoglycaemic attack or a controlled fast, with careful correlation with symptoms. C-peptide is filtered by the glomeruli and caution should be exercised in patients with reduced GFR as this may lead to elevated values independent of any changes in pancreatic status. C-peptide may also be helpful in classifying some patients, when there is uncertainty as to whether they have type 1 or type 2 diabetes.[11] The utility of C-peptide for assessing insulin resistance is limited and it is not recommended for this purpose.

Nutritional markers: Essential fatty acids, vitamins, iodine and trace elements

Essential fatty acids (EFAs) are divided into two main classes: omega-3 and omega-6. The shortest chain omega-3 essential fatty acid is linolenic acid, and the shortest omega-6 is linoleic acid.

The most well known longer chain EFAs are:

- Omega-6 – arachidonic acid (C20:4n6), a precursor to prostaglandins and leukotrienes
- Omega-3 – eicosapenatenoic acid (C22:5n3 – EPA) and docosahexaenoic acid (C24:6n3 – DHA) ('fish oils')

There is considerable literature on the biology and benefits of n3 and n6 EFAs, and increased intake of omega-3 rich foods has been reported to have beneficial cardiovascular and anti-thrombotic effects, as well as a wide range of other less well substantiated benefits. There are also some isolated reports that higher plasma levels of some EFAs in plasma and/or red cells are associated with better long-term outcomes, but randomised trial evidence using plasma levels as a marker is currently limited.

EFA testing is technically difficult and very expensive. This test is not appropriate for patients who are considering or taking EFA supplements. Based on current evidence, knowing the detailed composition of EFAs in plasma and red cells was not considered sufficient to justify publically funding such requests at this time. Targets to guide treatment are not clearly established, correlation with tissue levels is imperfect, and there is potential for confusion due to the range of other biological and dietary influences. Achieving an appropriate balance of EFAs is important in some limited clinical settings, such as patients with severe liver disease or short bowel syndrome on intensive nutritional support. An EFA test would be appropriate in this setting.

Vitamins B1 (thiamine), B2 (riboflavin), and B6 (pyridoxine)
Plasma levels of these vitamins are sometimes requested as part of an overall nutritional or wellness screen. However, clinically significant deficiency is rare in New Zealand, except in the context of significant malnutrition or malabsorption, and/or liver disease (e.g. alcoholism). All of these vitamins are water soluble with very limited storage in tissues such as fat, hence plasma levels will be very influenced by recent short-term intake.

The assays are all expensive and there are significant pre-analytical factors of collection, processing and storage to consider which, if not addressed correctly, will invalidate the result. Even if the patient is suspected to have a deficiency, testing is often unhelpful as the turnaround is slow. The clinical response to vitamin supplementation is more helpful in confirming the diagnosis, and is the only way to prove that symptoms leading to the suspected diagnosis were related to deficiency of that particular vitamin.

Patients who have had bariatric surgery are predisposed to vitamin and trace element deficiency, in some cases leading to short and long-term neurological complications, including Wernicke's encephalopathy, polyneuropathy and visual defects. Post-operative monitoring of nutritional status is considered appropriate in this situation and requests for vitamin B1 and B6 are approved.[12] Measurement of vitamin B6 (pyridoxine) is justified in a specialist setting, when investigating a patient with raised homocysteine levels.

Vitamin D has a central role in bone and calcium metabolism and vitamin D tests were developed for investigation of abnormalities of calcium metabolism as well as metabolic bone disorders, such as rickets and osteomalacia. In recent years an association has been reported between low vitamin D levels and a very wide range of disorders (cancers, cardiovascular disease, diabetes, autoimmune disorders and infectious diseases). However, a causal link has yet to be demonstrated for any of these conditions.[13–15]

Despite this, the number of requests for vitamin D tests has increased dramatically, with many patients who get reasonable sun exposure and who are otherwise at relatively low risk, wishing to know their vitamin D level.

A comprehensive literature review for the Ontario Ministry of Health concluded that there is little evidence that it is useful to test vitamin D concentrations in patients without symptoms of metabolic bone disease.[16]

It is not necessary to routinely measure vitamin D in patients with low bone density. It is reasonable to routinely provide vitamin D supplements (1.25 mg or 50,000 IU cholecalciferol per month), without testing vitamin D, to frail housebound or institutionalised elderly people, or those in the community who avoid sunlight for cultural or medical reasons.

Requests for a vitamin D test should clearly indicate a high risk of vitamin D/calcium abnormalities for investigation, e.g:

- Rickets or osteomalacia, known osteoporosis, abnormalities of calcium/phosphate metabolism, raised ALP with likely bone cause
- Cystic fibrosis, special diets (e.g. PKU), renal transplant, anticonvulsant use
- Children (16 years and under) and refugees
- Prior to treatment with interferon for hepatitis C

 For further information see: "Vitamin D supplementation: navigating the debate". BPJ 36 (Jun, 2011).

Vitamin K is a fat-soluble vitamin important in the post-translational modification (gamma-carboxylation) of a

number of proteins, importantly some clotting factors (II, VII, IX and X), and also certain bone proteins. Measuring vitamin K levels directly is rarely helpful except in limited specialist settings.

People at risk of vitamin K deficiency include those with fat malabsorbtion (e.g. chronic pancreatitis, cystic fibrosis, parenteral nutrition) and some neonates. However, a vitamin K test is not indicated as part of the general investigation of nutritional status and possible malabsorption.

The appropriate investigation of patients with clotting disorders due to possible vitamin K deficiency is the direct assessment of clotting status (raised prothrombin time and, if more severe, raised activated partial thromboplastin time). Echis ratio (a further test of clotting) may also sometimes be helpful. Plasma levels of individual clotting factors can also be measured if required.

Coenzyme Q10 (CoQ10, vitamin Q, ubiquinone) is important in mitochondrial oxidative metabolism and energy production, as well has having natural antioxidant effects. The most clearly established reason for measurement is the investigation of rare inborn metabolic defects, in which there may be primary or secondary CoQ10 deficiency.

Plasma CoQ10 measurement has been suggested to be useful in statin-induced myopathy, heart failure and neurological disorders such as Parkinson's disease. There is biological rationale for an intracellular deficiency of CoQ10 as a factor in these conditions. However, the correlation between plasma and intracellular (e.g. muscle biopsy) levels of CoQ10 is limited. Since CoQ10 is also mostly carried in the lipid fraction, statin treatment will inherently lower CoQ10 levels independent of those in tissues. Therefore this test is not recommended for this purpose.

Some evidence suggests that low CoQ10 predicts worsened mortality in heart failure and achieving a higher level may be associated with a better outcome in patients taking supplements. However, other trials have suggested no benefit and the value of measuring CoQ10 in these conditions at this time awaits further evidence.[17, 18]

For these reasons the group recommended CoQ10 measurement should be restricted to Cardiologists, Neurologists and Paediatricians managing patients with the above disorders.

Although it has been advocated, the use of CoQ10 measurement and treatment in chronic fatigue syndrome has weak evidence-base.

Urine iodine levels reflect recent iodine intake and vary widely from day to day depending on recent food intake; even a patient with relatively low body stores can have normal excretion if analysed within two to three days of an iodine-rich meal (foods rich in iodine include most seafood and seaweed, eggs/poultry, milk and sometimes soy products). Routine urine iodine testing has no established role in general practice, and there is no evidence that it leads to any beneficial outcomes in patients who are appropriately monitored for hypothyroidism and appropriately supplemented in pregnancy. Routine inclusion of iodine in a vitamin supplement (but not iodine testing) has been recommended in women who are pregnant by the Royal Australasian College of Obstetricians and Gynaecologists.[19]

The median urine iodide level in a population can be used as an index of population iodine status, however, urine iodine excretion (both spot urine iodine creatinine ratio and 24h excretion) has very low predictive value for iodine deficiency in an individual patient. WHO guidelines for population medians do not apply to individual subjects and will grossly over-diagnose iodine deficiency if misapplied in this way.[20] At least ten urine iodine collections are needed to provide a reasonable estimate of iodine status.[21] The earliest functional evidence of iodine deficiency is a rise in TSH, which can be treated with iodine supplementation.[21]

Currently the only clearly established use of measuring urine iodine in individual patients is in the assessment of patients undergoing radioiodine treatment, where high urine iodine suggests poor thyroid radioiodine uptake and reduced treatment efficacy. It is also sometimes helpful in the evaluation of patients with hyperthyroidism.

Zinc, copper, and selenium, mercury, chromium and cobalt. Unless there is a high pre-test probability of deficiency (i.e. a pre-disposing condition, such as gastrointestinal disease), or toxicity (e.g. workplace exposure) it is rarely necessary to measure plasma copper, zinc, selenium or blood mercury in patients in general practice. Deficiencies of zinc or selenium do not occur in people who consume a reasonable diet and have normal gastrointestinal function.

Measurement of these trace elements may be useful in the management of patients predisposed to deficiency by malnutrition and/or gastrointestinal disorders and especially in patients taking parenteral nutrition.

Measurement of plasma and urine copper levels are also useful in the diagnosis and management of Wilson's Disease (clinical details should state "? Wilson's Disease" or "raised LFTs") and in rare genetic disorders of copper metabolism (e.g. Menke's syndrome).

These tests are also helpful in cases of zinc, copper and selenium poisoning, and cases of suspected poisoning are an indication for referral. Measurement of whole blood and urine mercury are of value in monitoring workplace exposure and when mercury poisoning is suspected.

Measurement of serum cobalt and chromium is indicated in patients with concern over possible overexposure. The most common situation is patients with a metal-on-metal joint prosthesis where there is concern over possible deterioration of the joint surfaces, and who may present with symptoms such as pain, swelling, limping or trouble walking, or noise coming from the joint. If cobalt and chromium levels are abnormally elevated, it is recommended to repeat the tests after three months. If levels from the second test remain abnormally elevated, discussion with the Orthopaedic Surgeon is recommended.

For further information see: "Testing serum cobalt and chromium in people with metal-on-metal hip replacements". Best Tests (Dec, 2012).

High levels of cobalt and chromium can also occur in people working with ceramics or metals, excessive supplement intake or renal impairment. Urine testing is more appropriate than serum for assessing chronic occupational exposure.

Evidence was not considered sufficiently robust to justify the public funding of measurement of plasma zinc or the zinc/copper ratio in patients with depression, autism, other mental health disorders or chronic fatigue syndrome. Results of these tests are often misleading because low plasma zinc and raised copper levels are non-specific changes commonly seen in inflammatory states and chronic disease.

The presence of amalgam dental fillings or symptoms of fatigue, depression, cognitive decline etc. are not sufficient indications for measurement of blood or urine mercury levels. The major determinant of blood mercury is dietary fish intake, and amalgam fillings do not cause a clinically significant increase in blood mercury levels.[22]

Tumour markers

These include:

- Acid phosphatase
- CEA
- CA125, C15-3, CA19-9, CA72-4

Apart from acid phosphatase (Page 12), no formal restrictions have been placed on these tests at this time (Tier 1), however, guideline recommendations for requesting them have been developed.

The guidelines recognise the value of these tests for monitoring known malignancies of specific types in specific clinical settings. They can also be useful for diagnosis in patients with a high probability of cancer at presentation, e.g. CA125 in patients presenting with a suspicious ovarian mass, and can provide prognostic information.

Virtually none of the typical tumour markers are completely specific for malignancy, or for a particular type of malignancy. For example, while often thought of as useful in ovarian cancer, CA125 can also sometimes be raised in other malignancies such as pancreas, lung, breast, endometrium and non-Hodgkins lymphoma. It can also be raised in a wide range of benign disorders such as acute and chronic liver diseases, acute and chronic pancreatitis, rheumatoid arthritis, ulcerative colitis, endometriosis, menstruation, non-malignant ascites and pleural effusions and SLE. Similarly, while a very high CEA is strongly suspicious for malignancy, it can be raised in a wide range of cancers (e.g. gastrointestinal, lung, thyroid, breast), and also in benign diseases such as hepatitis.

The role of most soluble tumour markers in screening is still under evaluation but they are not currently recommended for this purpose in the general population based on insufficient large trial evidence for benefit.

As an example of the recommendations, the indications for measurement of CA125 are:

- Patients with symptoms or signs associated with high suspicion of ovarian cancer: persistent continuous or worsening unexplained abdominal or urinary symptoms, pelvic mass
- Case detection in patients at high risk of familial ovarian cancer
- At diagnosis of ovarian cancer to provide prognostic information
- After treatment to monitor response and detect relapse

However, measurement of CA125 is not indicated for:

- Investigation of non-specific symptoms, when probability of malignancy is low
- Screening of asymptomatic low risk population (in a low risk patient a mildly raised result is much more likely to be a false positive rather than a true positive)
- Investigation of other suspected malignancies

Lipid and cardiovascular disease related tests

Apolipoproteins B (ApoB) and A1 (ApoA1). These tests measure the protein component of lipid particles, LDL (ApoB) and HDL (ApoA1) respectively. Since there is only one ApoB or ApoA1 molecule per particle, they give an estimate of particle concentration rather than total cholesterol concentration in those particles.

At present there are no restrictions on requesting these tests as the demand for them is very low, and there is little evidence that they are being inappropriately ordered.

A number of epidemiological studies (but not all) suggest that these tests, and their ratio, may be marginally more predictive than lipid measurements themselves. They may identify some patients with genetic dyslipidaemias, and possibly help identify residual risk in patients on aggressive statin treatment.

These tests are significantly more expensive than lipid tests and while there measurement is improving, they are less well standardised internationally. Their advantage of being able to be measured in the non-fasting state is of limited practical value as non-fasting lipid tests themselves are usually reliably interpreted in most patients.

For further information see: "Fasting may be unnecessary for lipid testing", Best Tests Nov, 2013.

Lipoprotein (a) is a weak independent risk factor for premature coronary artery disease and thrombosis in the general population. Lp(a) levels are mainly genetically determined, change little over time, and are poorly responsive to diet or to lipid-lowering treatment. There is very limited evidence to support whether Lp(a) reduction reduces the incidence of cardiovascular events.

Based on current evidence, the group considered that measuring Lp(a) is not indicated as part of routine cardiovascular risk assessment in primary care.[23] If the clinical approach is otherwise clear based on other risk factors, then measuring Lp(a) has little additional value. The group recommended that requests for Lp(a) be funded (once only per patient) when requested by Cardiologists, as part of a specialist lipid/metabolic clinic, or with prior Chemical Pathologist approval.

Measurement should be limited to certain uncommon situations, particularly:

- Patients in whom assessment using traditional Framingham risk markers may be unreliable, e.g. an unexpectedly early personal history of CVD, or significant family history in the absence of clear Framingham risk factors
- Where measurement may influence the decision of whether or not to start the patient on pharmacological treatment based on other risk factors

For further information, see: "Assessing cardiovascular risk: what the experts think". BPJ 33 (Dec, 2010).

Lipoprotein electrophoresis was historically used to classify patients with likely familial dyslipidaemias (Frederickson classification), with interpretation being based on the staining pattern and intensity of different lipid fractions. However, this classification is now rarely used, electrophoresis is expensive and there are other clinical and laboratory means of recognising primary lipid disorders (e.g. apolipoprotein measurements, genetic tests). The group considered that lipoprotein electrophoresis should only be funded in specific clinical circumstances when requested by Cardiologists, Endocrinologists/metabolic specialists or Internal Medicine specialists.

The major remaining application of electrophoresis is when considering the rare diagnosis of type III dysbetalipoproteinaemia (broad beta or remnant removal

disease). Such patients have palmar xanthomas and increased concentrations of apoB-containing remnant particles (VLDL remnants, IDL).

High sensitivity CRP. Inflammation is now considered to play an important role in atherosclerosis. In well, asymptomatic patients the baseline level of CRP (referred to as high sensitivity CRP or hs-CRP) is thought to reflect the underlying level of inflammation and to have a graded association with CVD risk. There is epidemiological evidence linking levels of CRP with levels of cardiovascular risk, however, recent data has suggested that the risk is not as strong as originally stated. Genetic studies also fail to support a clear causal link of hs-CRP with cardiovascular disease. The group recommended that hs-CRP is funded when requested or pre-authorised by a Cardiologist, specialist lipid, metabolic or cardiovascular disease clinic or a Chemical Pathologist.

It is thought that hs-CRP is able to refine CVD risk in people rated at intermediate risk with traditional risk factors, and thereby re-categorise them above or below a treatment threshold. However, no current guideline (including local guidelines) recommends using hs-CRP as part of routine risk assessment. The American Heart Association suggests that this use be at the physician's discretion, especially in the context of deciding whether or not to prescribe a statin.

Recent data has suggested that using the value for hs-CRP in the Reynolds modification of the Framingham equation does not sufficiently alter risk in most patients at intermediate risk to be cost-effective.[24] The current risk calculator used in New Zealand also does not allow data for hs-CRP to be used.

There is also debate about the validity of the main intervention trial (Jupiter trial) that has been quoted to support the use of stratification by hs-CRP to guide treatment with statins. Further analyses of this and other large randomised trials shows the relative benefit from statin treatment is similar regardless of initial CRP level, i.e. the test does not identify a unique group that is likely to benefit.[25, 26]

Homocysteine is a sulphur-containing amino acid interconverted with methionine in a very important cycle of intermediary metabolism (methylation cycle), in which folate and vitamin B12 are required co-factors. Deficiency of folate and vitamin B12 may be associated with raised homocysteine, but measurement of these vitamins directly is generally considered adequate to assess the patient's nutritional status.

Population evidence shows raised plasma homocysteine levels to be associated with long-term cardiovascular risk, however, intervention trials using B vitamin supplementation (folate, B12, B6) to lower homocysteine have been disappointing, suggesting such supplementation may be associated with worse outcomes.[27] It is therefore most likely that mild/borderline homocysteine elevation is not itself causative of vascular disease, but rather may be a marker of other more complex predisposing nutritional factors. Regardless, since modifying homocysteine has been proven to be of little benefit its measurement as a cardiovascular risk marker was not considered sufficient to justify public funding.

Measuring plasma homocysteine is indicated when a monogenic disorder of methionine and homocysteine metabolism is suspected, e.g. patients with early or atypical thrombosis (including presentations such as retinal vein thrombosis), and when homocystinuria is otherwise suspected on clinical grounds.

Homocysteine elevation has also been suggested to be a marker of long-term risk of neurodegenerative diseases, such as Alzheimer's disease. A recent systematic review suggested there may be a weak association between raised homocysteine and dementia risk, but the evidence was of very low quality.[28] As with vascular disease, there was no proof of causal relationship, and no proof that lowering homocysteine mitigates this risk. Raised homocysteine is also associated with other factors which are themselves known to increase long-term dementia risk, such as diabetes, renal impairment, and advancing age.

Outdated tests

The following tests have been replaced in favour of other tests with greater clinical utility in most situations.

Prostatic acid phosphatase. For the diagnosis and monitoring of prostate cancer this test has been almost entirely superceded by PSA, which has much higher sensitivity for early disease, better correlation with tumour burden and treatment response and is more sensitive in identifying residual disease. Acid phosphatase is also more affected by prostatic hyperplasia (BPH) and digital rectal exam (DRE) than PSA. International guidelines have therefore not recommended its use, as in the large majority of patients it has no proven clinical benefit in addition to PSA.[29, 30]

Prostatic acid phosphatase is raised in certain uncommon disorders such as Gaucher's disease, however, other markers are preferred. It has also been used historically as a marker of bone resorption, but has been replaced by other markers with better biological and analytical performance.

The group recommended measurement of acid phosphatase when referred or pre-authorised by an Urologist, Internal Medicine Specialist, Paediatrician or Haematologist (or when pre-approved by a Chemical Pathologist).

Creatine kinase MB (CKMB). This isoenzyme of CK is present in highest concentration in heart muscle, but is also widely present at lower concentrations in skeletal muscle. It was widely used historically in the diagnosis of myocardial infarction. However, troponin (T or I) testing is far more sensitive and specific and has a much wider diagnostic window, with detection of myocardial injury generally before CKMB is increased and for up to 10 – 14 days. Recent guidelines, both internationally and from the New Zealand Cardiac Society, recommend troponin as the marker of choice in the investigation of patients presenting with possible acute coronary syndrome.[31–33]

CKMB testing has been suggested to be useful in the evaluation of possible reinfarction, but with modern troponin assays a change in troponin is usually reliable. In some patients where there may be an analytical issue with a particular troponin assay, an alternative (either Troponin T or I, or a different manufacturer's assay) will usually solve the problem, avoiding the need for CKMB testing.

Faecal fat. Although used historically for identifying and monitoring patients with steatorrhoea, this is a poor screen as typically over 90% of pancreatic function must be lost before it becomes elevated. It is also a very unpleasant test for both the patient and laboratory. Most laboratories no longer offer faecal fat testing.

Measuring fat content in a small faeces sample can be performed by measuring a "steatocrit", or by visualising fat droplets using a fat stain (this detects the large majority of patients with moderate/severe fat absorption). Other tests such as faecal elastase are both more sensitive and less onerous for evaluating pancreatic enzyme insufficiency. The only remaining use of faecal fat estimates (as steatocrit) is in specialist settings, e.g. as a means of quantitating the degree of fat malabsorption in patients on close monitoring of replacement regimens.[34]

Fructosamine. For a wide range of reasons, both biological and analytical, fructosamine is an inferior test compared with HbA_{1c} for monitoring patients with diabetes. It has a much shorter window of monitoring glucose levels, has greater biological variation, and is affected by albumin turnover (especially significant proteinuria) and hydration status. International evidence for the long-term prognostic value of HbA_{1c} is far greater and treatment targets are much better established.

Fructosamine should only be measured when a reliable HbA_{1c} result cannot be obtained, e.g. in situations of altered haemoglobin turnover (e.g. ongoing active blood loss or venesection) and with certain uncommon haemoglobin variants. If a HbA_{1c} analytical interference is identified then other HbA_{1c} methods without interference can usually be found, which is the preferred approach (if in doubt the laboratory should be contacted to discuss).

In the rare situations where fructosamine testing is indicated, there is little value in measuring it more often than monthly.

Tests with insufficient evidence

These tests lack sufficient evidence to justify funding their analysis under any circumstances.

Red cell magnesium (RBC Mg). Plasma magnesium is considered to be adequate for assessment of magnesium status and there is insufficient evidence to justify the additional expense of RBC Mg measurement for any clinical purpose. Evidence linking red cell magnesium to chronic fatigue syndrome was felt to be unconvincing.[35, 36]

Salivary progesterone measurement has been advocated as a means of monitoring transdermal progesterone treatment in peri- and post-menopausal women. Serum progesterone levels in such women are very low, reflecting perhaps the poor systemic absorption of progesterone creams through the skin. The evidence base to justify public funding of the salivary progesterone test was considered insufficient by the group.[37]

Salivary testosterone levels add little clinical utility to a serum testosterone measurement. Levels in saliva are very low and in current assays the precision at these levels also hampers interpretation.

Underutilised, but expensive tests

The following tests have increasing evidence for their clinical utility when requested within appropriate clinical guidelines, but are relatively expensive.

In some cases tests were recognised as being very good tests in specific clinical circumstances and, even though expensive, were probably underutilised. However, there were also situations where their clinical utility was limited and when the temptation to request them should be avoided.

BNP and NTProBNP is an example of such a test.

It is recommended that BNP or NT-ProBNP is requested in the following situations:

- Exclusion of heart failure as a cause of unexplained breathlessness and other non-specific symptoms
- Management of anti-heart failure treatment (secondary role only, usually for difficult to treat patients). There were no formal restrictions recommended for non-cardiologists, but it is recommended that repeat testing occur no sooner than two weeks between tests and, additionally, no more than four tests per year, per patient (more frequent need than this suggests excessive use or need for specialist involvement)

These tests have high negative predictive value for the exclusion of undiagnosed heart failure in patients presenting with non-specific symptoms and not already taking anti-heart failure treatment. Conversely, a clearly high result supports the diagnosis of heart failure and also carries adverse prognosis, independent of other variables (although in most acute cases this is clinically obvious through other means). However, mild-moderate elevation does not exclude the possibility of some other cause of breathlessness besides, or in addition to, heart failure. These tests also do not completely avoid the need for echocardiography, which provides other important information on cardiac structure and function, such as cardiac valve anatomy and (regional) myocardial contractility and relaxation.

The value of BNP and NTProBNP is much less well established for guiding ongoing anti-heart failure treatment. While a rise or fall can sometimes help guide treatment, proof of outcome benefit is much more limited and at present these tests have a secondary role only. NHF/NZGG guidelines do not specifically restrict use in this setting but have not encouraged it and NICE guidelines (UK) recommend their use be restricted to challenging patients under specialist management.

It takes at least two weeks for a new equilibrium level to be established and repeat measurement within this time frame is not recommended. Patients with heart failure who are difficult to manage should be referred for specialist review.

The Laboratory Schedule Test List and Laboratory Test Guidelines are available from: **www.dhbsharedservices.health.nz**

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The New Zealand Laboratory Schedule and Test Guidelines: **Microbiological and Serological Tests**

In October, 2013, the New Zealand Laboratory Test Schedule was published to provide consistent guidance and ensure uniform availability of tests across all District Health Boards (DHBs). The new Schedule divides tests into Tier 1 and Tier 2 to indicate whether all referrers can order the test, i.e. Tier 1, or whether a test must be ordered in conjunction with another health professional with a particular area of expertise, i.e. Tier 2. In this third article of an ongoing series we focus on the new Laboratory Schedule and Guidelines in relation to microbiological and serological tests for infectious diseases.

General Practitioners have access to more than 500 different laboratory tests in New Zealand. From this range the average General Practitioner requests over 4000 tests each year.[1] With this number of tests available, and this volume of testing, selecting the right test, for the right patient, at the right time can be challenging. Emerging evidence, changing guidelines, new testing methods and the ability of infectious organisms to evolve relatively quickly means that best practice inevitably changes with time.

The Laboratory Test Schedule and Laboratory Test Guidelines are available from: **www.dhbsharedservices.health.nz/Site/Laboratory/Laboratory-Schedule-Review-Project.aspx**

How was the infectious diseases section created?

A microbiological and serological Subgroup was formed to review tests for infectious diseases. This was made up of clinical microbiologists (both hospital and community) and public health specialists who examined the currently available tests and made recommendations as to which health professionals required access to each test. The Subgroup will continue to review the infectious diseases section of the Schedule regularly.

For further information see: "The New Zealand Laboratory Schedule and Test Guidelines: What does it mean for general practice?", BT (Nov, 2013).

Important points to note for microbiological and serological tests

The microbiological and serological test section of the Laboratory Schedule includes the following features:

- Alerts have been added to tests for notifiable infections to remind clinicians when notification to the Medical Officer of Health is required
- Tests for organisms causing infectious diarrhoea are now labeled by the suspected organism, rather than by the test that is used to identify them
- The practice of "sentinel testing" has been introduced
- Situations where "screening" tests will not be funded have been specified
- Outdated or unnecessary tests have been removed from the Schedule, where appropriate

Guidance has been provided for some tests in the microbiological and serological Laboratory Schedule to help clinicians request the most appropriate test. These recommendations are based on New Zealand and/or international best practice. Further guidance is likely to be added to the Schedule in future reviews.

Clinicians are invited to provide feedback by suggesting areas where additional information would be helpful. To provide feedback on the Schedule email: **ALLDHBs@dhbsharedservices.health.nz**

Tier 1 and Tier 2 tests for infectious diseases

The Tier 1 category makes the following tests more accessible:

Faecal antigen testing for *Helicobacter pylori* is now considered the most appropriate test for *H. pylori* infection. Previously, faecal antigen testing for *H. pylori* was only funded for hospital laboratories despite most of the requests for this test being made by General Practitioners.

For further information see: "The changing face of *Helicobacter pylori* testing", (Page 20).

The interferon gamma release assay (IGRA, Quantiferon gold test) for tuberculosis exposure or latent tuberculosis infection is now recommended to identify patients who are at high risk of developing active tuberculosis, in preference to older tuberculin tests, e.g. the Mantoux test. The IGRA has greater specificity than tuberculin testing and requires only one patient visit to the clinic. IGRA testing for latent tuberculosis is particularly recommended in the following patients: BCG-vaccinated people, immunocompromised people, e.g. those taking corticosteroids or methotrexate, high risk people who may not attend a second consultation or where a second visit is impractical.[2] IGRA testing in children aged under seven years is not currently recommended.[2] The Mantoux test can still be used to diagnose latent tuberculosis infection and is the preferred test in children aged under seven years.[2] The guideline to the microbiological and serological Laboratory Schedule can provide further information to clinicians when requesting a test for tuberculosis.

Nucleic acid amplification tests (NAAT) to detect *Bordetella pertussis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are Tier 1 tests. Unlike culture tests that were previously used, NAAT tests only need a sample of DNA, and do not require viable bacteria to produce a positive result. Results are also available within hours, compared to cultures which may take three to 12 days.[3] NAAT testing also has the advantages over serology testing of not requiring the patient to have mounted an immune response in order to produce a positive result and of not being complicated by immunisation or past infection.

Influenza virus testing has been included as a Tier 1 test when assisting public health authorities in defining the epidemiology of large scale outbreaks. Previously this was possible but was not recognised in testing guidelines. Under normal circumstances this test may only be requested in primary care after consultation with a public health specialist. The Schedule also has the flexibility to allow other tests to be changed from Tier 2 to 1 as required.

The Tier 2 category will have little effect on general practice

The creation of a Tier 2 category for microbiological and serological testing will not have a significant impact on clinicians in the community as many of the tests in this category were already restricted to specific situations.

The following are examples of Tier 2 tests:

Reflex testing, which occurs automatically when the need for a second test is identified by the laboratory after an initial positive result. For example, when a test for *Toxoplasma gondii* is performed, if the initial test for IgG is positive, and clinical information suggests that this may be an acute infection, the sample is sent for avidity testing to determine if the IgG is a response to a past or recent infection. Screening Gram-negative bacilli that are resistant to cephalosporins for extended β-lactamase production is another example of reflex testing.

Some tests that require invasive sampling by a specialist clinician are classified as Tier 2, e.g. biopsies for *H. pylori* culture and susceptibility testing.

Tests for uncommon pathogens, e.g. arboviruses, are now classified as Tier 2. When considering requesting tests for uncommon pathogens a discussion with an Infectious Diseases Specialist or Clinical Microbiologist may be helpful in assessing the likelihood of a pathogen being present or in interpreting the results of the test. The Tier 2 category promotes consultation in less common situations and improves the quality of requests and the interpretation of test results.

Alerts for notifiable infections

The microbiological and serological Laboratory Schedule now includes an alert column to remind clinicians when notification to a Medical Officer of Health is required, e.g. a positive *Salmonella*, *Shigella*, *Yersinia*, or *Campylobacter* faecal culture. This feature was introduced to increase notifications and to improve understanding of when notification is required.

The Schedule also contains some footnotes relating to case definitions of notifiable diseases, e.g. defining a probable case of pertussis as opposed to a confirmed case.

Tests for faecal pathogens are now specified by pathogen

Test for organisms causing infectious diarrhoea are now labeled in the Schedule by the suspected organism, rather than by the test that is used to identify them. This change was made to encourage clinicians to include clinical information when requesting tests and to allow laboratories to choose the most appropriate test. Listing the patient's risk factors, e.g. recent overseas travel, helps laboratories to optimise testing.

For example, previously, when investigating infectious diarrhoea, if a request for enteric pathogens was made the laboratory performed microscopy and culture, however, different laboratories might culture for different organisms as there was no standardisation in which cultures would be performed. Now clinicians may request the "*Salmonella, Shigella, Yersinia, Campylobacter culture*" test for these common pathogens and additional testing can be added by the laboratory on the basis of clinical information provided.

Sentinel testing may be appropriate in some DHBs

The microbiological and serological Laboratory Schedule allows for DHBs to request health professionals to participate in the reporting of local antimicrobial susceptibility profiles, i.e. sentinel testing, to assist prescribers in the use of empiric antimicrobial treatment. This practice enables laboratory validation of local antibiotic guidelines for the treatment of common conditions. Examples where sentinel testing may

provide useful information in local susceptibility include:

- Females with uncomplicated cystitis, who are generally treated empirically, may have urine samples tested to determine local patterns of antibiotic susceptibility. This was suggested by the Subgroup in response to the introduction of increasingly resistant urinary pathogens, and because the susceptibility of *Escherichia coli* isolates varies geographically.
- *Neisseria gonorrhoeae* is now generally detected by NAAT and therefore susceptibility data is not available in every case
- *Streptococcus pneumoniae* is a common respiratory pathogen with a susceptibility profile that is hard to predict

It is anticipated that sentinel testing will improve the use of tests to diagnose and test for infections and promote the rational use of antimicrobials. Local sentinel testing is not recommended unless initiated by a DHB. Participation in the ESR national surveillance programme of antimicrobial resistance remains important to monitor changes at a national level.

When are "screening" tests not funded?

The microbiological and serological Laboratory Schedule now outlines situations when tests are not funded. This will make it clear for laboratories and DHBs under which situations tests will not be funded, when they are negotiating contracts. Tests are not funded in the following situations:

- Occupational testing, e.g. pre-employment drug testing
- To provide evidence of immunity for travel purposes
- Providing information for insurance or for visa applications
- Tests required by sports groups, e.g. testing for prohibited substances in athletes or proof of HIV status to obtain a professional boxing license
- Testing pre- or post-vaccination, e.g. hepatitis A testing to determine a patient's immunity before or after vaccination

Tests that are no longer necessary have been removed

Microbiological and serological tests which were not considered necessary have been removed from the schedule include:

- Chlamydia IgG tests have not been found to be useful for the routine diagnosis of Chlamydia infections. NAAT is considered a better test for patients suspected of having a Chlamydia infection.
- *H. pylori* serum antibody tests were routinely used to test for *H. pylori*. This test has been superseded by the use of *H. pylori* faecal antigen tests using monoclonal antibodies. A guideline will be released to assist clinicians in the use of this test.
- Hepatitis C antibody immunoblot and hepatitis C confirmatory immunoblot have been replaced by hepatitis C NAAT tests for viral detection and confirmation of patients with active infection
- TORCH screening for perinatal infections in newborn infants is no longer recommended and is not funded. Individual tests should be ordered when a congenital infection is suspected.
- Typhoid serology is not funded because culture for *Salmonella typhi* is considered to be a better test

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The New Zealand Laboratory Schedule and Test Guidelines: haematology tests

The New Zealand Laboratory Schedule has been created to provide consistent guidance and ensure uniform availability of tests across all District Health Boards (DHBs). The new Schedule divides tests into Tier 1 and Tier 2 to indicate whether all referrers can order the test, i.e. Tier 1, or whether a test must be ordered in conjunction with another health professional with a particular area of expertise, i.e. Tier 2. In addition, clinical guidelines are provided on the use of some tests. In this article we focus on the haematology tests in the schedule.

It is estimated that 85% of clinical decisions involve laboratory investigations. The objective of the New Zealand Laboratory Schedule is to make the most relevant tests available, and to provide guidelines on their optimal use.

The schedule was created by an overall steering group, managed by DHB Shared Services, with subgroups formed for each area of clinical speciality. The haematology subgroup is led by Dr Stephen May and made up of clinical and laboratory haematologists, with representation from around the country. The subgroup continues to meet to consider new investigations available as well as reviewing indications for older tests.

The haematology tests are ranked in Tier 1 and Tier 2 tests

Tier 1 tests may be requested by any registered medical practitioner as well as other practitioners who are able to request investigations, e.g. midwives.

Tier 2 tests are specialist tests whereby the referrer needs appropriate vocational registration or credentialing to order the test. Tier 2 test are also able to be ordered by General Practitioners on the advice of a relevant specialist.

Guidelines on selected haematology tests

Erythrocyte sedimentation rate (ESR) (Tier 1)

ESR has historically been used in clinical medicine as a measure of inflammation. However, it has significant limitations in terms of measurement accuracy. In addition, ESR is affected by numerous physiological variables and by factors other than inflammation, such as haemoglobin and plasma protein levels.

Despite its limitations, ESR may have some advantages in the assessment of the following conditions:

- Systematic lupus erythematosis
- Rheumatoid arthritis
- Kawasaki disease
- Rheumatic fever
- Hodgkin lymphoma
- Temporal arteritis
- Inflammatory bowel disease in children (initial assessment)

ESR should not be used to screen for plasma cell dyscrasias. If these conditions are suspected, protein electrophoresis and immunofixation or serum free light chain assays (see below) should be used.

C-reactive protein (CRP) is the preferred investigation for the assessment for a possible inflammatory or infective disorder. It is seldom appropriate for both ESR and CRP to be requested together.

While ESR and CRP are no longer routinely requested together for most conditions, either marker (or both) can be raised in giant cell arteritis (temporal arteritis) and given the significant potential for morbidity in people with giant cell arteritis, it is recommended that both are requested in the initial presentation. For further information see: "Giant cell arteritis: Always keep it in your head", BPJ 53 (Jun, 2013).

Serum free light chains (Tier 2)

The symptoms of multiple myeloma may be classical (e.g. bone pain) or non-specific. If multiple myeloma is suspected, a practical approach is to first request serum protein electrophoresis. If an increase in immunoglobulins is found, or the test is normal, but clinical suspicion remains, the need for further testing should be discussed with a Haematologist or other relevant specialist.

Serum free light chain assays can detect elevated levels of light chains (of immunoglobulin) in the blood, even when those levels are undetectable by serum protein electrophoresis. In a serum free light chain assay, both free kappa (κ) and lambda (λ) chains are measured and the ratio is calculated. Excessive free κ or λ increases the likelihood a of monoclonal plasma cell disorder.

The International Myeloma Working Group guidelines suggest that a serum free light chain assay is used for prognostic purposes in all patients with:

- Monoclonal gammopathy of unknown significance (MGUS)
- Smouldering multiple myeloma
- Active multiple myeloma
- Amyloidosis

The test is also indicated for patients with:

- Suspected myeloma, MGUS or amyloidosis
- Unexplained renal impairment
- Unexplained proteinuria
- Unexplained peripheral neuropathy

Follow-up testing is recommended no more frequently than every three months, unless the patient is on active chemotherapy.

For further information see: "Making sense of serum protein bands", Best Tests (Jul, 2011).

Chronic lymphocytic leukaemia (CLL) investigations (Tier 1)

Early B-cell chronic lymphocytic leukaemia is the most common type of adult leukaemia. It mainly affects people aged over 50 years (median 65 years), and patients are asymptomatic in the early stages with the only feature being a peripheral lymphocytosis. Diagnosis of chronic lymphocytic leukaemia (CLL) is based on cell marker studies (flow cytometry), along with clinical assessment.

Consider CLL or other lymphoproliferative disorders if the patient has persistent lymphocytosis of $> 5 \times 10^9$/L for more than three months.

1. Discuss with the Haematologist if cell marker studies are required for persistent unexplained lymphocytosis
2. Refer to the Haematology Outpatient Department if the referral criteria are met (see below); this usually signals advanced or progressive disease. Otherwise, regular monitoring (full blood count) in general practice is indicated; initially every three to six months, then yearly if stable or slow.

Referral criteria are outlined in full in the Laboratory Test Guidelines. The criteria include:

- Age < 55 years, with progressive disease
- Significant symptoms, e.g. significant weight loss, fatigue, night sweats
- Advanced stage of disease
- Disfiguring lymphadenopathy or hepatosplenomegaly
- Recurrent infections
- Haemolytic anaemia
- Lymphocyte count which has doubled in less than six months and is $> 30 \times 10^9$/L

Haemoglobinopathy investigations (Tier 1)

The most significant haemoglobinopathies/thalassaemias are: sickle cell disease, beta thalassaemia and alpha thalassaemia ("CIS" inheritance pattern). Although there are currently no specific referral criteria on the laboratory schedule for patients suspected of having one of these conditions, discussion with a Haematologist is recommended.

There is no formal haemoglobinopathy screening currently undertaken in New Zealand, therefore investigations are done on an *ad hoc* basis (as a once only investigation) for:

1. Investigations of hypochromic microcytic blood pattern when iron deficiency has been excluded
2. High risk ethnic groups, e.g. Middle Eastern, African, Pacific peoples
3. Follow up of family studies
4. Investigations of abnormal haemoglobins during other investigations, e.g. an abnormal haemoglobin found incidentally while testing HbA_{1c} for diabetes

For further information on investigating haemoglobinopathies/thalassaemias in patients with microcytic anaemia, see: "Anaemia on full blood count: investigating beyond the pale", Best Tests (Sep, 2013).

Inherited thrombophilia testing (Tier 1 and 2)

Thrombophilia testing is of limited utility and should not be used as a screening test. However, it is indicated in the following situations:

- Idiopathic venous thromboembolism in patients aged less than 45 years
- Warfarin induced skin necrosis
- Children presenting with purpura fulminans
- Siblings of patients with homozygous factor V Leiden
- Homozygous PT20210A or compound heterozygotes for these mutations
- Thrombosis in unusual sites, e.g. cerebral, mesenteric or portal

In all other situations testing should only be undertaken after consultation with a Haematologist or as part of a clinical trial.

The need for any investigation is dependent on the usefulness of the result and if there will be no change in clinical management as a result of the investigation, then it is not indicated.

For further information, see: Baglin T, Gray E, Greaves M, *et al*. Clinical guidelines for testing for heritable thrombophilia. British Journal of Haematology 2010;149:209-20.

Additional considerations for testing

Any testing should be requested as a result of, or to provide evidence of, a clinical condition, or to monitor chronic conditions or exclude clinically significant differential diagnoses.

Tests on the Laboratory Schedule should not be used for screening purposes outside a formal screening programme. Pre-employment screening is excluded from funding, as is testing for immigration purposes, or tests required prior to travel, although these tests can be purchased from an IANZ accredited laboratory.

The Laboratory Test Schedule and Laboratory Test Guidelines are available from: **www.dhbsharedservices.health.nz/Site/Laboratory/Default.aspx**

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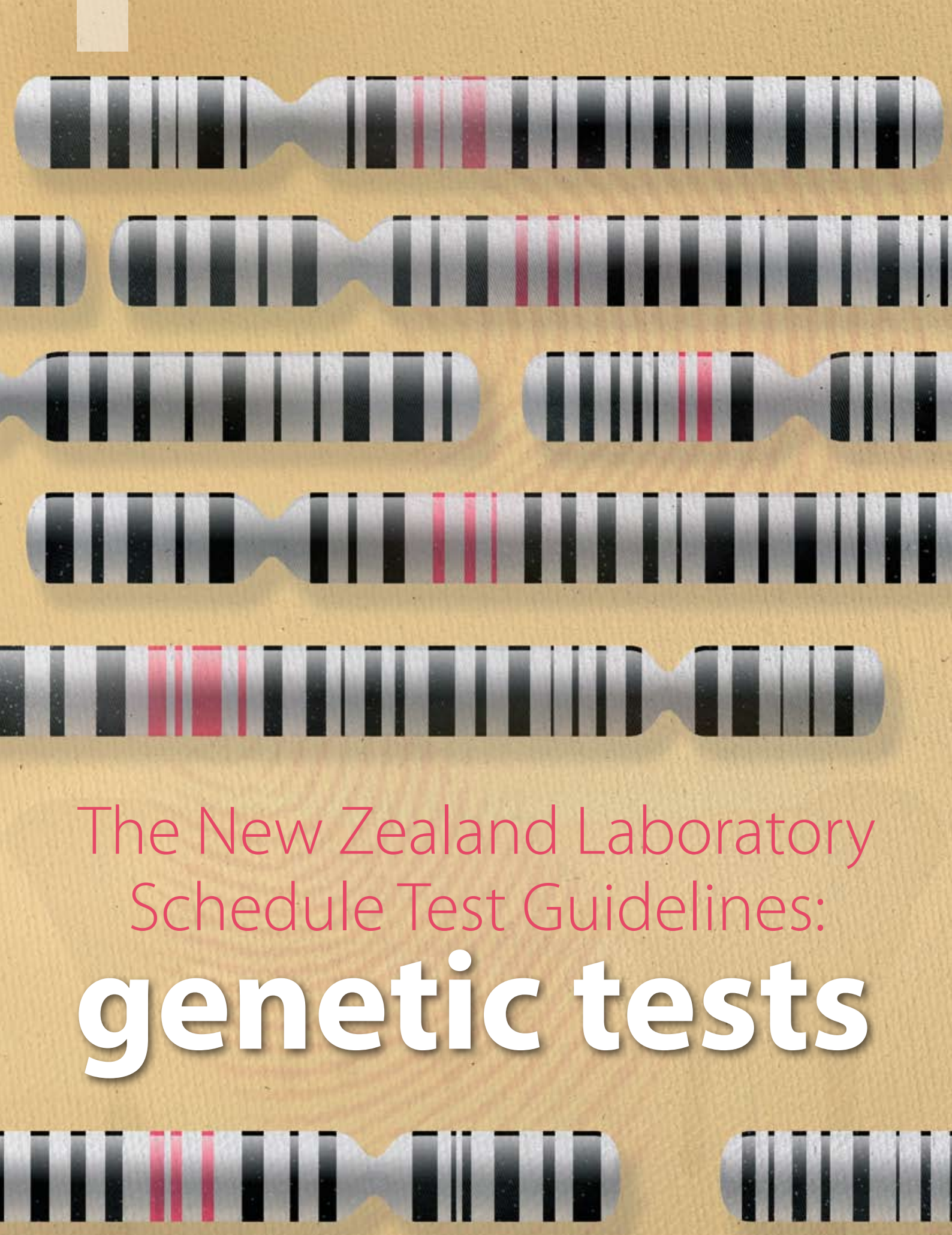


The New Zealand Laboratory Schedule Test Guidelines: genetic tests

The New Zealand Laboratory Schedule provides clinicians with consistent guidance when considering requesting laboratory tests. It will ensure the uniform availability of tests across District Health Boards (DHBs) in the future. Tests are divided into Tier 1, which all referrers can order, and Tier 2, meaning that the test must be ordered in conjunction with another health professional with a particular area of expertise. In addition, clinical guidance is provided on the use of some tests. In this article, with the assistance of Dr Joanne Dixon (leader of the Laboratory Schedule genetics subgroup), we focus on the genetic tests in the Schedule.

The role of primary care in genetic testing

Genetic testing can provide patients with information that may affect them for the rest of their lives, and potentially those of their family/whānau for generations to come. Given the potential significance of genetic testing it is important that requests for tests are appropriate and that patients are given sufficient information to make informed decisions before testing occurs. Equally, it is important that clinicians are able to provide this advice, to interpret the results of genetic tests correctly, and to support patients who are affected by genetic disorders.

Most general practitioners request only a few genetic tests. However, primary care does have a role in identifying patients who may benefit from genetic testing. For example:

- Testing to confirm a diagnosis, e.g. in a patient with abnormal iron metabolism suggestive of hereditary haemochromatosis
- Detecting the presence of a gene associated with a familial cancer syndrome, e.g. Lynch syndrome (hereditary non-polyposis colorectal cancer) in a person with a strong family history of colorectal cancer
- In rare cases, genetic testing may be useful to exclude a diagnosis, e.g. to avoid the necessity of performing small bowel biopsy in a young patient with suspected coeliac disease when the results of serology are equivocal

The New Zealand Laboratory Schedule

The Guidelines for genetic testing were developed by the Genetics Subgroup, led by Dr Joanne Dixon. The group included genetic diagnostic laboratory directors (LabPlus, Canterbury Health Laboratories and Wellington Regional Genetics laboratory) and clinicians.

The information about genetic tests in the Laboratory Schedule is divided into:

- Commonly requested genetic tests
- Genetic biochemistry
- Genetic haematology
- Genetic immunology
- Cyto-molecular genetics
- Genetic oncology

The majority of genetic tests listed on the New Zealand Laboratory Schedule are Tier 2. This means that as local DHBs choose to adopt the Schedule general practitioners will need authorisation from a clinician with relevant genetic experience before a request for testing is accepted.

There are several genetic tests available on the Schedule as Tier 1 tests, e.g. genotyping for hereditary haemochromatosis and testing for the absence of HLA-B27 when excluding ankylosing spondylitis, which can be requested by general practitioners without specialist authorisation. However, many genetic tests are for rare disorders and are only available through international laboratories via Genetic Health Services New Zealand (GHSNZ) clinicians. It is therefore generally recommended that all patients be discussed with a relevant clinician when considering the need for genetic testing. This also ensures that testing is appropriate and that patients have access to genetic counselling.

 For further information on the New Zealand Laboratory Schedule see: **www.dhbsharedservices.health.nz/Site/Laboratory/Laboratory-Schedule-Review-Project.aspx**

 For further information about GHSNZ, see: "Genetic Health Services New Zealand: what you need to know", Page 11.

Guidance on selected genetic tests

Genetic testing can provide clinical information of varying degrees of usefulness depending on the type of test that is requested, and the personal and family history of the patient. The different types of genetic testing that general practitioners need to have a broad knowledge of can be divided into:

1. **Diagnostic testing** to confirm a diagnosis, e.g. hereditary haemochromatosis in patients with elevated transferrin saturation.

2. **Pre-symptomatic testing** for a patient with a family history of a disorder that is caused by a single gene with full penetrance, i.e. all people with the gene will eventually display symptoms, e.g. Huntington disease.[1]

3. **Predictive testing** to determine whether a patient has a significantly increased lifetime risk of developing a condition due to the presence of a single gene, e.g. the **BR**east **CA**ncer gene (BRCA) for breast and ovarian cancer in females.[1]

4. **Carrier testing** to determine if a patient has a recessive gene for a condition, e.g. cystic fibrosis.

5. **Susceptibility testing** to determine if a combination of genetic variations results in a patient having an increased lifetime risk of developing a condition, e.g. diabetes or schizophrenia. However, these types of tests have limited clinical application as the relevant conditions often have multi-factorial causes.[1]

Specific examples of conditions encountered in primary care where genetic testing may be appropriate are provided below.

Hereditary haemochromatosis – an example of diagnostic testing

Hereditary haemochromatosis is predominantly found in people of European ancestry. This disorder causes an increase in iron absorption from the intestine due to a defect in hepcidin, the hormone which regulates iron homeostasis.[2] Iron deposits accumulate in the liver, pancreas, heart, joints, skin and gonads, which can cause serious damage if a person is untreated.

It is estimated that as many as one in ten people of European ancestry carry one copy of the gene for hereditary haemochromatosis, but the condition is rare among people of African or Asian ancestry.[3] Genetic testing for hereditary haemochromatosis is therefore unlikely to be clinically useful in people who do not have European ancestry.

Genotyping for hereditary haemochromatosis is available as a Tier 1 test where local guidelines permit. However, it is recommended that the patient is discussed with a gastroenterologist, haematologist or internal medicine specialist, or alternatively with GHSNZ.

The early symptoms of hereditary haemochromatosis are non-specific, including lethargy, arthralgia and abdominal pain.[2] Late complications include diabetes and peripheral arthritis. **Testing for hereditary haemochromotosis should only be considered in patients who have biochemical evidence of abnormal iron metabolism**, i.e. elevated fasting transferrin saturation of 45% or higher or elevated fasting serum ferritin concentration >300 ng/mL in males or >200 ng/mL in females, once more common causes of altered iron metabolism have been excluded.[4, 5] Elevated ferritin may be associated with inflammation due to infection, autoimmune conditions, cancer, excessive alcohol use and/or fatty liver, which can also cause transferrin levels to be raised.[2]

N.B. Fasting serum ferritin provides a more accurate marker of the total amount of iron stored in the body. Consuming some foods, e.g. iron-fortified breakfast cereals, can influence serum ferritin levels.

GHSNZ recommends that genetic testing of asymptomatic family members of an affected individual should only be undertaken following the recommendation of a clinician with relevant genetic experience or after discussion with a genetic counsellor.

There are two principle mutations in the HFE gene that can cause hereditary haemochromatosis: C282Y and H63D. Most people with haemochromatosis will be homozygous with the genotype HFE C282Y/C282Y, meaning they have two copies of the most common mutation for the condition; approximately one in 200 people in New Zealand have this genotype.[6] However, not all people with this genotype will develop haemochromatosis; the clinical penetrance is estimated to be 60 – 70%.[7] It has been estimated that in the United Kingdom a general practitioner with 1000 patients can expect to have approximately two patients with clinical hereditary haemochromatosis.[8]

A small number of people who are heterozygous carriers for the HFE gene (i.e. one copy of a mutant gene) will have elevated serum iron markers, and some will develop iron overload, but this does not result in significant iron deposition. Genetic testing of patients with suspected hereditary haemochromatosis ensures that this small proportion of patients do not undergo the intensive management that is required for patients with haemochromatosis and significant iron deposition (see below).

Approximately 5% of people with haemochromatosis carry the genotype HFE C282Y/H63D.[2] This is a compound heterozygous genotype where a person has copies of two different disease-causing mutations. Patients with a compound heterozygous genotype require management and treatment similar to that of patients with hereditary haemochromatosis who have a homozygous (HFE C282Y/C282Y) genotype.

Patients with hereditary haemochromatosis are treated by phlebotomy with blood removed once or twice per week to achieve a target ferritin level of < 50 micrograms/L, followed by maintenance treatment to keep ferritin levels between 50 – 100 micrograms/L.[2] Patients who commence phlebotomy treatment before they develop liver cirrhosis are likely to have a normal life expectancy.[2] Patients with haemochromatosis have an increased risk of osteoporosis and periodic DEXA scans are recommended.[2]

bpac[nz] will be publishing a more detailed article on the diagnosis and management of hereditary haemochromatosis in 2015.

Huntington disease – an example of presymptomatic testing

Huntington disease is a progressive neurodegenerative disorder that is ultimately fatal. It is inherited in an autosomal dominant pattern (only one copy of the abnormal gene needs to be present for the disease to be expressed), therefore there is a 50% chance that a person with an affected parent will develop the condition. The genetic test for the gene (HTT) that causes Huntington disease is more than 99% sensitive, because a single mutation accounts for the vast majority of cases.[9] People with Huntington disease have an expanded CAG repeat in the HTT gene which causes an abnormally long polyglutamine section in the huntingtin protein (N.B this is the correct spelling of the protein).[10] This results in an abnormal conformation of the mutant protein that is thought to cause selective neuronal toxicity within the striatum.[10] The prevalence of Huntington disease in Australia is reported to be 6 – 12 cases per 100 000 people.[11] There is limited data on the prevalence of Huntington disease in New Zealand.

Diagnostic testing for the Huntington disease mutation is classified as Tier 2 and should be requested by a neurologist, geriatrician or internal medicine specialist through GHSNZ. Pre-symptomatic testing in families with a history of Huntington disease is arranged by GHSNZ, and is not available for patients aged under 18 years. A positive Huntington test result is a life-changing event that requires careful management and support from both genetic counsellors and clinicians. Affected people may choose not to conceive, or they may wish to pursue prenatal testing for Huntington disease. People with children may feel anxious that they could have passed the disease-causing gene on. A positive test may influence a person's financial and career decisions and may affect relationships with their partner or siblings. For these reasons, many people who have a family history of Huntington disease prefer the uncertainty of not being tested.[12]

The mean onset of Huntington disease is age 40 years, with death occurring within 15 – 20 years of onset.[10] Patients who have developed Huntington disease can be identified by a progressive deterioration of motor control and cognitive function. Chorea is often seen early and is characterised by involuntary writhing movements. Later bradykinesia, incoordination and rigidity are more severely disabling.[10] There is currently no known cure for Huntington disease.

Inherited cancer syndromes – examples of predictive testing

There are many genes in which a mutation can allow the growth and replication of normal cells to escape usual control systems. In some situations these mutated genes can be passed on to an affected person's children. These include tumour suppressor genes, e.g. the BRCA mutation, oncogenes and mismatch repair genes. However, familial cancer syndromes are relatively rare. Patients who have been diagnosed with cancer, with a significant family history of cancer, may be referred for genetic testing by the clinician who is managing their treatment or by a general practitioner. General practitioners act as "gate-keepers" for asymptomatic people who are concerned that they may be affected by a familial cancer syndrome. A general practitioner with 1000 patients can expect to have 15 – 17 patients with a hereditary predisposition to cancer.[12]

If a person has a strong history of cancer in their family, especially if family members developed cancer before the age of 50 years, then it is reasonable to consider referring the patient to GHSNZ for counselling to determine their risk. It may be useful to discuss the patient with a relevant clinician, such as an oncologist or gastroenterologist, before considering a referral. Referral of families to GHSNZ for genetic assessment should include a three generation family tree which identifies family members who have been affected by cancer. If a familial mutation has not been previously identified, genetic testing must begin with DNA from an affected family member, in order to reduce false negative results.

Familial colorectal cancer

Autosomal dominant inheritance is estimated to account for 5 – 10% of cases of colorectal cancer.[13] Lynch syndrome (hereditary non-polyposis) is the most common hereditary colorectal cancer syndrome. A sample of 500 patients treated consecutively for colorectal cancer found that 3.6% had Lynch syndrome, of which 44% were diagnosed before age 50 years.[14] Each of these patients had at least three relatives with Lynch syndrome. Females with Lynch syndrome also have an increased risk of developing endometrial and endometrioid ovarian cancers.

Familial adenomatous polyposis (FAP) is caused by a mutation in a tumour suppressor gene and accounts for less than 1% of colorectal cancers. One in 5000 to 7000 people have FAP.[15]

If a patient has a personal or family history of colorectal cancer that is suggestive of a familial cancer syndrome then referral to the New Zealand Familial Gastrointestinal Cancer Service (see below) is recommended. Patients with an appropriate tumour histology and family history will then be referred to GHSNZ for mutation screening as required.

For further information visit: **www.nzfgcs.co.nz**

For further information see: "Surveillance of people at increased risk of colorectal cancer", (BPJ 44, May 2012).

Familial breast cancer and ovarian cancer

The predominant genetic abnormality that increases the risk of females developing breast and/or ovarian cancer is the presence of BRCA1 or BRCA2. It is thought that these mutated genes are present in approximately 5% of patients with breast cancer and 15% of patients with high-grade epithelial ovarian cancer.[16] Additional factors in a patient's family history that may indicate an increased risk for the development of cancer include: [17]

- Bilateral breast cancer
- Male breast cancer
- High-grade epithelial ovarian cancer*
- Jewish ancestry
- Sarcoma in a relative younger than age 45 years
- Glioma or childhood adrenal cortical carcinomas

* N.B. Borderline mucinous ovarian cancer is not associated with BRCA gene mutations

Women who are positive for a BRCA1 or BRCA2 mutation can be offered more frequent breast screening, as well as beginning screening at a younger age, e.g. having a mammogram every year, beginning at age 25 to 35 years. Hormonal therapy, e.g. tamoxifen, as well as prophylactic mastectomy, may be considered by some women as risk-reducing treatment options. Bilateral salpingo-oophorectomy (removal of the ovaries and fallopian tubes) is strongly recommended for patients who are BRCA mutation carriers, as increased frequency of monitoring has not been shown to result in improved long-term survival or earlier detection.

Examples of carrier testing that may be encountered in primary care

Every person is an asymptomatic carrier of a number of recessive genes that could potentially be passed on to their biological children. If their partner is also a carrier for the same autosomal condition there will be a one in four chance for each of their children having the genotype associated with the condition.

Thalassaemia (haemoglobinopathies) testing

The thalassaemias are the most common single gene disorders worldwide.[12] They are autosomal recessive blood disorders characterised by the abnormal production of one or more of the four protein chains (alpha or beta) that make up haemoglobin. Every person has four copies of the alpha globin gene and two copies of the beta globin gene.[18] Alpha thalassaemias are usually due to deletions of alpha globin genes, and beta thalassaemias are usually due to mutations in the beta globin genes.

Worldwide, approximately one in 20 people carry a gene for thalassaemia and it is thought that this provides protection against malaria.[18] The prevalence of genes causing alpha thalassaemia is increased among Māori and Pacific peoples,

as well as people of Chinese, South East Asian, Southern European, Middle Eastern, Indian subcontinent and African ancestry.[18] Deletion of a single gene (silent alpha thalassaemia) results in a mild decrease in mean cell volume. People with silent alpha thalassaemia are not generally anaemic. Deletion of two genes (alpha thalassaemia trait) causes a more marked microcytosis and hypochromia, but any anaemia is usually mild. People with deletion of three genes (haemoglobin H disease) are almost always anaemic with severe microcytosis and may require intermittent blood transfusions. Deletion of all four alpha globin genes (alpha thalassaemia major) results in fetal hydrops and is generally incompatible with survival.

There is an increased number of carriers for beta thalassaemia among people of Middle-Eastern, Southern-European, Indian subcontinent, Central and South-Asia and African ancestry.[18] People with a single mutated gene generally have mild anaemia with marked hypochromic microcytosis. Mutation of both genes (beta thalassaemia major) results in a severe transfusion-dependent anaemia. Because beta globin synthesis only starts around the time of birth, this will usually become apparent during the first year of life.

Haemoglobin electrophoresis has traditionally been used as the initial investigation for patients suspected of carrying a gene for thalassaemia.[19] Following electrophoresis, patients who have clinical signs consistent with thalassaemia or have a family history of thalassaemia should be referred to GHSNZ for genetic counselling before genetic testing is considered.

Patients who are carriers for thalassaemia do not require treatment.[12] Where both parents are carriers of a potential thalassaemia-causing gene, genetic testing during pregnancy may be appropriate and can be discussed with a haematologist or with GHSNZ.

For further information see: "Anaemia on full blood count: investigating beyond the pale" (BT Sept, 2013).

Cystic fibrosis testing

Cystic fibrosis is the most common autosomal recessive paediatric disease, although adults are increasingly affected as survival rates improve.[12] Approximately 1 in 20 – 25 people carry a mutation in a gene on chromosome 7 (CFTR) that can cause cystic fibrosis; approximately 1 in 2500 people of European ancestry develop the condition.[12] The mutation causes an abnormality in a membrane ion channel, resulting in impaired chloride and sodium transport across the epithelium and thick, viscous secretions. Cystic fibrosis mainly affects the lungs, but also involves other organs such as the pancreas, liver and intestines.[12]

Screening for immunoreactive trypsin (IRT) occurs routinely at birth as part of the Newborn Metabolic Screening Programme (formerly known as the heel prick or Guthrie test), and a test for mutations in CFTR is performed in infants with IRT above a threshold level. This is reported to detect 95% of infants born with cystic fibrosis.[20] Infants who have only one copy of the altered CFTR gene (heterozygotes) may also have a positive cystic fibrosis screening test. Analysis of the salt content of the infant's sweat is then used to confirm a diagnosis of cystic fibrosis.[20]

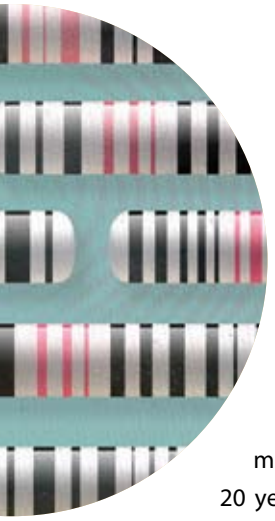
CF carrier testing is appropriate for patients with a family history of cystic fibrosis. If both prospective parents are genetic carriers it is recommended that they discuss their reproductive options with a genetic counsellor at GHSNZ. Prenatal testing for cystic fibrosis during pregnancy is available for couples who are both carriers for cystic fibrosis. Pre-implantation genetic testing may also be available to screen embryos for cystic fibrosis.

The clinical features of cystic fibrosis are malaise, failure to thrive, chronic respiratory problems, malabsorption, pale bulky stools, jaundice, pancreatic dysfunction and some males may be infertile due to a congenital absence of the vas deferens.[12] A sweat test may be considered for patients who have clinical features of cystic fibrosis, regardless of whether or not newborn screening was performed.[20]

In general, treatment of cystic fibrosis involves maintaining adequate nutrition and preventing and limiting the impact of chest infections.[12] Patients with cystic fibrosis often use bronchodilators followed by hypertonic saline solution via nebuliser.[12] Pancreatic enzyme supplements may also be used in some patients.[21] Physiotherapy is beneficial in clearing the patient's airways.[12]

Muscular dystrophy (myotonic dystrophy)

Duchenne muscular dystrophy and a rarer less severe variant, Becker muscular dystrophy, are X-linked recessive conditions and are therefore more common in males.[12] Duchenne muscular dystrophy affects approximately one in 3500 males and Becker muscular dystrophy affects approximately one in 30 000 males.[22] The mutation that causes the condition occurs in the gene coding for the protein dystrophin, which connects muscle fibres to the extracellular matrix; this is the largest gene on the X chromosome.



Both forms of muscular dystrophy are characterised by increasing weakness of proximal muscles as muscle tissue is progressively replaced by connective tissue.[12] Duchenne muscular dystrophy is generally diagnosed between the ages of two to five years and is progressive from this point.[12] Respiratory problems are the main cause of death for people with Duchenne muscular dystrophy, and this often occurs by age 20 years.[12] People with Becker muscular dystrophy have a similar, but much later and slower, onset of symptoms. A very small number of females who are carriers for muscular dystrophy will display muscle weakness.[22]

Genetic counselling should be offered to female patients of reproductive age who have a family history of muscular dystrophy; this can be arranged by GHSNZ. A focus of genetic counselling in this situation will be to determine the likelihood that the female is a carrier of a faulty dystrophin gene. The patient's family tree is used in the following way:[22]

- If a female has an affected son and an affected brother, uncle or cousin, then it is certain that she has passed on the faulty gene to her son
- If a female has an affected son, but no other affected relatives then she may be a genetic carrier, but there is also the possibility that the mutation may have occurred for the first time when the son was conceived
- A female who has two affected sons and no other relevant family history is most likely a genetic carrier

A constantly elevated serum creatine kinase (CK) level is consistent with a person being a genetic carrier for muscular dystrophy, however, approximately one third of genetic carriers do not have an elevated level.[22] Measuring serum creatine kinase levels is therefore of limited clinical value in this situation. If a female is confirmed as a carrier for muscular dystrophy then genetic counselling offers her the opportunity to discuss her personal risks and that of other family members. Pre-implantation genetic testing may also be available to screen embryos for muscular dystrophy.

Women who are genetic carriers for muscular dystrophy can experience changes in cardiac function and may benefit from referral to a cardiologist for assessment of cardiac function.[22]

There is no treatment for muscular dystrophy although corticosteroids can delay disease progression.[12]

Genetic testing is rarely useful to exclude or support a diagnosis

There are several genetic tests available to general practitioners in the Laboratory Schedule that relate to conditions with non-specific symptoms. These have limited clinical usefulness in the context of primary care; it is recommended that these tests only be requested in specific situations following consultation with GHSNZ.

Genetic testing for coeliac disease is rarely indicated

Coeliac disease is a systemic immune disease associated with gastrointestinal dysfunction and highly variable non-gastrointestinal features. The prevalence of coeliac disease in New Zealand adults is estimated to be approximately 1%.[23] There is a strong genetic component to coeliac disease with more than half of affected people having at least one other affected family member.[4] Most people who have coeliac disease have coeliac-associated antibodies and specific pairs of variations in two human leukocyte antigen (HLA) genes, HLA DQA1 and HLA DQB1.

Genetic testing for patients with suspected coeliac disease is available as a Tier 1 test under the immunology section of the Laboratory Schedule, however, the genetic section of the Schedule recommends that test requests be restricted to paediatricians, immunologists and gastroenterologists, i.e. Tier 2. This is because tissue transglutaminase antibodies (TGA) are the preferred test when investigating patients with suspected coeliac disease. Following a positive antibody test the diagnosis is generally confirmed with duodenal biopsy. In the rare situations when the results of serological tests are equivocal, and a duodenal biopsy is relatively contraindicated, e.g. in a young child, it may be appropriate to test for genetic variation, as exclusion of at-risk genotypes may mean that it is unnecessary to perform this procedure. However, as only 3% of patients with one or both of HLA DQA1 and HLA DQB1 will develop gluten intolerance,[24] the presence of these alleles is not diagnostic for coeliac disease, but their absence essentially excludes a diagnosis of coeliac disease.

Testing asymptomatic family members for genetic markers of coeliac disease is not indicated.

For further information see: "Investigating the Gut: Coeliac disease" (BT Mar, 2010).

Ankylosing spondylitis

Ankylosing spondylitis is an uncommon cause of back pain typically seen in patients aged in their mid-20s.[4] The condition

is more frequent in males and is caused by a mixture of genetic and environmental factors; most of which have yet to be confirmed.[4]

The prevalence of ankylosing spondylitis is higher in North America (0.32%) and Europe (0.24%) than in Asia (0.17%), and is lowest in Latin America (0.1%) and Africa (0.074%).[25]

The clinical features of ankylosing spondylitis as well as spinal abnormalities on radiology are used to diagnose patients with ankylosing spondylitis. The presence of HLA B27* confers susceptibility to ankylosing spondylitis, however, testing for the presence of HLA B27 has limited clinical value. This is because approximately 8 – 10% of people with Caucasian ancestry are HLA B27 positive and more than 90% of these people will never develop the condition.[26] A negative test result for HLA B27 can be useful as approximately 90% of people with ankylosing spondylitis (some estimates are as high as 98%) are HLA B27 positive and a negative result makes a diagnosis much less likely.[26]

Family testing for variations in HLA B27 is not indicated in asymptomatic family members of an affected patient.

* The products of the human leukocyte antigen (HLA) genes play an important role in the immune system by binding proteins resulting from the breakdown of self-cells or foreign pathogens and presenting them to T cells.

Further reading about genetic testing

Testing in children - American Academy of Paediatrics: **http://pediatrics.aappublications.org/content/131/3/620.full.pdf+html**

Cardiac inherited diseases – Cardiac Inherited Diseases Group: **www.cidg.org/webcontent/cidg/Home/tabid/53/Default.aspx**

Gastrointestinal Cancers – NZ Familial Gastrointestinal Cancer Registry: **www.nzfgcr.co.nz/home**

Policy on pre-symptomatic testing in children and young adults (Human Genetics Society of Australasia: **www.hgsa.org.au/documents/item/244**

Policy on pre-symptomatic and predictive testing for genetic disorders (Human Genetics Society of Australasia): **http://www.hgsa.org.au/documents/item/272**

DNA storage requirements: **www.geneticheathservice.org.nz**

Genetic Information (Centre for Genetic Information, NSW Government): **www.genetics.edu.au**

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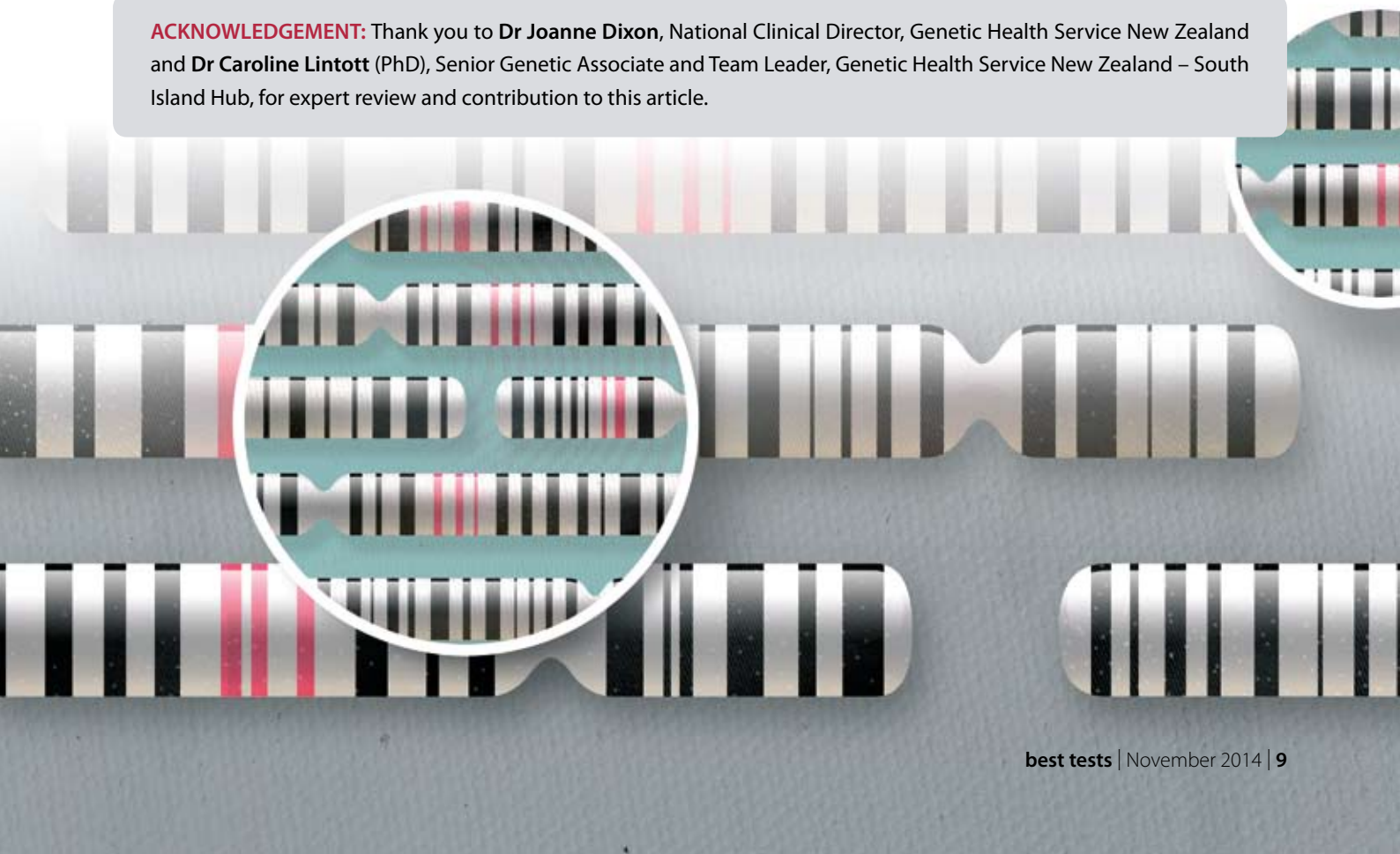

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