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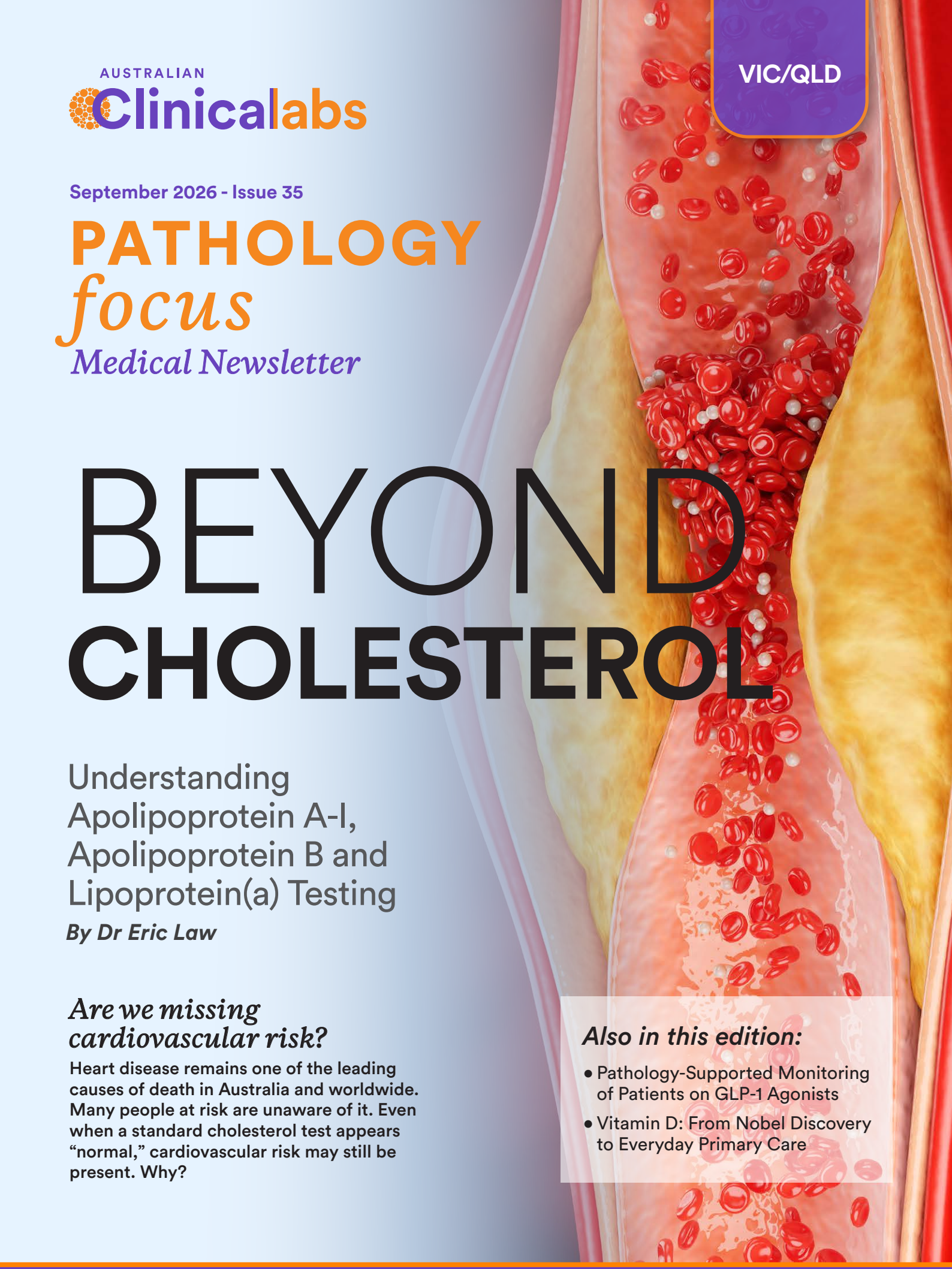

BEYOND CHOLESTEROL

Understanding Apolipoprotein A-I, Apolipoprotein B and Lipoprotein(a) Testing

By Dr Eric Law

Are we missing cardiovascular risk?

Heart disease remains one of the leading causes of death in Australia and worldwide. Many people at risk are unaware of it. Even when a standard cholesterol test appears "normal," cardiovascular risk may still be present. Why?

Also in this edition:

Cardiovascular Risk Beyond the Standard Lipid Panel

Cardiovascular disease remains one of Australia's leading causes of death, accounting for approximately one in four deaths nationally. In everyday clinical practice, risk assessment relies heavily on the standard lipid panel: total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol and triglycerides.

LDL-C, however, measures the amount of cholesterol carried within LDL particles, not the number of atherogenic particles themselves. This distinction matters. Two individuals with identical LDL-C levels may have very different numbers of LDL particles circulating in the bloodstream. Because atherosclerosis is driven by the entry and retention of lipoprotein particles within the arterial wall, particle number carries strong biological relevance; hence, apolipoprotein testing offers a practical way to explore this dimension of risk.

Apolipoprotein B: Measuring Atherogenic Particle Burden

Apolipoprotein B (ApoB) is the major structural protein of LDL and other atherogenic lipoproteins. Critically, each atherogenic particle contains exactly one ApoB molecule, so ApoB concentration directly reflects the total number of circulating atherogenic particles.

Why the Difference Matters

Consider two patients with the same LDL-C level (see Figure 1). The first has fewer, larger LDL particles, each carrying more cholesterol but with fewer opportunities to penetrate and become retained in the arterial wall. The second has many smaller LDL particles, each carrying less cholesterol. Total LDL-C is identical, yet the greater particle count increases the probability of arterial wall penetration and retention, driving plaque formation.

> *"...accumulating evidence suggests ApoB and non-HDL cholesterol may be superior to LDL-C in predicting atherosclerotic cardiovascular disease risk."*

In Scenario 1, there are fewer, larger LDL particles, each carrying a greater amount of cholesterol. Because there are fewer particles (low LDL-P), there are fewer opportunities for particles to enter and become retained within the arterial wall. In Scenario 2, there are many small LDL particles, each carrying less cholesterol. Although total LDL-C is the same, the number of circulating LDL particles (high LDL-P) is greater. This distinction underpins the clinical value of measuring ApoB, which reflects total LDL particle number rather than cholesterol content.

The Royal College of Pathologists of Australasia (RCPA) notes that ApoB serves as a measure of atherogenic lipoprotein burden, and accumulating evidence suggests that ApoB and non-HDL cholesterol may be superior to LDL-C in predicting atherosclerotic cardiovascular disease risk.

This becomes particularly relevant when LDL-C and ApoB are discordant. A patient may have an LDL-C within the target range but an elevated ApoB, indicating a higher particle burden, and cardiovascular risk appears to align more closely with ApoB in these cases.

The RCPA recommends ApoB targets of less than 1.00g/L for individuals at high cardiovascular risk and less than 0.80 g/L for those at very high risk.

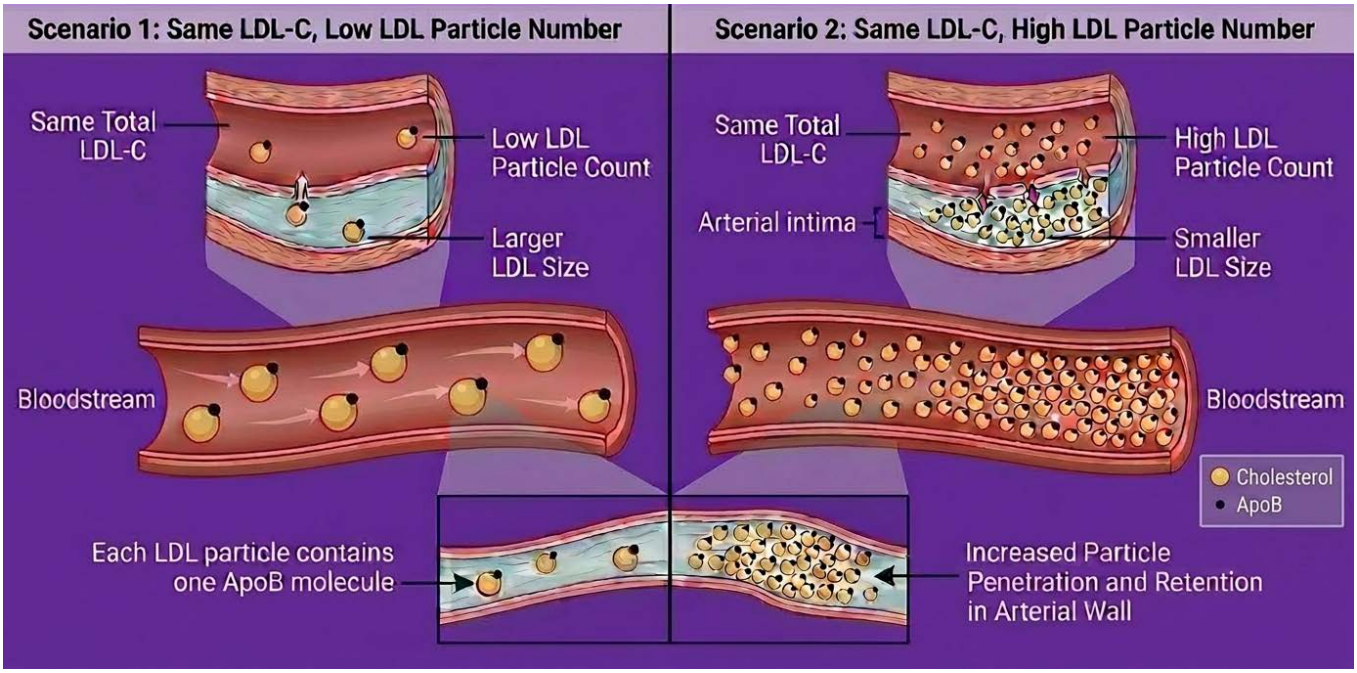

Figure 1. Schematic diagram: LDL-C vs LDL particle number (LDL-P)

Apolipoprotein A-I: The Protective Counterpart

Apolipoprotein A-I (ApoA-I) is the principal structural protein of HDL cholesterol and plays a central role in reverse cholesterol transport, facilitating the removal of cholesterol from peripheral tissues and its return to the liver for processing.

As the RCPA notes, ApoA-I and HDL levels are inversely related to atherosclerotic cardiovascular disease risk. While ApoA-I is less commonly used than ApoB in routine clinical decision-making, it represents the protective side of the lipoprotein profile. Reduced ApoA-I levels are commonly associated with an increased risk of atherosclerosis and coronary artery disease. Although measuring ApoA-I concentration offers useful information for cardiovascular risk assessment, the functional integrity of the protein is equally important. In states of chronic, significant inflammation, ApoA-I may undergo oxidative modification or become dysfunctional, diminishing its protective effects on the vascular endothelium.

HDL cholesterol (HDL-C) is well known and is commonly used in cardiovascular risk assessment, but it measures only the cholesterol content within HDL particles, not the number of particles present. Apolipoprotein A-I (ApoA-I), the main structural protein of HDL, provides a better estimate of HDL particle concentration and reflects reverse cholesterol transport capacity. As a result, two individuals with the same HDL-C may differ in HDL particle number or composition, and potentially in cardiovascular risk. ApoA-I measurement may be helpful when lipid markers are discordant, when calculating the ApoB/ApoA-I ratio, or when additional risk stratification is needed. Nonetheless, HDL-C is sufficient for routine assessment in most patients, and ApoA-I testing is generally reserved for specific clinical situations rather than used routinely.

A helpful way to understand the difference is to think of HDL cholesterol (HDL-C) as measuring the amount of cholesterol being transported, while ApoA-I reflects the main structural protein that makes up the HDL particles themselves, similar to estimating how many carriers are available to transport that cholesterol. Two people may have comparable HDL-C levels but differ in the number or composition of their HDL particles.

This distinction can matter, as HDL particle numbers may, in certain situations, provide additional information about cardiovascular risk beyond cholesterol content alone. Measuring ApoA-I can therefore offer complementary insight when refining CVD risk assessment. ApoA-I levels generally track with HDL levels, and low ApoA-I concentrations are associated with a higher risk of cardiovascular disease.

The ApoB/ApoA-I ratio captures the balance between atherogenic and protective particles. A higher ratio reflects a predominance of atherogenic lipoproteins relative to HDL-associated protective capacity and has been associated with increased cardiovascular risk in large cohort studies.

Lipoprotein(a): An Inherited and Independent Risk Factor

Lipoprotein(a), or Lp(a), is composed of an LDL-like particle attached to apolipoprotein(a), a protein encoded by the highly polymorphic LPA gene. A defining characteristic of Lp(a) is the marked variability in the size of apolipoprotein(a), driven by differences in the number of kringle IV type 2 repeat sequences within the gene. This genetic polymorphism results in more than 40 isoforms of apolipoprotein(a), leading to substantial inter-individual variation in Lp(a) particle size and circulating concentration.

Lp(a) has been identified within the arterial wall and is thought to contribute to the development of atherosclerosis. Because apolipoprotein(a) is structurally similar to plasminogen, Lp(a) may interfere with normal fibrinolysis and promote a pro-thrombotic state. Elevated circulating Lp(a) levels are associated with earlier onset of atherosclerosis and an increased risk of stroke. When high Lp(a) concentrations occur alongside elevated LDL cholesterol, the risk of coronary heart disease increases markedly, by approximately six-fold.

Lp(a) concentrations are predominantly genetically determined and generally remain stable over a person's lifetime. They are not significantly influenced by age, sex, diet, physical activity or most conventional lipid-lowering therapies. Elevated Lp(a) is recognised as an independent, inherited causal risk factor for atherosclerotic cardiovascular disease, calcific aortic valve stenosis, cardiovascular mortality and all-cause mortality.

Because of the marked isoform variability of Lp(a), measured Lp(a) levels—as well as reference intervals and clinical cut-offs—depend on the assay methodology used. Please refer to the reference ranges and commentary found on your pathology report.

Australian commentary has increasingly highlighted Lp(a) as an under-recognised contributor to cardiovascular risk. Lp(a) is described as a "mystery cholesterol" absent from routine lipid testing, meaning it may go undetected in standard assessments. The FH Australasia Network estimates that approximately one in five individuals has elevated Lp(a), and first-degree relatives of affected individuals have a 50% likelihood of sharing the trait.

When Apolipoprotein Testing May Be Informative

ApoB and Lp(a) testing are particularly valuable when the standard lipid panel does not fully account for a patient's clinical picture. Situations worth considering include:

- Premature cardiovascular disease or a strong family history of early events
- Residual risk despite LDL-C at target
- Suspected familial hypercholesterolaemia
- Discordance between LDL-C and non-HDL cholesterol

Additional testing should meaningfully refine risk stratification and support clinical decision-making, as it complements rather than replaces established risk tools (see Table 1 on following page).

Article continues over page

Table 1. Interpretation of Apolipoprotein and Lipoprotein(a) Results

Marker	Abnormal Finding	Potential Clinical Implication
ApoB	High	Increased atherogenic particle burden
ApoA-I	Low	Reduced protective lipoprotein capacity
ApoB/ApoA-I	High	Higher net atherogenic balance
Lp(a)	High	Increased cardiovascular risk

Clinical Implications

Management decisions should remain individualised and guided by current clinical guidelines. In general terms:

- Elevated ApoB supports optimisation of LDL-lowering strategies to reduce total atherogenic particle burden.
- Elevated Lp(a) underscores the importance of aggressively addressing all modifiable risk factors, including LDL-C, blood pressure, glycaemic control and smoking, given the absence of approved Lp(a)-specific therapies in routine practice.

Standard lipid testing remains fundamental to cardiovascular prevention. Apolipoprotein testing extends this foundation by shifting the focus from cholesterol quantity to particle number, particle balance and inherited risk. Interpreted thoughtfully and in clinical context, ApoB, ApoA-I and Lp(a) can meaningfully refine risk assessment and support more individualised prevention strategies.

Conclusion

Cardiovascular risk assessment in Australia continues to rely primarily on the standard lipid panel. While LDL-C remains central to prevention strategies, it reflects cholesterol mass rather than the number of circulating atherogenic particles. Increasing evidence and contemporary expert consensus highlight that particle number, reflected by ApoB, may more closely align with atherosclerotic risk, particularly when traditional markers appear discordant.

ApoA-I provides insight into the protective component of the lipid profile, and the ApoB/ApoA-I ratio captures the balance between atherogenic and anti-atherogenic forces. Lipoprotein(a), meanwhile, represents a distinct and inherited risk factor that is not detected on routine lipid testing.

Together, these markers deepen our understanding of lipid biology and help explain why some patients experience cardiovascular events despite “acceptable” LDL-C levels, while others with similar cholesterol values remain event-free. Importantly, apolipoprotein testing is not intended to replace absolute cardiovascular risk assessment tools, but to refine them when clinical uncertainty, discordance or inherited risk is suspected.

KEY MESSAGES

- ✓ LDL-C measures cholesterol content, not particle number. ApoB provides a direct estimate of total atherogenic particle burden.
- ✓ Discordance matters. When LDL-C and ApoB differ, cardiovascular risk appears to align more closely with ApoB.
- ✓ ApoA-I reflects protective HDL biology, and the ApoB/ApoA-I ratio captures overall lipoprotein balance.
- ✓ Lp(a) is an inherited, independent cardiovascular risk factor affecting approximately one in five individuals and is generally stable over a lifetime.
- ✓ A single Lp(a) measurement is usually sufficient, particularly in those with premature cardiovascular disease or strong family history.
- ✓ Management remains focused on aggressive control of modifiable risk factors, especially LDL-C, blood pressure, smoking, and glycaemic status, given the current absence of widely available Lp(a)-specific therapies.

In clinical practice, apolipoprotein testing should be used thoughtfully; not routinely for all patients, but strategically when it adds clarity. Moving beyond cholesterol quantity to particle number and inherited risk enables a more nuanced and individualised approach to cardiovascular prevention.

How to Order Apolipoprotein Testing with Clinical Labs

Request form instructions:
Complete the Clinical Labs general pathology request form.

What to request:

- Apolipoprotein A-I or apoA-1
- Apolipoprotein B or apoB
- Lipoprotein(a) or Lp(a)

Note: If both apoA-1 and apoB are requested, the apoA-1/apoB ratio will be provided.

Clinical notes recommendation:
Include cardiovascular risk and family history, if known.

Specimen required:
SST (serum)

Test cost:
There are no MBS item numbers available for these tests. Current out-of-pocket pricing and payment information is available at pay.clinicallabs.com.au. Note that ACL offers discounted pricing when ApoA1 and ApoB are requested together.

Supplementary tests:
Lipid Profile+HDL

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About the author:

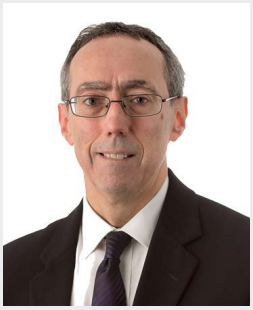
Dr Eric Law

MBChB BMedSc (1st Hon) PhD FHKAM (Pathology) FHKCPath FRCPA FRCPath FAACB IFCAP

Lab: Bella Vista (NSW)
Speciality: Chemical Pathology
Areas of Interest: Chemical Pathology, Biochemical Genetics, Inherited Metabolic Disorders
Phone: 1300 134 111
Email: eric.law@clinicallabs.com.au

Dr Law completed his medical science degree (first honor) and MB ChB at the Chinese University of Hong Kong and obtained his PhD from the University of Hong Kong. He is a Fellow of the Royal College of Pathologists of Australasia (FRCPA), with scope of practice in both Chemical Pathology and Genetic Pathology. Additionally, he is a Fellow of the Australian Association of Clinical Biochemists (FAACB), the Royal College of Pathologists (FRCPath), an International Fellow of College of American Pathologists (IFCAP), Hong Kong College of Pathologists (FHKCPath), Hong Kong Academy of Medicine (FHKAM(Pathology)) and the Faulty of Science (Research), RCPA. His areas of interest include chemical pathology, inherited metabolic disorders, clinical metabolomics and medical teaching.

Local pathologist:



Dr David Deam

MBBS MAACB FRCPA

Lab: Clayton (Vic)
Speciality: Chemical Pathology
Areas of Interest: Endocrine function testing, protein abnormalities, laboratory automation
Phone: (03) 9538 6777
Email: david.deam@clinicallabs.com.au

Dr Deam graduated with Honours in Medicine from Monash University in 1978 and obtained his FRCPA in 1985, following postgraduate training in Biochemistry at the Royal Melbourne Hospital. After several posts in Chemical Pathology at the Royal Melbourne Hospital and the Royal Women's Hospital, he was appointed Head of Chemical Pathology at the Royal Melbourne in 1996. He joined Gribbles Pathology (now Australian Clinical Labs) in 1998. Dr Deam has played an active role in teaching scientific, nursing and medical staff at both undergraduate and postgraduate levels and has been an examiner for the Australasian Association of Clinical Biochemists as well as the Royal College of Pathologists of Australasia. Dr Deam's research interests and publications include work on thyroid function testing, various aspects of diagnostic protein measurement and the rational use of biochemical tests.

Pathology-Supported Monitoring of Patients on GLP-1 Agonists:

A Practical Guide for General Practitioners

By Associate Professor Chris Barnes

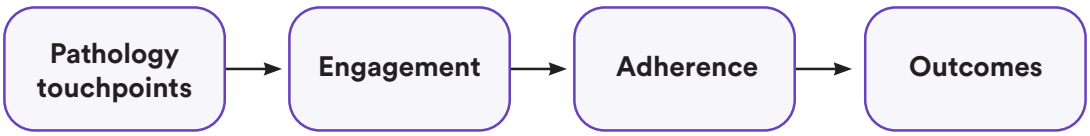
GLP-1 receptor agonists represent a major advance in the management of obesity and type 2 diabetes, delivering significant weight loss, improved glycaemic control and cardiovascular benefit.[1,2]

As use expands into primary care, general practitioners (GPs) are central to initiating and monitoring therapy. However, these benefits are accompanied by important considerations, including reduced nutritional intake, gastrointestinal side effects, and rare complications such as pancreatitis and gallbladder disease.[3,4]

In parallel, there is increasing use of unregulated GLP-1 products, often sourced online or marketed as "GLP-1 peptides," which may be ineffective or unsafe. This further reinforces the role of GPs in guiding evidence-based care.

Pathology testing is therefore critical, not only for safety monitoring but also as a structured tool to support patient engagement and optimise outcomes. Emerging real-world evidence highlights that adherence to GLP-1 therapy is highly variable, with discontinuation commonly driven by cost, side effects and inadequate support structures.[5]

GLP-1 Pathway



The Role of the General Practitioner

GPs provide longitudinal care and are best placed to monitor effectiveness and tolerability, detect complications early, identify nutritional deficiencies, and counsel patients on safe, regulated therapies.

Importantly, pathology testing can be used as an engagement tool, helping to reinforce adherence, provide objective feedback on progress, support shared decision-making and identify early risks and intervene when necessary. This positions pathology as both a clinical and behavioural tool, supporting improved outcomes.

Recommended Laboratory Investigations

Baseline Assessment

Prior to initiation, establish baseline metabolic and nutritional status. Recommended baseline tests:

Baseline investigations		
• HbA1c / fasting glucose	• Thyroid function (TSH ± fT4)	• Lipid profile
• Renal function (U&E, eGFR)	• Iron studies	• Vitamin D / calcium
• Liver function tests	• Vitamin B12 / folate	

Nutritional Monitoring During Weight Loss

Reduced intake increases the risk of micronutrient deficiency, particularly with rapid weight loss (>5–10%). Recommended monitoring:

Early Phase Monitoring (rapid weight loss):	Maintenance Phase (6-12 monthly):	Earlier testing if symptomatic:
• FBC • Iron studies • B12 / folate • Vitamin D / calcium	• Repeat "Early Phase Monitoring" ± albumin	• Fatigue • Hair loss • Dizziness

Monitoring for Pancreatitis

Pancreatitis is rare but important.[4] Test if the symptoms below are present. If lipase is elevated, stop therapy and manage urgently.

Symptoms	Pathology Monitoring
• Persistent epigastric pain • Radiating back pain • Nausea/vomiting	• Serum lipase (preferred) • Serum amylase

Gallbladder and Hepatobiliary Monitoring

Rapid weight loss increases gallstone risk.[3] Investigate for the following symptoms:

Symptoms	Pathology Monitoring
• RUQ pain • Post-prandial symptoms • Abnormal LFTs	• LFTs (ALP, GGT) • Ultrasound if indicated

Integrating Pathology into Practice

A structured approach is beneficial for patient investigations, consisting of baseline testing, maintenance monitoring (6-12 months) and symptom-triggered testing. Pathology can also be used proactively to track progress, reinforce adherence, detect early complications and reduce treatment-related risk. This enables pathology to support health benefits while minimising adverse outcomes.

Conclusion

GLP-1 agonists are transforming obesity care, with GPs at the centre of safe implementation. This role is increasingly important given the rise of unregulated GLP-1 products. Pathology testing underpins effective care by enabling baseline risk assessment, early and ongoing nutritional monitoring, detection of complications, and patient engagement and adherence. A structured, evidence-informed approach allows GPs to maximise benefit while minimising harm.

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About the author:

Assoc. Prof. Chris Barnes

MBBS FRACP FRCPA

Lab: Clayton (Vic)
Speciality: Haematology
Areas of Interest: Paediatric haematology, non-malignant haematological conditions including thrombosis and bleeding disorders
Phone: (03) 9538 6777
Email: chris.barnes@clinicallabs.com.au

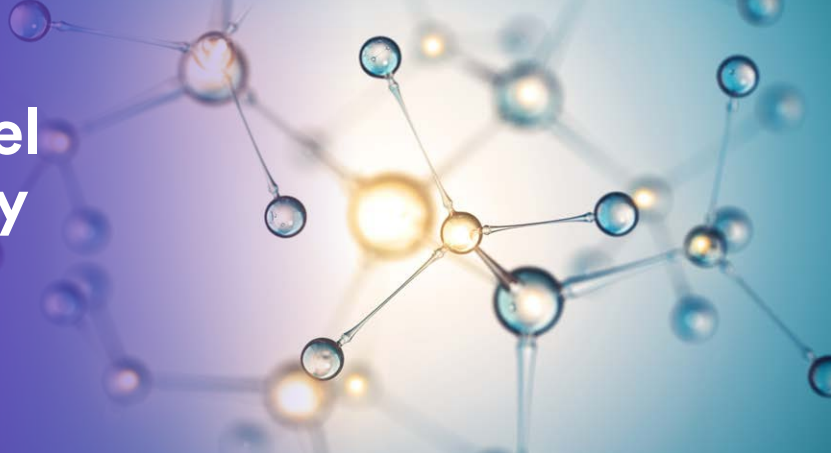
Associate Professor Chris Barnes is the National Director of Haematology and provides strategic direction nationally for haematology at Clinical Labs. He is a clinical and laboratory-trained haematologist who has been part of Melbourne Haematology and has worked with Clinical Labs (and previously Healthscope) for several years. A/Prof Barnes also works at the Royal Children's Hospital and is director of the Haemophilia Treatment Centre. He has experience in both management and leadership positions. A/Prof Barnes has an active clinical research interest and is also director of Melbourne Haematology (Clinical) and Melbourne Paediatric Specialists.

Available as Single-Click Test Profiles within Best Practice, MedicalDirector Clinical, Genie, Gentu and eResults!

Search for the following test profiles in Clinical Recommendations/ Clinical Details or the main ACL eOrdering test list:

- **GLP-1 Monitoring (Baseline)**
- **GLP-1 Monitoring (Early Phase Nutritional)**
- **GLP-1 Monitoring (Nutritional Maintenance)**
- **GLP-1 Monitoring (Pancreatitis)**
- **GLP-1 Monitoring (Gallbladder & Hepatobiliary)**

Vitamin D: From Nobel Discovery to Everyday Primary Care



By Dr Eric Law

Abstract

Vitamin D occupies a distinctive position at the intersection of nutrition, photobiology and endocrinology. First identified in the early twentieth century as the antirachitic factor responsible for preventing rickets, it is now recognised as a secosteroid hormone exerting genomic effects through a nuclear receptor. Its scientific journey spans industrial public health crises, sterol chemistry, ultraviolet photochemistry, molecular endocrinology and more. For many of us, Vitamin D remains relevant in skeletal health, falls prevention and selected high-risk populations. Revisiting its discovery and biochemical evolution illustrates how a subtle ultraviolet-induced modification of a cholesterol derivative continues to influence everyday clinical decision-making.

Introduction: A Disease of Industrialisation

Long before Vitamin D was recognised as a vitamin, its precursor molecules were absorbing ultraviolet radiation in primitive eukaryotic organisms inhabiting the ancient oceans. Ergosterol, a Vitamin D_2 precursor and fungal membrane sterol present in early phytoplankton, underwent photochemical transformation when exposed to sunlight, an evolutionary adaptation that may have provided protection against ultraviolet damage. Hundreds of millions of years later, a closely related photochemical principle would prove central to understanding one of the most devastating diseases of industrial childhood.

At the turn of the twentieth century, rickets was endemic in industrialised cities across Europe and North America. Rapid urbanisation had drawn families into densely populated, smoke-laden environments where narrow streets and coal-derived smog obscured direct sunlight. In some industrial centres, autopsy studies suggested that as many as 80–90% of children exhibited evidence of rickets. The skeletal manifestations were striking: bowed legs, widened wrists, rib deformities, growth retardation and profound muscular weakness. Pelvic deformities in affected girls later contributed to life-threatening complications in childbirth. The link between industrialisation and skeletal deformity was unmistakable, yet the biological mechanism remained mysterious.

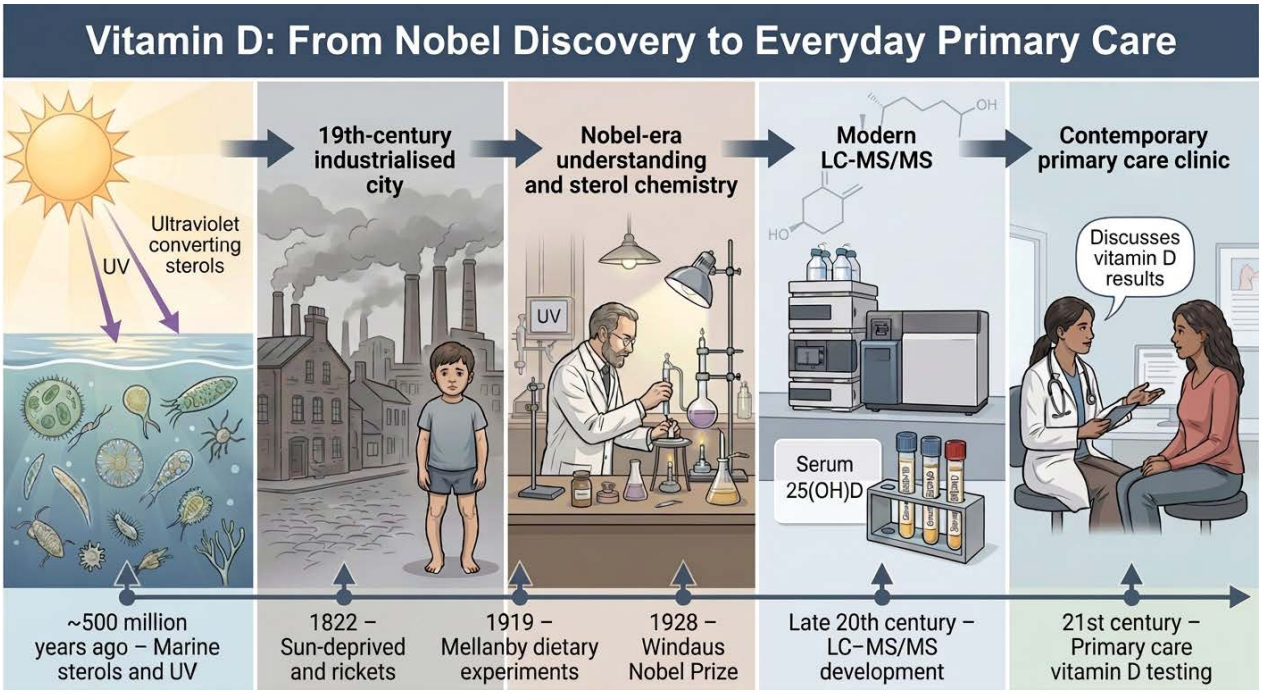
Clinical observation preceded biochemical explanation. In 1822, the Polish physician and chemist Jędrzej Śniadecki noted that children in sun-deprived Warsaw developed rickets, whereas rural children exposed to sunlight did not. Later, in 1890, Theobald A. Palm recognised the geographic relationship between limited sunlight and the high incidence of rickets in urban Britain. Collectively, these observations laid the groundwork for the discovery of the "sunshine vitamin". Meanwhile, fishing communities had long administered cod liver oil to prevent the disease, which was later recognised as a rich source of Vitamin D. In 1919, Sir Edward Mellanby demonstrated experimentally that rickets could be induced in porridge-fed, caged dogs through dietary restriction and reversed with cod liver oil, implicating a nutritional deficiency. Professor Elmer McCollum, later referred to by *TIME* magazine as "Dr Vitamin" for his discovery of Vitamin A in 1913 and Vitamin B in 1915, subsequently established that the antirachitic factor was a new and distinct vitamin, coined Vitamin D in 1922.

Ultraviolet radiation soon emerged as a critical component in elucidating the pathogenesis and treatment of rickets. Dr Kurt Huldschinsky demonstrated that exposure of affected children to mercury arc lamps resulted in radiographic mineralisation of long bones, thereby providing compelling evidence that ultraviolet irradiation induced a cutaneous photochemical reaction generating a circulating antirachitic factor. The subsequent structural elucidation of this factor was advanced through developments in sterol chemistry. Cholesterol, initially identified in human gallstones, was subsequently and unexpectedly recognised as central to the biochemical framework of Vitamin D. Dr Adolf Windaus subsequently demonstrated that ultraviolet irradiation of a cholesterol precursor yielded the antirachitic compound, thereby establishing Vitamin D as a sterol-derived molecule activated by photochemical transformation. In recognition of his work elucidating the relationships among sterols, vitamins and bile acids, and determining their structures, Dr Windaus was awarded the Nobel Prize in Chemistry in 1928.

The Sterol Backbone: Structure Determines Function

What began as an investigation into a crippling disease of industrial childhood ultimately revealed a profound biological principle: sunlight can convert a membrane sterol into an endocrine signal. From ancient marine photochemistry to the smoke-darkened streets of industrial Europe, Vitamin D emerged as a molecule that links environment, molecular structure and hormonal regulation in a single elegant pathway. To understand Vitamin D further, one must begin with sterols.

Sterols are a subgroup of steroids and a class of lipids characterised by a four-ring carbon core, a hydroxyl (–OH) group at the C3 position and typically a side chain at



C17. All steroids share a common tetracyclic framework of four fused carbon rings (A, B, C and D), known as the cyclopentanoperhydrophenanthrene nucleus, the classical steroid backbone. Rings A, B and C are six-membered, while ring D is five-membered. This four-ring system is believed to enhance chemical stability and optimise function. Cholesterol is a sterol, whereas cortisol, estradiol, testosterone and aldosterone are steroid hormones derived from the same structural framework.

Vitamin D (D3) originates from 7-dehydrocholesterol, a cholesterol derivative synthesised in the skin. When exposed to ultraviolet B radiation (approximately 290–315 nm wavelength), a photochemical reaction cleaves the bond between carbon atoms 9 and 10 in the B ring. This reaction opens the B ring. The resulting molecule is not a classical steroid but a secosteroid (from the Latin *secare*, "to cut"). The A, C and D rings remain intact, but the opened B ring transforms a rigid membrane sterol into a conformationally flexible signalling molecule capable of endocrine activity. A single ultraviolet-induced structural modification thus generates profound physiological consequences.

From Photochemical Product to Active Hormone: Metabolism, Molecular Forms and Measurement

Vitamin D_3 (cholecalciferol), synthesised in the skin, is biologically inert. It functions as a prohormone and must undergo sequential hydroxylation to become hormonally active.

The first hydroxylation occurs in the liver, mediated primarily by Vitamin D 25-hydroxylase (CYP2R1), producing 25-hydroxyvitamin D [25(OH)D]. This metabolite represents the principal circulating form and the usual laboratory marker used to assess Vitamin D status in clinical practice. Its half-life of approximately 2-3 weeks reflects its role as a systemic reservoir rather than an active hormone.

The second hydroxylation occurs predominantly in the kidney, where 25(OH)D is converted by 1α-hydroxylase (CYP27B1) into 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (calcitriol), the active hormone with a half-life of around 4-6 hours. This step is tightly regulated by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), serum calcium and phosphate concentrations, thereby integrating Vitamin D metabolism into the classical endocrine feedback mechanisms that maintain mineral homeostasis.

Extrarenal expression of CYP27B1 in immune cells, the placenta and other tissues enables local (paracrine or autocrine) production of calcitriol, adding further physiological complexity. Catabolism occurs via 24-hydroxylase (CYP24A1), which inactivates both 25(OH)D and calcitriol. Pathogenic variants in CYP24A1 impair degradation and may lead to hypercalcaemia and hypercalciuria, a clinically relevant genetic disorder of Vitamin D metabolism (also known as infantile hypercalcemia type 1, an autosomal recessive condition). Understandably, detailed exploration of genetic bone diseases and inherited Vitamin D disorders would take us well beyond the intended scope of this article.

The link between sunlight, Vitamin D and bone health represents a distinctive physiological adaptation. Unlike other vitamins obtained primarily through diet, Vitamin D can be synthesised in the skin from a cholesterol precursor following ultraviolet B exposure and subsequently acts as a hormone regulating gene transcription. This light-dependent mechanism provides an environmental cue that supports calcium absorption and skeletal mineralisation, processes essential for maintaining structural integrity in terrestrial life. The uniqueness of this photochemical–endocrine pathway explains why Vitamin D, unlike other vitamins, plays such a central and irreplaceable role in bone health.

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Molecular Diversity: D_2, D_3 and Epimers

Vitamin D exists in multiple biochemical forms reflecting its metabolic progression. These include **Vitamin D_3 (cholecalciferol),** derived from animal sources or by conversion of 7-dehydrocholesterol in the skin, and **Vitamin D_2 (ergocalciferol)**, derived from plant and fungal sources.

Both forms undergo identical hepatic and renal activation pathways. Although structurally similar, D_2 and D_3 differ slightly in their side-chain configuration (D2 includes an additional double bond between C22 and C23 and a methyl group at C24), which may influence pharmacokinetics.

Metabolism also produces epimeric variants, most notably **C3 epimers** such as **3-epi-25(OH)D**. Epimers are stereoisomers differing in configuration at a single chiral carbon—that is, carbon 3 of the A ring, where the usual hydroxyl group is flipped "downward" instead of "upward". While structurally similar to 25(OH)D, the subtle but biologically significant spatial orientation of the hydroxyl group can reduce receptor affinity (e.g. the geometry of the hydrogen bond) and biological potency. Of note, **C3 epimers** can be found in neonates and infants; in adults, their clinical significance appears limited but analytically relevant.

Analytical Considerations

Clinical assessment measures **serum 25(OH)D** (unit: nmol/L), not 1,25-dihydroxyvitamin D (for which concentrations are typically measured in pg/mL rather than ng/mL). This distinction is crucial: 25(OH)D reflects cumulative Vitamin D stores, whereas $1,25(OH)_2D$ is tightly regulated and often normal even in deficiency.

Most routine immunoassays measure total 25(OH)D but may not reliably distinguish between D_2, D_3 and epimers. Cross-reactivity can modestly influence results, particularly in paediatric populations. Liquid chromatography–tandem mass spectrometry (LC–MS/MS), when appropriately configured, can differentiate $25(OH)D_2$, $25(OH)D_3$ and epimers with greater specificity.

In addition, routine immunoassays may not fully distinguish the C3-epimer from standard 25(OH)D. In most healthy adults, this analytical limitation has minimal clinical impact. However, it may become relevant in neonatal assessment, rare disorders of Vitamin D metabolism, research settings or when laboratory results appear discordant with the clinical picture. Where clinically indicated, specialised LC-MS/MS testing with separation of individual Vitamin D species can be arranged. Please discuss this with a pathologist or relevant specialist if further clarification is required.

Genomic Signaling: The Vitamin D Receptor

Calcitriol exerts its biological effects by binding to the Vitamin D receptor (VDR), a ligand-activated transcription factor belonging to the nuclear receptor superfamily, which also includes receptors for glucocorticoids, mineralocorticoids, oestrogens, progesterone and thyroid hormone. In brief, nuclear receptors are specialised proteins inside cells that bind to small molecules such as hormones and act as transcription factors to "switch on" or "switch off" the genes.

> *"The uniqueness of this photochemical–endocrine pathway explains why Vitamin D, unlike other vitamins, plays such a central and irreplaceable role in bone health."*

Beyond the classical role in calcium and bone homeostasis, 1,25-dihydroxyvitamin D regulates the expression of numerous genes across a wide range of tissues, including immune cells, skeletal muscle, the vasculature and reproductive organs. Upon ligand binding, the VDR forms a heterodimer with the retinoid X receptor (RXR) and interacts with Vitamin D response elements (VDREs) in the promoter regions of target genes. This complex recruits coactivator or corepressor proteins, leading to chromatin remodelling and regulation of gene transcription. At the time of writing, the exact number of genes targeted or influenced by Vitamin D remains unknown. Estimates suggest it may be in the hundreds to over a thousand. Some examples include:

- Intestinal calcium and phosphate absorption
- Bone remodelling and mineralisation
- Suppression of parathyroid hormone
- Renal tubular calcium handling
- Cellular proliferation and differentiation

Vitamin D Testing in Practice: From Research to Clinical Laboratory

Given the central role of Vitamin D in calcium homeostasis, bone metabolism and its broader effects on immune, muscular and cardiovascular function, disturbances in Vitamin D status can have important clinical consequences. Both deficiency and excess may impact skeletal health and contribute to a range of systemic conditions. Therefore, modern guidance recommends selective, risk-based testing rather than routine population screening for Vitamin D status. Measurement of serum 25-hydroxyvitamin D [25(OH)D] is considered appropriate in individuals with specific risk factors or clinical indications. More information is available in standard guidelines and professional position statements.

A serum 25(OH)D threshold of 50 nmol/L is commonly used to define adequacy for bone health, particularly at the end of winter, in recognition of seasonal variation. According to the Royal College of Pathologists of Australasia (RCPA; *Vitamin D – Interpretation of Results*, accessed 22 July 2026), the following categories apply:

- **Vitamin D adequacy**: ≥50 nmol/L at the end of winter (levels may need to be 10-20 nmol/L higher at the end of summer to account for seasonal decline)
- **Mild deficiency**: 30-49 nmol/L
- **Moderate deficiency**: 12.5-29 nmol/L
- **Severe deficiency**: <12.5 nmol/L

Vitamin D toxicity is characterised by hypercalcaemia, hypercalciuria and nephrocalcinosis. Hypercalcaemia is uncommon until serum 25(OH)D concentrations exceed approximately 220 nmol/L and is more typically reported at levels above 500 nmol/L.

If laboratory results are discordant with the clinical presentation, or fail to respond appropriately to supplementation, measurement by LC-MS/MS may be considered for more specific assessment.

Conclusion: A Molecule Illuminated by Sunlight

Vitamin D reminds us that even the most familiar terms in everyday conversation may reflect intricate and elegant physiology, and that much of modern clinical practice remains grounded in chemistry, quite literally illuminated by light. Nevertheless, in routine practice, this remarkable cascade of photochemistry and endocrine regulation is ultimately distilled into a single numerical value (nmol/L) on an otherwise unremarkable laboratory report.

The modest figure, while clinically practical, signifies far more than a threshold to correct or a target to attain. It represents a finely regulated biological system shaped by evolution, environment and human behaviour. To recall the science behind the number is to restore perspective: even the most routine test result reflects molecular precision, physiological balance and the enduring interplay between sunlight and human biology.

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How to Order Vitamin D Testing with Clinical Labs

Request Form Instructions:

- Note the reason for testing to meet Medicare eligibility criteria for bulk-billing in the 'Clinical Notes' section of a Clinical Labs General Pathology Request Form.
- Blood samples can be collected at any Clinical Labs collection centre.

Repeat Testing:

- Due to the long half-life, repeat testing should not be performed earlier than 3 months after commencing Vitamin D supplementation or changing the dose.

Additional Tests:

- Serum calcium, phosphate and parathyroid hormone levels may assist in interpreting the Vitamin D result within the context of overall calcium homeostasis.
- If osteoporosis is present, assessment of bone turnover markers (fasting C-terminal telopeptide of type 1 collagen [CTX] and procollagen type 1 N-propeptide [P1NP]) may be considered to monitor bone turnover in response to therapy.

About the author:

Dr Eric Law

MBChB BMedSc (1st Hon) PhD FHKAM (Pathology) FHKCPath FRCPA FRCPath FAACB IFCAP

Lab: Bella Vista (NSW)
Speciality: Chemical Pathology
Areas of Interest: Chemical Pathology, Biochemical Genetics, Inherited Metabolic Disorders
Phone: 1300 134 111
Email: eric.law@clinicallabs.com.au

Local pathologist:

Dr David Deam

MBBS MAACB FRCPA

Lab: Clayton (VIC)
Speciality: Chemical Pathology
Areas of Interest: Endocrine function testing, protein abnormalities, laboratory automation
Phone: (03) 9538 6777
Email: david.deam@clinicallabs.com.au

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