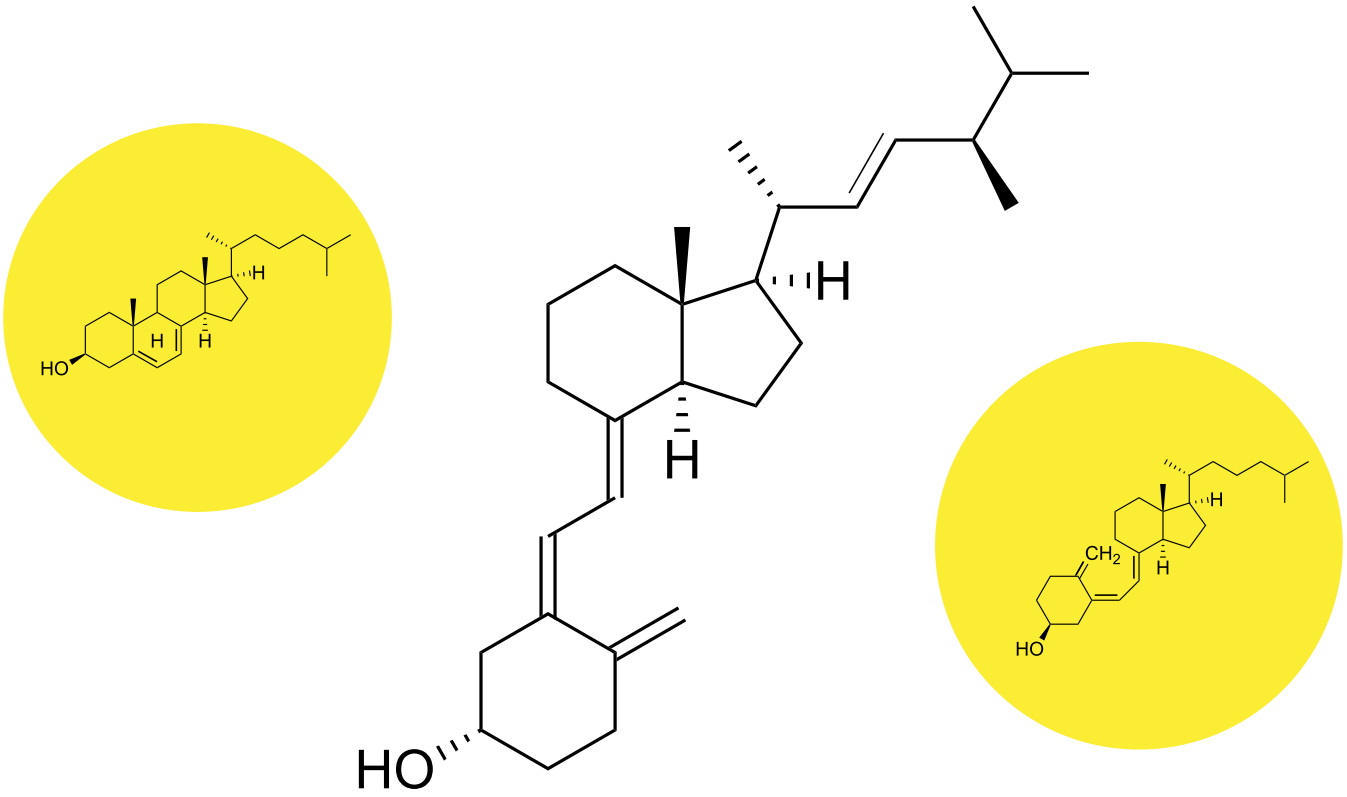

Now is the Time for

Higher Quality Vitamin D Testing

ThermoFisher SCIENTIFIC

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LC-MS/MS:
The Gold Standard Technology in Vitamin D Testing

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is rapidly becoming a gold standard technology for selected clinical laboratory testing. This analytical technique offers advantages in accuracy over many traditional immunoassays and considerable potential multiplexing of a broad range of analytes. LC-MS/MS is already established as the reference method for the Vitamin D Standardization-Certification Program (VDSCP) and is finding favor in a number of clinical specialties such as endocrinology, therapeutic drug monitoring and drugs of abuse testing. LC-MS/MS excels at small molecule, peptide and protein marker identification.

However, despite its excellent specificity, few clinical diagnostic labs are able to use LC-MS/MS testing. This is mostly due to its complexity, need for high-level user expertise and lack of standardization across widely varying lab-defined instrument and test method setups.

Advantages of using LC-MS/MS to measure 25(OH)D [25-hydroxy vitamin D] are that it can reduce or eliminate the specificity and interference issues that are observed with immunoassay measurement, the reagent cost is minimal, and multiple analytes can be measured in one method. Some challenges of LC-MS/MS are the complexity of this instrumentation and, in the majority of cases, the methods have to be developed and fully validated by the laboratory as laboratory-developed tests.[1]

Developed in close collaboration with mass spectrometry experts and vitamin D researchers, the Thermo Scientific™ Cascadion™ SM 25-Hydroxy Vitamin D Assay brings LC-MS/MS testing to routine clinical laboratories. In conjunction with the easy-to-use Thermo Scientific™ Cascadion™ SM Clinical Analyzer, Thermo Fisher Scientific delivers a turnkey assay with off-the-shelf convenience for both expert users and laboratories without LC-MS/MS experience. It is easy to implement versus laboratory-developed tests (LDTs) and offers faster turnaround times in comparison to batch workflows. The fully supported single-vendor commercial platform combines total LC-MS/MS integration and automation with an intuitive graphical user interface and bi-directional Laboratory Information System connectivity to maximize uptime, traceability and standardization for vitamin D sufficiency testing.

Vitamin D in Health and Disease

Vitamin D chemistry

Vitamin D is a steroid prohormone, not a vitamin. As dietary sources are very limited, the largest source of vitamin D is from the action of sunlight on the skin. Ultraviolet radiation turns sterols already present in the skin into precursors for vitamin D, and liver enzymes then convert these precursors into the storage or active forms of vitamin D.[2]

Most vitamin D is found in its storage forms, 25-OH vitamin D_2 and D_3, at levels up to 1,000-fold higher and with a longer half-life than the active form, 1,25-$(OH)_2$-vitamin D_2.[2] This makes total 25-OH vitamin D (D_2 and D_3 combined) the analyte of choice for measuring vitamin D sufficiency. Vitamin D_2 (ergocalciferol) is found in plant sources and supplements, and is the major form of vitamin D prescribed in the US.[1,2]

Vitamin D is essential in maintaining bone health and calcium balance. Low levels cause impaired bone mineralization leading to osteomalacia and osteopenia. Such loss of mineralization can result in fractures and bone loss, and in children, the characteristic skeletal deformities known as rickets.[3] Chronic deficiency of vitamin D can also result in muscle weakness and twitching, lightheadedness and other signs of nervous system disruption (related to the loss of ionic calcium in body fluids).[4] However, a vast amount of emerging data, albeit primarily observational, are highly supportive of a wider role for vitamin D in overall health and disease management. Not only is the receptor found in the cell nucleus of many different tissue types, but vitamin D directly or indirectly affects around 3% of the human genome.[4] Vitamin D insufficiency may be associated with chronic conditions such as cancer, autoimmune disease, cardiovascular disease and diabetes.[4] Research into the roles and significance of vitamin D has exploded in recent years, with more than 78,000 papers on this topic now listed in PubMed, and vitamin D researchers now have their own academic conferences.

High-risk patient populations for deficiency or insufficiency

Regular vitamin D screening and supplementation are critical for members of a variety of subpopulations. Diseases such as chronic kidney disease, irritable bowel disease, cystic fibrosis, hyperparathyroidism and liver failure can impact the production, absorption and distribution of vitamin D, leading to symptoms of insufficiency in addition to the normal effects of these diseases. Similarly, people with osteoporosis and other illnesses related to calcium and vitamin D absorption require regular monitoring and supplementation as part of their treatment. Many medications, including commonly prescribed drugs such as glucocorticoids and antifungals, also affect vitamin D metabolism. Pregnancy comes with increased need for vitamin D in the service of fetal health. Perhaps most prevalent, melanin prevents ultraviolet light from turning other sterols into vitamin D precursors just as it protects against skin cancer, putting certain ethnic populations at higher risk of insufficiency.[3] The Endocrine Society maintains a detailed series of supplementation guidelines for certain at-risk populations and recommends regular screening to ensure that their vitamin D levels remain in range. These people rely on accurate vitamin D assays, a solid foundational knowledge of insufficiency cut-offs, and proper classification for the management of their health.

These are not insignificant populations, especially in aggregate. For example, focusing on just a few of these:

- 44 million people in the US alone have osteoporosis. Across the US, Europe and Japan, there are a total of 75 million people.[5]
- The prevalence of chronic kidney disease is about 14% in the general adult population; there are about 661,000 people in the US with kidney failure.[6]
- About 4.9 million people in the US have diagnosed liver disease[7] and 29 million people in the European Union have chronic liver diseases.[8]

Indications for 25(OH)D measurement (candidates for screening) from the Endocrine Society Clinical Practice Guidelines[3]

Physical	Conditions	Disease states	Medications	Granuloma-forming disorders
Pregnant and lactating women	Rickets (in children)	Hepatic failure	Antifungals	Sarcoidosis
Older adults with a history of falls	Osteomalacia	Chronic kidney disease	Antiseizure medication	Tuberculosis
Older adults with a history of non-traumatic fractures	Osteoporosis	Some lymphomas	Glucocorticoids	Histoplasmosis
Individuals with darker skin		Crohn's disease	Anti HIV	Coccidioidomycosis
Obesity			Cholestyramine	Berylliosis

- Various malabsorption syndromes afflict more than 7 million people in the US. In the European Union about 2.5 to 3 million people live with just one of those conditions: irritable bowel disease.[9]
- Roughly 3.4 million people in the US[10] and about 6 million in Europe[11] have epilepsy, and many of them are being treated with anti-seizure medications.

Testing patients for 25-hydroxyvitamin D has become more common in the past 10 years, but the methods used to measure this analyte have specificity and standardization issues. These issues have implications for clinicians in interpreting the results in the context of the currently available guidelines that use cut-off concentrations for determining a patient's vitamin D status.[1]

Recommendations for screening, clinical cut-offs and supplementation

Unfortunately, there is limited consensus as to what constitutes vitamin D insufficiency or deficiency. The recommended cut-offs vary between professional societies and geographic locations. Below is a table showing the variation in the current vitamin D cut-off levels recommended by various professional societies and national healthcare systems:

Status (ng/mL)	ANZBMS[a] ESAOA[b] (2013)	SFCN[c] (2012)	IOF[d] (2010)	Canadian Medical Assoc. Osteoporosis[e] (2010)	IOM[f] (2011)	GRIO[g] (2018)	US Endocrine Society[h] (2011)
Deficiency	<5	<10	NA	<25	<12	<10	<20
Insufficiency	5–10 10–20	<10–20	NA	25–75	12–20	11–30	21–29
Sufficiency	>20	>20	>28-32	NA	>20	>30	30–100
Toxicity Risk	NA	NA	NA	>100	>50	NA	>100

a: Australian New Zealand Bone and Mineral Society (Vitamin D and health in adults in Australia and New Zealand: a position statement, Med J Australia 2012;196:686-687), **b:** Endocrine Society of Australia, Osteoporosis Australia (Source: Osteoporosis Australia, Vitamin D position statement – Reviewed October 2013), **c:** Swiss Federal Commission for Nutrition (Source: Quack Lötscher, et. al., Originally published at Bern, Switzerland: Federal Office of Public Health), **d:** International Osteoporosis Foundation (Source: Published on International Osteoporosis Foundation, https://www.iofbonehealth.org), **e:** Canadian Medical Association-Osteoporosis (Source: Hanley, MD, et. al., CMAJ, 182(12), Sept. 7, 2010) **f:** Institute of Medicine US (Source: A. Catherine Ross, et. al., Dietary Reference Intakes for Calcium and Vitamin D, Institute of Medicine of the National Academies, 2011), **g:** French Group of Research and Information about Osteoporosis (Source: In Press - Karine Briot, et. al., Joint Bone Spine (2017), **h:** US Endocrine Society (Source: Holick, et. al., JCEM, 97(7), June 6, 2011).

Conversion factors: ng/mL→nmol/L for 25-OH vitamin D_3 is 2.496; ng/mL→nmol/L for 25-OH vitamin D_2 is 2.423.

In a recent review of the guidelines for > 40 countries, the author concluded that there is still no consistency with which vitamin D deficiency is defined in terms of 25(OH)D concentrations, and although it is recommended to give a daily supplement of vitamin D to infants in their first year of life, and to the elderly, there is no consensus on the dosage.[1]

The Endocrine Society guidelines suggest that 20 ng/mL or lower is considered vitamin D deficiency and 21–29 ng/mL be considered insufficiency.[3] By this standard, at least 20% of elderly Americans, 13% of Europeans,[12] and 50% of Hispanic-American and African-American children are vitamin D insufficient or deficient, and levels worsen during winter. Even apparently healthy students are susceptible, with 32% at a Boston hospital showing vitamin D deficiency.[13] Other researchers are more critical, and propose that the levels considered unhealthy in the above recommendations are actually safe. Widespread worry about vitamin D levels among patients and clinicians alike has made vitamin D testing and supplementation routine for many in the general population, despite the Endocrine Society and other medical authorities explicitly stating that there is insufficient evidence to mandate routine screening of people who are neither symptomatic nor at-risk. The debate was lively enough to prompt a New York Times article in April 2017.[14] All of these dynamics sometimes confound what has been established: **that well-respected clinical practice guidelines recommend high-risk groups have their vitamin D levels tested and, if necessary, managed**.

Vitamin D toxicity

Although rare, vitamin D toxicity is a serious and life-threatening medical condition. Patients with vitamin D toxicity report vomiting, excessive urine production, unusual thirst, encephalopathy, and renal dysfunction related to excessive calcium in the blood, and extreme cases can induce nerve dysfunction and kidney stones.[13] Vitamin D toxicity is virtually unheard-of outside of patients receiving intensive vitamin D supplementation, such as is in the treatment of a deficiency. Perhaps most noteworthy, a vitamin D toxicity outbreak in Denmark was traced to a liquid supplement that contained 75 times the indicated level of vitamin D, according to Danish health authorities.[15]

Other vitamin D toxicity incidents followed from inappropriate prescriptions or patients taking excessive amounts in search of additional health benefits, and have led to the hospitalization of dozens of children and adults.[15] Careful monitoring protects patients from the danger of crossing from sufficiency to toxicity via changes to their supplementation regimens. However, this monitoring is only as good as the upper detection limits of vitamin D assays and consensus on where those limits are. Unfortunately, definitions of vitamin D toxicity are even more variable than those for insufficiency, and the comparative rarity of vitamin D toxicity means that the demand for assays that are accurate at high concentrations is much lower.

Toxicity associated with inaccurately manufactured and labeled vitamin D supplements is a globally reported problem, and levels between 100 and 200 ng/mL in the blood can result from inappropriate or erroneous vitamin D administration.[17]

Conversion factor: ng/mL⟶nmol/L: 2.496

A serum concentration consistently >200 ng/mL is considered to be potentially toxic.[18] Most reports suggest a toxicity threshold for vitamin D of 10,000 to 40,000 IU/day and serum 25(OH)D levels of 200–240 ng/mL (500–600 nmol/L), but the Food and Nutrition Board in the Health and Medicine Division of the National Academies of Sciences, Engineering and Medicine has concluded that serum 25(OH)D levels above approximately 50–60 ng/mL should be avoided, as even lower serum levels (approximately 30–48 ng/mL) are associated with increases in risk for a variety of negative health outcomes.[19] An August 2010 publication, a Concise Review for Clinicians, in the Mayo Clinic Proceedings observed that a 25-OH vitamin D level of 80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism with normal renal function, but that most patients with vitamin D toxicity have levels greater than 150 ng/mL.[20]

Immunoassays, Variability and Bias

Contributing to the inconsistent guidelines is the fact that common immunoassays are both variable and subject in varying degrees to cross-reactivity or interference from a number of substances, some inherent in the human body and others from the outside.

Variability between assay methods and between laboratories using the same methods may range from 10% to 20%, and classification of samples as 'deficient' or 'nondeficient' may vary by 4% to 32%, depending on which assay is used.[21]

One publication described a study aimed at determining the ability of four (4) different immunoassays available in the US to detect 25(OH)D_2 in the serum of healthy individuals compared with an LC-MS/MS assay that is certified by the VDSCP. The authors found that each of the four immunoassays had a mean negative bias compared with the 25(OH)D_2 concentration measured by LC-MS/MS, with a smallest bias of –7% and a largest bias of –16%.[1,22]

We evaluated three automated immunoassays against the first FDA-cleared CDC/NIST-traceable LC-MS/MS method. The observed percent cross-reactivities for 25(OH)D_2 were 115% (Centaur™), 52% (Cobas™), and 44% (Architect™). We estimate that 8% of our population has >20 ng/mL 25(OH)D_2, thereby compromising the accuracy of 25(OH)D results in >3,000 samples annually.[23]

One study investigated the cross-reactivity of a 25(OH)D immunoassay for 24,25(OH)$_2$D compared with an LC-MS/MS method. They found that adjusting the 25(OH)D measurement for the concentration of 24,25(OH)$_2$D in the sample reduced the significant positive bias of some of the immunoassays compared to the LC-MS/MS assay, but increased the negative bias of other immunoassays.[1,24]

Another study measured the concentration of Vitamin D Binding Protein (VDBP) in patient samples along with the 25(OH)D concentration, and found an inverse correlation between the two values in four out of the five immunoassays that were used. LC-MS/MS assays usually have a sample preparation procedure before analysis that disrupts the binding of 25(OH)D to VDBP and therefore enhances the specificity of the measurements and ensures that all of the 25(OH)D is being quantified.[1,25]

Sources of assay inconsistency

Below is a table highlighting the major sources of variation observed between vitamin D assays, (adapted from French).[1]

Calibration	Especially prevalent with different LC-MS/MS assays as laboratories tend to make their own calibrators. But immunoassay manufacturers also make their own calibrators, and because they use different predicate devices for the 510(k) application to the FDA, assays may be significantly biased in calibration compared with one another.
Ability of a method to detect both 25-OH vitamin D_2 and 25-OH vitamin D_3	Different immunoassay methods tend to have varying levels of specificity for 25-OH vitamin D_2 and 25-OH vitamin D_3.
Ability of a method to measure only 25-OH vitamin D_2 and 25-OH vitamin D_3	The various forms of vitamin D may have only small variations in structure, making it challenging to design antibodies that only detect the correct forms. An example of one of these forms is 3-epi-25(OH)D_3, the C-3 epimer of 25(OH)D_3 that can be quantified by 25(OH)D immunoassays as 25(OH)D_3. Another vitamin D metabolite is 24, 25-dihydroxyvitamin D (24,25(OH)$_2$D). The concentrations of 24,25(OH)$_2$D vary from 2% to 20% of total 25(OH)D. Therefore, if they are incorrectly measured as 25(OH)D, they could falsely increase a patient's 25(OH)D sufficiency.
Complete dissociation from the Vitamin D Binding Protein (VDBP)	Automated immunoassays do not have a sample preparation step before analysis, but they do address disruption of the 25(OH)D bound to VDBP, commonly by onboard sample pretreatment step(s).

The issues related to calibration and standardization of vitamin D assays are not confined to immunoassay methods. Although LC-MS/MS is a more specific technology, some methods do not use a reference method procedure and laboratories running LC-MS/MS methods often make their own calibrators in-house.[1]

Vitamin D Standardization-Certification Program (VDSCP) assay performance guidelines

To address the issues of calibration and standardization, in 2013 the National Institute of Standards and Technology (NIST) released Standard Reference Material SRM972a, which contains serum samples with certified concentrations of 25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3, and 24,25-dihydroxy vitamin D_3 (24,25$(OH)_2D_3$). The concentrations for this SRM were determined by measurement on candidate reference measurement procedures by LC-MS/MS at NIST and the Centers for Disease Control and Prevention (CDC).[26–28] The VDSCP, a joint effort between the University of Ghent, the CDC, and the NIST have codified precision and accuracy benchmarks against which vitamin D immunoassays and other methods can be judged and compared. To meet the VDSCP's standards, a vitamin D assay must show a coefficient of variation (CV) at or below 10% (precision) and a bias of 5% or lower (accuracy).[23] Many common commercial assays do not meet these standards, often exhibiting variation in excess of 20%.[29]

Another source of interference with certain 25-OH vitamin D immunoassays is the common supplement biotin.

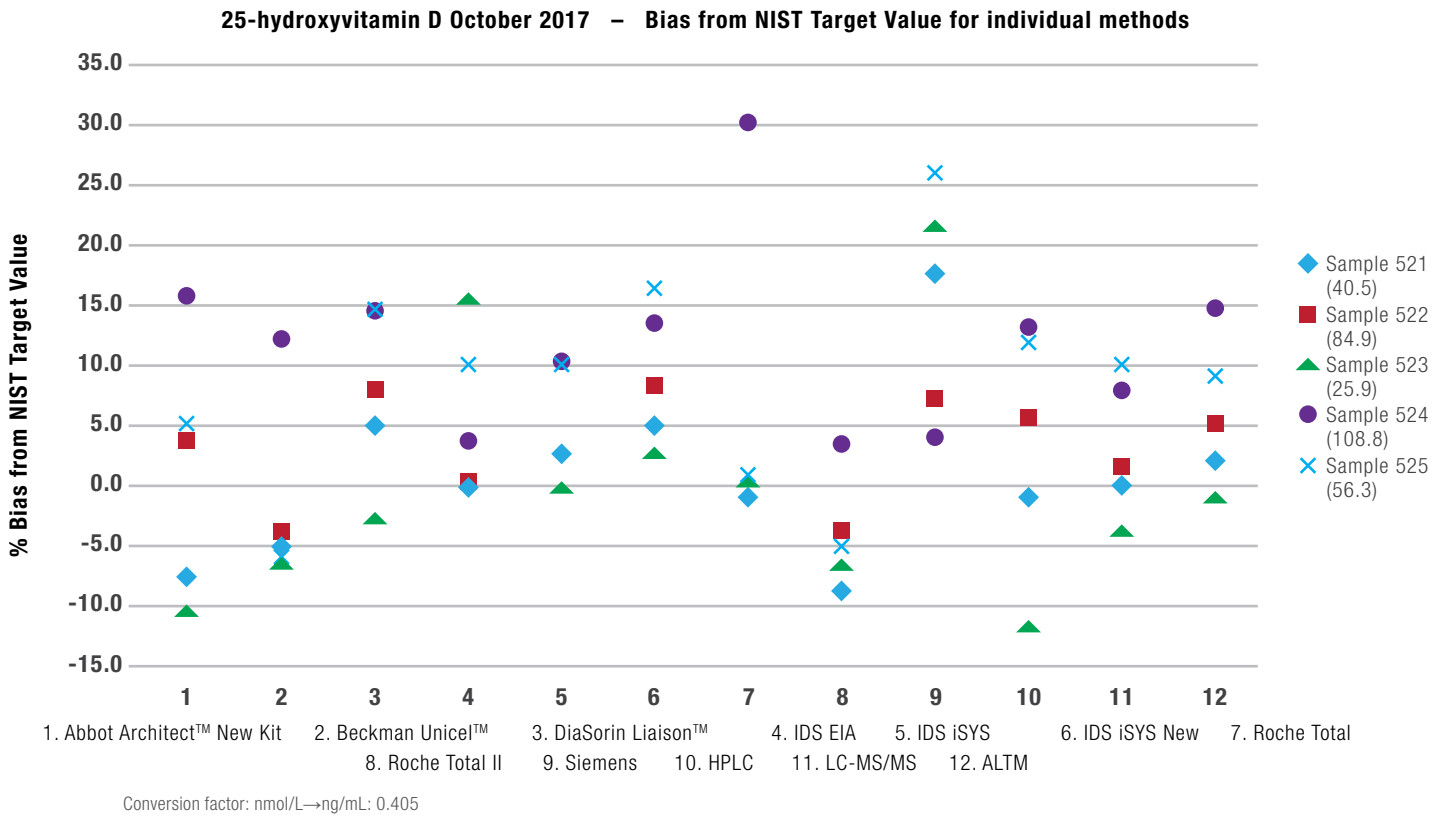
> Biotin ingestion was associated with falsely increased 25 OH vitamin D results by a mean of 9.25 ng/mL using a streptavidin/biotin based immunoassay product from one of the major IVD [in vitro diagnostic] vendors.[30]

The US FDA issued a Safety Communication in 2018 warning that biotin may interfere with certain lab tests, with recommendations for consumers, healthcare providers and lab personnel.[31] Impacted IVD manufacturers have responded with educational information and countermeasures for the applicable assays. Although these measures help avoid the effect of biotin supplementation on patient results, adhering to them can be inconvenient for the patient, the doctor and the lab. Conversely, due to the differences in the measurement technique used for LC-MS/MS, biotin does not interfere with these methods.

The inconsistency between vitamin D assays, especially immunoassays, means that patient results are inconsistent, and this contributes to the confusion over the need for supplementation. The same patient can be told their vitamin D levels are healthy or unhealthy depending on which laboratory performs the test. The inconsistency also contributes to the controversy around the proper levels of vitamin D considered sufficient for health. If the same test can produce readings 32% higher or lower in another lab, then separate studies of vitamin D levels in healthy and unhealthy populations can also differ significantly, with different labs concluding that different vitamin D levels are associated with good versus ill health.

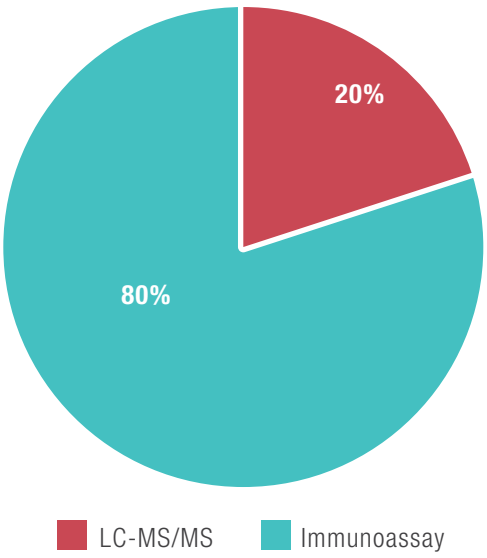
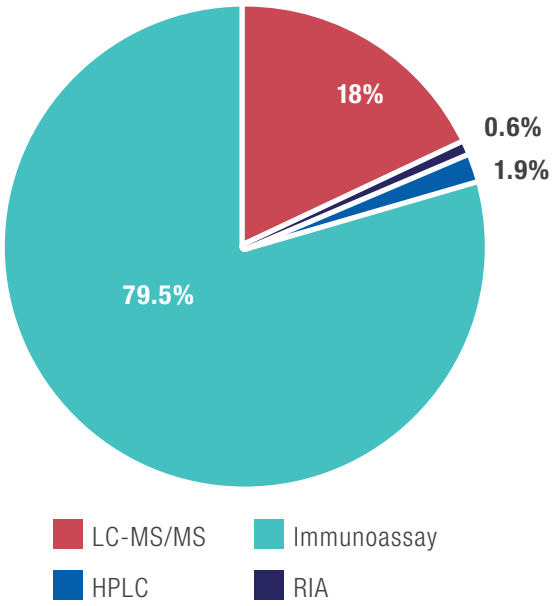
> The major issue of having 25(OH)D methods that do not produce consistent results is that the ranges that are used to define deficiency and sufficiency, for example, are based on cut-off concentrations. If the methods are not standardized in measurement, it makes interpreting 25(OH)D concentrations difficult for clinicians and patients. Further, it impedes the implementation of any type of clinical practice guidelines in vitamin D supplementation and 25(OH)D status recommendations.[1–3,32]

The chart below illustrates a comparison of mean % bias versus the NIST target value for a number of different commercial immunoassays as well as high-performance liquid chromatography (HPLC) and LC-MS/MS measurements. These data have been reported by the Vitamin D External Quality Assessment Scheme (DEQAS), a vitamin D proficiency–testing program. Notice that the mean biases for the LC-MS/MS methods are much closer together around the target values than those for the immunoassays. This indicates that these measurement tools are much more consistent, despite the lack of 3-epimer resolution for some LC-MS/MS LDTs, which is known to result in a positive bias upwards of 10%. Also of note, all of the samples in this survey contained only endogenous 25-OH vitamin D_3, not including an analysis of 25-OH vitamin D_2 recovery for the assay methods.[19] (Figure from 2017 DEQAS report.)[32]



Conversion factor: nmol/L→ng/mL: 0.405

Recent results from two 25-OH vitamin D proficiency testing surveys, the 2017 College of American Pathologists (CAP) Accuracy-Based Vitamin D survey B and the January 2018 DEQAS survey, revealed the following results across many participating laboratories using a variety of vitamin D testing methods:

	2017 CAP Accuracy-Based Vitamin D Survey B	January 2018 DEQAS Survey
Participating Labs:	342	842
Assay Method Distribution:		
Proficiency Sample:	Target value of **22.29 ng/mL** total 25(OH)D, but containing 0.36 ng/mL of 25(OH)D_2, 18.63 ng/mL of 25(OH)D_3, and 1.2 ng/mL of 3-epi-25(OH)D_3	Target value of **19 ng/mL** total 25(OH)D
Results:	The different mean results for this sample ranged from **9.8 to 28.5 ng/mL**	The reported concentrations varied from **15.18 to 26.06 ng/mL**, with a median of 19.2 ng/mL

(Data adapted from French).[1]

Extent of variation and clinical implications

The issues with immunoassays, both in terms of which data have historically been collected and the quality of those data, are particularly pronounced for people in some of the currently defined high-risk groups, as well as other groups of interest in current vitamin D research. Many immunoassays were not developed with these groups in mind, leading to greater cross-reactivity and less trustworthy results. The US Preventive Services Task Force has identified collecting accurate data on these and other under-studied at-risk populations as critical to determining whether existing vitamin D benchmarks need to be adjusted and whether broad-scale vitamin D supplementation should go forward.[21] Getting more and broader LC-MS/MS data on vitamin D is a critical part of that mission, since as a measurement methodology, LC-MS/MS is not subject to the same non-specific interactions as immunoassay technology.

In 2011, a study was undertaken by the VDSP to determine the baseline of 25(OH)D assay performance with both immunoassays and LC-MS/MS assays in different laboratory environments. They found that 6 out of 8 LC-MS/MS assays passed the VDSCP criteria of ≤ 10% CV and bias of ≤ 5%. However, only 4 out of 8 immunoassays passed the ≤ 10% CV criterion, and only 3 out of 8 passed the ≤ 5% bias requirement.[1,33]

In summary, variable and inconsistent test results have been a barrier to achieving global consensus on deficiency versus insufficiency levels as well as strong clinical evidence proving the apparent role of low levels of vitamin D in many diseases. Clinical and research labs need to be able to effectively screen patients, especially high-risk ones, for vitamin D status to show deficiency, insufficiency, sufficiency or toxicity, producing reliable results that can guide clinicians for therapeutic intervention where required.

What Does Higher Quality Vitamin D Testing Look Like? Why the Cascadion System?

Any attempt at integrating LC-MS/MS into clinical testing requires input from the end users themselves. Developed with close attention to customer feedback and expert opinions, the Cascadion SM Clinical Analyzer breaks barriers between LC-MS/MS and the clinical lab. As the first all-in-one LC-MS/MS solution designed to meet the needs of clinical laboratories, the Cascadion SM Clinical Analyzer offers a fully supported, automated single-vendor commercial platform that combines gold standard accuracy with ease of use for the routine lab.

Specificity from gold standard methodology

Using the Cascadion SM 25-Hydroxy Vitamin D assay on the Cascadion Clinical Analyzer, LC-MS/MS analysis avoids interferences and cross-reactivity commonly seen with antibody-based immunoassay testing, allowing it to return only 25-OH vitamin D data.[35] Validation studies show less than 10% interference from other circulating factors such as bilirubin, cholesterol and hemoglobin, and vitamin D metabolites such as di-hydroxy Vitamin D and C_3 epimers.

The C_3 epimers are important to consider in vitamin D assay design. These can cause overestimation of vitamin D status in pediatric and some adult patients due to interference during analysis. The Cascadion SM 25-Hydroxy Vitamin D Assay is designed to eliminate the C_3 epimers via chromatographic separation, and recognizes only 25-OH vitamin D_2 and D_3 when reporting total levels.

3-epi-25(OH)D_3 [is] the C_3 epimer of 25(OH)D_3 that can be quantified by 25(OH)D immunoassays as 25(OH)D_3 and can be present at up to 17% of total 25(OH)D [depending on the age of the person from whom the sample is collected]. This epimer can also be measured as 25(OH)D_3 in LC-MS/MS methods if it is not chromatographically separated from 25(OH)D_3 before it enters the mass spectrometer.[1,35–39]

Less Than 10% Interference[35]	No of compounds tested
Endogenous and Exogenous Compounds	26
Compounds with Similar Chemical Structures and Metabolites	20
Compounds with Possible Mass Spectral Overlap	89

Wide measurement range and precision consistent with VDSCP Program recommendations[35]

Precision and Measurement Range	25-Hydroxy Total Vitamin D	25-Hydroxy Vitamin D_2	25-Hydroxy Vitamin D_3
Assay Precision (Total 25-OH Vitamin D - D_2 + D_3)			
Total %CV (n=84 for each concentration tested)	2.3–7.6%	3.2–6.1%	2.9–7.6%
Range of Mean Values (ng/mL)	5.95 - 114.61	5.24 - 104.54	5.95 - 102.94
Analytical Measurement Range			
ng/mL	3.4–264	3.4–132	3.4–132
nmol/L		8.24–319.83	8.49–329.47
Spike Recovery Using Patient Sample Matrix (90–110%)		20, 50, 70 ng/mL	20, 50, 70 ng/mL

Method Comparison[35]	n	Slope	Intercept	R
Cascadion SM 25-Hydroxy Vitamin Assay vs. CDC VDSCP Reference Method	115	1.04	-1.582	0.9966

Also among the many endogenous and exogenous compounds and compounds with similar chemical structure and metabolites tested with the Cascadion SM Vitamin D Assay was the common supplement biotin, discussed earlier in the Immunoassays, Variability and Bias section above. The Cascadion assay was not affected by high levels of biotin up to 3500 ng/mL, based on a testing criteria of < ±10% from the target 25-OH vitamin D concentration.[35] The DEQAS proficiency testing survey has also begun to offer a sample with a high level of biotin (Sample 545 containing approximately 586 ng/mL of biotin), which was included in their October 2018 Report for 25-OH vitamin proficiency testing results. The Cascadion SM 25-OH Vitamin D assay was a participant in that survey.

Traceability and standardization

The Cascadion SM 25-Hydroxy Vitamin D Assay incorporates validated controls, calibrators and internal standard reagents, all supplied by a single vendor for consistent testing across laboratories. In addition, the assay components and analyzer are manufactured by Thermo Fisher Scientific as medical devices and are compliant with applicable IVD regulatory requirements.[35,42–43]

All vitamin D results are easily traceable to specific lots of the consumables and disposables used to generate that test. This traceability will help save laboratory time with quality assurance and certification.

The Cascadion SM Vitamin D Assay is also traceable to the NIST vitamin D standard reference materials, as listed in the Certificates of Analysis for the calibrators and controls.[35]

Customer experience[41]: The precision of the Cascadion SM Vitamin D Assay has been tested in an actual routine clinical laboratory during a beta evaluation of the Cascadion system conducted at Frimley Park Hospital, Berkshire and Surrey Pathology Services during the summer of 2018. In that study, within-batch coefficients of variation (CVs) for vitamin D_3 of 1.51% to 5.10%, and between-batch CVs for both vitamin D_2 and D_3 ranging from 2.96% to 5.38%, were observed. All of these CVs are well below the precision criteria recommended by the CDC/Ghent VDSCP of <10%.

Accuracy

The Cascadion SM Assay's accuracy was established through a method comparison study using 115 patient samples with CDC VDSCP reference method assigned values, ranging from 5.3 to 119.3 ng/mL.[35]

Customer experience[45]: As part of a beta evaluation of the Cascadion system, the Viollier AG clinical laboratory located in Allschwil, Switzerland, assessed the performance of the Cascadion SM 25-OH Vitamin D Assay with four vitamin D External Quality Control (EQC) programs. The table below shows the EQC programs included in this evaluation, along with a summary of the % biases observed compared to the target values.

The average % difference between vitamin D_3 and vitamin D_2 CDC results and Cascadion assay results, respectively, was 3.7% and 1.7%, which is well within the VDSCP program recommendation of <5%.[45] For 98.3% and 100% of the individual patient results obtained for vitamin D_2 and D_3, respectively, the difference between the Cascadion assay and CDC result was <20%.

Customer experience[45]: The Viollier AG clinical lab's beta evaluation compared the Cascadion SM Total Vitamin D Assay, containing 25-OH vitamin D total, 25-OH vitamin D_3 and 25-OH vitamin D_2, against the routine method, the chemiluminescence immunoassay used with the Abbott Architect™ i200SR. The method comparison included a total of 2,000 patient samples. The results yielded an R^2 of 0.9235 and a regression equation of y = 1.0879x – 7.8826. They concluded, "In general, there is a good correlation between the Cascadion SM assay results and our routine methods. However, there is scattering for high values (>60 ng/mL). Looking at the EQCs results for these, it suggests that Cascadion SM analyzer is more precise and accurate than the current routine method."

Conversion factor: ng/mL - nmol/L: 2.496

EQC Program Results[45]	Number of EQC Samples Evaluated	Vitamin D Concentrations (nmol/L)	% Biases Observed Across EQC Samples
National Institute of Standards and Technology (NIST)	2	158.7 & 287	4.3% & 0.7%
DEQAS	6 (1 D_2-only & 1 D_3-only)	31.8–87.7	1.3%–7.8%
Centre Suisse de Contrôle de Qualité (CSCQ)	2	59.8 & 113	0.3% & 0.9%
Referenz-Institut für Bioanalytik (RfB)	2	35 & 42.3	3.4% & 2.0%

Conversion factor: nmol/L→ng/mL: 0.405

Summation

With established guidelines recommending screening for defined high-risk populations and with the Vitamin D Standardization and Certification Program's assay performance guidelines, some clinicians now specifically request measurement by LC-MS/MS. In addition, many research studies strongly indicate a broader role for vitamin D in a variety of other chronic diseases.

Clinical diagnostic laboratories need to be well positioned to take advantage of this situation by offering accurate and timely results for clinicians and their patients. The Cascadion SM 25-Hydroxy Vitamin D Assay delivers continuous, accurate, consistent vitamin D testing in an easy-to-use format, breaking the barriers between LC-MS/MS and the clinical lab for patient safety and service.[41, 45]

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