

Vitamin D

Laboratory Testing Methods

FOR EDUCATIONAL USE



Circulating 25-hydroxyvitamin D is used to assess a patient's vitamin D status with 25-hydroxyvitamin D being the primary metabolite.^{1,2} According to DEQAS survey results, immunoassay methods perform as well as LC-MS/MS. Separate reporting of the 25-OH D₃ and 25-OH D₂ metabolites may be misleading to healthcare providers who might consider a low 25-OH D₃ or 25-OH D₂ value as an indicator of D deficiency despite the total 25-OH D result being within the sufficiency range.³

Laboratory methods for measuring vitamin D

There are two basic methods for measuring vitamin D: immunoassay and chromatography. The immunoassay techniques include radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA). The chromatographic approaches include high pressure liquid chromatography (HPLC) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). These chromatographic methods, also known as fractionated or direct detection, quantify 25-OH D₃ and 25-OH D₂ independently and add both values to get the total 25-OH D.

Chromatography technology provides a high probability that a molecule of interest will be correctly identified and quantitated because it is detected as a specific transition from one mass fragment to another. The fractionated technique is not without shortcomings.⁴⁻⁶ There have been numerous reports of discrepancies between the results of LC-MS/MS and immunoassays, with LC-MS/MS reporting serum values up to 40% higher than those reported using immunoassay.⁶ Singh et al. reports a potential problem with LC-MS in its relative inability to discriminate between 25-OH D₃ and its inactive isomer 3-epi-25-OH D₃.⁷ Additional challenges with using this method properly include the need for each laboratory to develop its own in-house standards, since there is as yet no certified reference material to test method accuracy. Furthermore a high-degree of expertise of laboratory personnel is required to conduct the complex procedure.⁴ Not all laboratories have the same standard operating procedures, and LC-MS/MS inter-laboratory values can vary by 20%.⁵ Aliquots of one sample sent to seven different laboratories using LC-MS/MS to measure vitamin D reportedly generated seven different results.⁶

Performance between immunoassay and fractionated methods are similar

Nevertheless, laboratories using their own developed fractionated tests imply the specificity afforded by chromatographic methods will deliver greater accuracy than immunoassays. According to the October 2008 distribution of DEQAS (Table 1), the inter-laboratory precision between HPLC and LC-MS/MS are similar to immunoassays.⁸

Method	Mean 25-OH D concentration, ng/mL (%CV)					
	N	Sample 341	Sample 342	Sample 343	Sample 344	Sample 345
25-Hydroxyvitamin D RIA – DiaSorin Inc.	40	20.8 (18%)	32.2 (18%)	42.6 (16%)	37.8 (21%)	62.3 (17%)
Liaison 25 OH Vitamin D, Total – DiaSorin Inc.	144	20.9 (15%)	30.3 (15%)	41.5 (14%)	31.0 (17%)	58.6 (15%)
25-Hydroxy Vitamin D RIA – IDS Ltd.	29	24.0 (15%)	35.8 (15%)	51.5 (15%)	39.1 (14%)	67.3 (14%)
25-Hydroxy Vitamin D EIA – IDS Ltd.	84	22.0 (12%)	33.4 (15%)	44.6 (17%)	34.8 (13%)	62.4 (16%)
25-Hydroxy Vitamin D EIA, Automated – IDS Ltd.	32	22.7 (12%)	34.4 (15%)	45.4 (16%)	36.4 (13%)	63.1 (12%)
HPLC	16	23.5 (25%)	37.7 (20%)	49.8 (21%)	38.9 (17%)	66.6 (18%)
LC-MS/MS	39	22.6 (15%)	37.8 (13%)	50.2 (13%)	39.6 (14%)	66.3 (18%)

Table 1: Method means for DEQAS samples distributed in October 2008.⁸

Data from Vitamin D external Quality Assessment Scheme (DEQAS, www.deqas.org) indicates that most participating labs use immunoassays to measure total 25-OH D (Figure 1).⁹ While the number of DEQAS participants has almost doubled since 2007, the proportion using LC-MS/MS has remained relatively constant at approximately 10%. The most commonly used reporting methods in the July 2009 DEQAS survey were immunoassays manufactured by DiaSorin, IDS, and LC-MS/MS (Figure 2).⁹ These 510(k) cleared diagnostic 25-OH D tests are under strict FDA control and monitoring for assay performance and reliability. Clinical reference laboratories are replacing these FDA-cleared tests with their own lab-developed LC-MS/MS that are diverse and not under FDA scrutiny. The reasons for this switch are the 'perceived' advantages of LC-MS/MS technology being more accurate, precise, specific, cost effective, and providing the separate determination of 25-OH D₃ and 25-OH D₂.

However, according to DEQAS data, the immunoassay method performs as well as LC-MS/MS (Table 1).⁸

DEQAS Proficiency Testing Program for 25-OH D Immunoassay and LC-MS/MS Methods

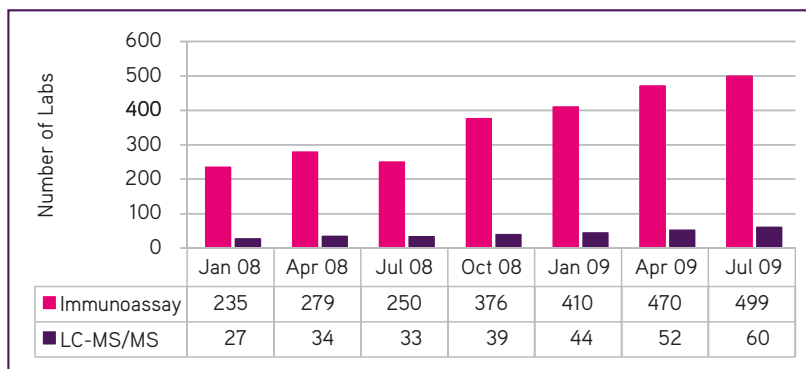


Figure 1: Number of labs using immunoassay and LC-MS/MS method participating in DEQAS survey.⁹

Vitamin D Assay Method

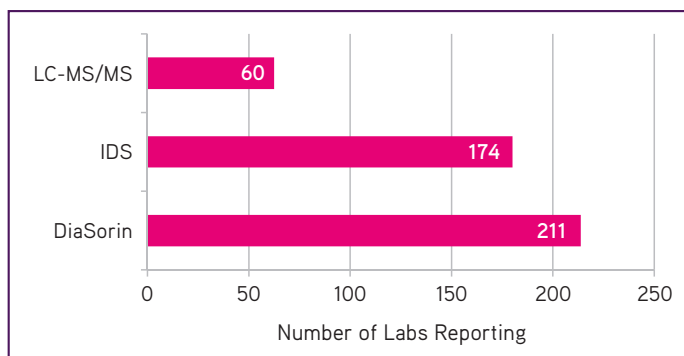


Figure 2: Vitamin D assay methods used in DEQAS survey.⁹

US FDA has cleared the following test kits for quantitative determination of 25-hydroxyvitamin D in human serum or plasma to be used in the assessment of vitamin D sufficiency (Table 2).

Product	Company	Approval Date
IDS-iSYS 25-Hydroxy Vitamin D	IDS Ltd.	06/2010
Vitamin D HPLC	Esa Biosciences Inc.	05/2008
Liaison 25 OH Vitamin D, Total	DiaSorin Inc.	10/2007
25-Hydroxy Vitamin D EIA	IDS Ltd.	05/2002
25-Hydroxy Vitamin D RIA	IDS Ltd.	09/1999
25-Hydroxy Vitamin D RIA	DiaSorin Inc.	11/1998

Table 2: Current available FDA 510(k) cleared 25-hydroxyvitamin D test kits – Adapted from www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm

Clinical reporting of circulating 25-OH D levels

Because of the nature of the fractionated method, laboratories that use this method must quantify 25-OH D₃ and 25-OH D₂ separately. Some of these reference laboratories advocate reporting separate values because they claim that doing so is superior to simply adding them together to create a total 25-OH D value.

The important objective in assessing vitamin D status is to obtain a measure of the total circulating 25-OH D, whether from an immunoassay or fractionated method. FDA has recently cleared a Vitamin D HPLC test kit for quantitation of total 25-OH D (Table 2) but not for detection of 25-OH D₃ and 25-OH D₂ individually. Approximately 99% of all patients who need vitamin D assessment will have almost no circulating 25-OH D₂. 25 OH D₂ level will be detectable only if the patient is undergoing therapy for vitamin D deficiency who are taking higher doses (approximately 50,000 IU) of vitamin D₂.³

Binkley et al. conducted a web-based survey via e-mail depicting a hypothetical 82 year old hip fracture patient history (in whom 25-OH D was measured) with three 25-OH D laboratory result scenarios (Table 3). In scenario 1, 25-OH D₃ level = 32 ng/mL and 25-OH D₂ level = <5 ng/mL. 14% (8/57, 45 physicians, 12 physicians assistants and nurse practitioners) of healthcare providers incorrectly identified the patient as vitamin D deficient.

In scenario 2, 25-OH D₃ = 16 ng/mL and 25-OH D₂ level = <5 ng/mL, all responding healthcare providers correctly identified this as vitamin D deficiency in need of vitamin D treatment. However, for the scenario 3, reporting 25-OH D₃ as less than 5 ng/mL and 25-OH D₂ as 40 ng/mL, 13 (23%) interpreted these results to indicate either vitamin D deficiency or vitamin D₃ deficiency requiring vitamin D treatment.

This separate reporting has been shown to confuse the clinician.³

Conclusion

The only way to determine whether a person is vitamin D deficient or sufficient is to measure their circulating level of 25-OH D. There are a variety of assay methods used to measure 25-OH D, immunoassay and chromatography. According to DEQAS survey, the immunoassay performs as well as LC-MS/MS test method. Lab-developed LC-MS/MS tests measure both 25-OH D₃ and 25-OH D₂ independently and report the individual results. Separate results may contribute to clinical misinterpretation. The total 25-OH D, i.e. 25-OH D₃ plus 25-OH D₂ is used by physicians to determine sufficiency for their patients.

Clinical Interpretation				
Scenario	Vitamin D Deficiency, treated with vitamin D n (%)	Vitamin D ₂ Deficiency, treated with vitamin D n (%)	Vitamin D ₃ Deficiency, treated with vitamin D n (%)	Sufficient Vitamin D, no treatment required n (%)
25-OH D ₃ : 32 ng/mL 25-OH D ₂ : <5 ng/mL	1 (2%)	7 (12%)	0 (0%)	49 (86%)
25-OH D ₃ : 16 ng/mL 25-OH D ₂ : <5 ng/mL	52 (93%)	2 (4%)	2 (4%)	0 (0%)
25-OH D ₃ : <5 ng/mL 25-OH D ₂ : 40 ng/mL	2 (4%)	0 (0%)	11 (19%)	44 (77%)
Following text accompanied the result:	Vitamin D, 25-OH D clinical reference value, measure as:			
	a) <10 ng/mL severe deficiency	b) 10-24 ng/mL mild to moderate deficiency	c) 25-80 ng/mL optimum concentration	d) >80 ng/mL toxicity possible

Table 3: Potential for clinical misinterpretation when both 25-OH D₂ and 25-OH D₃ are reported.³

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About IDS

IDS excels at developing and supporting scientifically advanced solutions that allow laboratories to efficiently perform and confidently report results for specialty diagnostic tests. For over 50 years, researchers and clinicians alike have turned to IDS for the specialty testing products and support they need to generate results they can trust.

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