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REVIEW ARTICLE

Vitamin D testing: advantages and limits of the current assays

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Abstract

Vitamin D deficiency and insufficiency has become a pandemic health problem with a consequent increase of requests for determining circulating levels of 25-hydroxyvitamin D [25(OH)D]. However, the analytical performance of these immunoassays, including radioimmunoassay and ELISA, is highly variable, and even mass spectrometric methods, which nowadays serves as the gold standard for the quantitatively determination of 25(OH)D, do not necessarily produce comparable results, creating limitations for the definition of normal vitamin D status ranges. To solve this problem, great efforts have been made to promote standardization of laboratory assays, which is important to achieve comparable results across different methods and manufacturers. In this review, we performed a systematic analysis evaluating critically the advantages and limits of the current assays available for the measure of vitamin D status, i.e., circulating 25(OH)D and its metabolites, making suggestions that could be used in the clinical practice. Moreover, we also suggest the use of alternatives to blood test, including standardized surveys that may be of value in alerting health-care professionals about the vitamin D status of their patients.

Introduction

Vitamin D deficiency and insufficiency has become a pandemic health problem mostly related to an inadequate sun exposure and few food sources that naturally contain vitamin D. There are in addition several risk factors that exacerbate the deficiency, including dark skin pigmentation, pregnancy, malabsorptive syndromes, obesity, and aging [1]. Historically, 25-hydroxyvitamin D [25(OH)D] was measured by either a manual radioimmunoassay (RIA) or a

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vitamin D-binding protein (VDBP) assay and many clinical studies that provide the scientific basis for current clinical recommendations are based on these results [2, 3]. Over the past decade, mounting evidence showed that vitamin D deficiency could be associated with an increased incidence and progression of many diseases [4], including osteoporosis [5], chronic diseases [6–8], cancer progression [9, 10], autoimmune, and type 2 diabetes [11, 12]. As a result of these observations, medical laboratories experienced a sharp rise in requests for 25(OH)D determination and in vitro diagnostic (IVD) companies developed automated immunoassays and enzyme-linked immunosorbent assays (ELISAs), which allowed laboratories to cope with the continuously increasing workload. Quickly, it became clear that the analytical performance of these immunoassays was highly variable [13–16]. Even mass spectrometric methods did not necessarily produce comparable results [13, 17].

These discordant results provided by different laboratories has created limitations such as the definition of normal 25(OH)D ranges and measurement errors may produce contradictory clinical conclusions and decisions. Nowadays, only a few studies compared directly the different assays, evaluating the different risk of possible bias in the measure of 25(OH)D levels. To solve this problem, great efforts have been made to promote standardization of laboratory assays, which is important to achieve comparable results across different methods and manufacturers [18].

In this review, we performed a systematic analysis evaluating critically the advantages and limits of the current assays available for the measure of vitamin D status, i.e., circulating 25(OH)D and its metabolites. We suggest which assay should be used for the evaluation of the vitamin D status as well as new methods that could be applicable in the clinical practice.

Methods: search strategy and selection criteria

A systematic approach was employed to search and review the relevant literature. The online databases PubMed (MEDLINE), Google Scholar, EMBASE, and the Cochrane Library were searched with the MeSH terms "Vitamin D" OR "Vitamin D Deficiency" OR "Ergocalciferols" OR "25-hydroxyvitamin D" OR "24,25-dihydroxyvitamin D" OR "calcitriol" OR "calcidiol" combined with the title keywords "Immunoassay" OR "chromatography" OR "LC-MS/MS" OR "Analytical" OR "C3-epimer" OR "Vitamin D Metabolism Ratio" OR "Mass screening" OR "Questionnaire" OR "Detection" OR "External Quality Assurance Scheme" OR "Standardization Program". Literature search were performed without language restriction up until September 2019 and also identified relevant articles from the reference lists of any retrieved papers. Studies were first evaluated by title, and availability of the full text identify the most relevant paper. Duplications, conference abstracts, editorials, and letters to the editor were excluded. Manuscripts not focused on the topic were later excluded.

25(OH)D and related metabolites

Vitamin D has a secosteroid structure similar to that of steroids but with a ruptured bond between atoms C_9 and C_{10}. Cholecalciferol (vitamin D_3) is the natural form of vitamin D produced in human skin after exposure to solar ultraviolet (UV) B (290–315 nm) radiation. Vitamin D3 is also available in some foods, including oily fish, cod liver oil, and sun-dried mushrooms, and fortified foods, including milk, orange juice, margarine, and cooking oil, and from supplements [10]. Vitamin D_2 (ergocalciferol) is of yeast fungal/plant origin, whereas vitamin D_3 is derived from animals. Vitamin D_3 and vitamin D_2 are transported in the bloodstream to the liver, where they are metabolized to 25-hydroxvitamin D_3 [25(OH)D_3, also known as calcifediol or calcidiol] and 25-hydroxvitamin D_2 [25(OH)D_2], respectively. Both of these 25(OH)D metabolites [designated as 25(OH)D], are converted in the kidneys to its active form 1α,25-dihydroxyvitamin D [1,25(OH)$_2$D], which binds strongly to the vitamin D receptor (VDR) in the target tissues [10, 19, 20]. It is found in very low concentrations in the blood and is more hydrophilic than 25(OH)D. Hence, the measurement of this dihydroxy metabolite is more challenging and requires pre-analytic manual extraction steps making the assays more laborious. Research and clinical practice related to acquired and inherited disorders of vitamin D metabolism have been hampered because of the difficulty of measuring its active form in the serum. In the clinical practice, the measurement of total 25(OH)D [25(OH)D_3 and 25(OH)D_2] is the gold standard measurement for vitamin D status due to its longer half-life and stability (25 days) and the relative abundance (i.e., ng/mL; 1 ng/mL = 2.496 nmol/L) in serum. Dihydroxylated bioactive forms are present in the blood at very low concentrations (i.e., pg/mL). 1,25(OH)$_2$D, which has a half-life of 4–6 h, is often normal or even elevated in vitamin D-deficient patients because of secondary hyperparathyroidism increasing the renal production of 1,25(OH)$_2$D.

Why 25(OH)D and its metabolites are difficult to measure

Total 25(OH)D is the widely accepted metabolite to measure for vitamin D status. Nonetheless, albeit many methodological improvements have been made, its determination is still a challenge [16, 18, 21]. The reasons include the

tandem determination of both 25(OH)D_2 and 25(OH)D_3 and the compulsory dissociation of the hydrophobic 25(OH)D from its carrier proteins (VDBP), albumin, and lipoproteins. On this note, it is particularly important to realize that, when using automated platform organic solvents, which infer the best dissociation, cannot be employed, consequently resulting in use of alternative releasing agents that result in an inferior dissociation of 25(OH)D from its binding proteins. This is particularly observed when analyzing samples from the pregnant women or those on estrogen therapy or patients with chronic kidney disease (CKD) [22–24]. Furthermore, it needs to be emphasized that 25(OH)D_2 and 25(OH)D_3 have dissimilar affinity constants for these binding proteins, as such only an efficient dissociation method will suffice to produce accurate recovery and quantification of both the forms. Resulting from an exponential increase in demand for 25(OH)D testing, automated immunoassays are favored and as obvious from the Vitamin D External Quality Assurance Scheme (DEQAS) participants, <1% of the laboratories use currently RIA (www.deqas.com). However, a number of studies have reported comparisons between different automated platforms and RIA, high-pressure liquid chromatography (HPLC), and liquid chromatography tandem mass spectrometry (LC-MS/MS), with poor agreement [13, 14, 25–31]. For this reason, new insight into the physiology and analytics of vitamin D may perhaps allow reconsideration of how we assess vitamin D status.

Assays for vitamin D metabolite measurements

Competitive binding methods and chemiluminescence immunoassays (CLIAs)

Competitive protein-binding assay (CPBA), RIA, and CLIAs are all binding assays. CPBAs were used for a short period of time and included organic solvent extraction and chromatography prior to assay. CPBAs measured both 25(OH)D_2 and 25(OH)D_3 and underestimated 25(OH)D at low levels and overestimated at high levels [32]. The techniques were withdrawn from the market due to various technical problems [33]. Initial RIAs used small samples and I^{125} as a tracer. In addition, they were not subjected to nonspecific interferences, were non-expensive, and also accurate. However, they required the use of radionuclides and some of those methods discriminated between 25(OH)D_2 and 25(OH)D_3 [4, 23, 32]. CLIAs are based on the dissociation of 25(OH)D from its binding protein (VDBP), then bound to the specific phase antibody and second to the addition of magnetic particles coated with antibody against a 25(OH)D-isolumino tracer. Then the unbound material is removed with a wash cycle. In the next step, the reagents are added to initiate the chemiluminescent reaction. The light signal is detected by a photomultiplier as relative light units; this measurement is inversely proportional to the concentration of 25(OH)D [34].

Commercial competing binding assays and immunoassays differ among each other according to the cross-reactivity with different vitamin D metabolites and when compared to chromatographic methods [35] (Table 1). Indeed, immunoassays do not detect 3-epi-vitamin D metabolites, although they may have cross-reactivity with the 24,25(OH)2D_3 and other vitamin D metabolites, which may increase the risk of bias [36]. The majority of immunoassays measures total 25(OH)D [25(OH)D_2 and 25(OH)D_3] levels, since they are not able to differentiate 25(OH)D_3 and 25(OH)D_2 (Table 1). Most of the used automated immunoassay methods (Abbott Architect, Beckman Dxi, DiaSorin Liaison, IDS iSYS, Roche, and Siemens) have an almost constant intra-assay coefficient of variation that ranges from 4.2% for the new Abbott Architect kits to 15–19% for the Siemens kit [37–42]. In addition, there is not always complete fidelity in the measurement of 25(OH)D_2 thereby underestimating the total 25(OH)D especially for patients who were on vitamin D_2 [43, 44]. To note, recently, it has been shown that the new assay from Roche (Elecsys® Vitamin D total II) had good reactivity toward 25(OH)D_2, with a cross-reactivity of 95.2% for 25(OH)D_2 and 92.6% when normalized to 25(OH)D_3, overcoming partly the problem of 25(OH)D_2 measurement [41].

Chromatographic methods

Chromatographic assays used to measure vitamin D metabolites include HPLC and LC-MS/MS. Initial studies on 25(OH)D were based on the use of HPLC, including a lipid extraction of the serum, after which the 25(OH)D fraction was submitted to HPLC and the UV absorption to measure 25(OH)D. This procedure was available only for research laboratories and was not routinely available for the clinical practice/scenario. However, HPLC-UV lacks the sufficient sensitivity for the measuring of low levels of 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$ [45]. On the other side, LC-MS/MS is a powerful analytical technique that combines the separating power of liquid chromatography with the highly sensitive and selective mass analysis capability of triple quadrupole mass spectrometry. LC-MS/MS measures both 25(OH)D_2 and 25(OH)D_3 and presents a high correlation with the HPLC ($r = 0.96$) [14]. LC-MS/MS has an excellent sensitivity for the measuring of analytes in a wide range of concentrations (0.07 pg/mL to 100 ng/mL), although it has a poor rate of production. For this reason, LC-MS/MS is considered as the reference technique used to measure 25(OH)D, yet requires an expert analyst [46]. Moreover, LC-MS/MS methods can measure several (in the same sample) vitamin D metabolites. Despite this concept, the technique

Table 1 Characteristics of commercial competing automated competing binding assays and immunoassays for 25(OH)D.

Automated systems	Marketed	Antibody/label	Characteristics/changes	Comments
Liaison 25-OH Vitamin D Total, DiaSorin CIL assay	Initially by Incstar, later by DiaSorin; Saluggia (VC), Italy	Sheep polyclonal/Acridinium	RIA kit. In 2004, it changed to a CIL assay to be used on the Liason analyzer. The DiaSorin 25(OH)D assay is a 2-step procedure that involves a rapid extraction of 25(OH)D and other hydroxylated metabolites from serum or plasma, followed by a competitive RIA procedure	The method has co-specificity for 25 $(OH)D_3$ and $25(OH)D_2$. The "3-epimer" of 25(OH)D is stated as having 1.3% cross-reactivity. The stock 25(OH)D solution is calibrated by UV spectrophotometry. https://www.diasorin.com/sites/default/files/allegati/menu_liaision_fam_coll_07.19.pdf
25-Hydroxyvitamin D EIA; Immunodiagnostic Systems (IDS)	Immunodiagnostic Systems (IDS); Bolton, UK; and Bensheim, Germany	Sheep polyclonal/Acridinium ester	Chemiluminescence method. Samples are subjected to a pretreatment step with NaOH to denature the DBP inside the IDS-iSYS Multi-Discipline Automated Analyzer. It provides measurement of total 25(OH)D [$25(OH)D_3$ and $25(OH)D_2$]	This assay is not aligned to the NIST Standard Reference Material. The measurement range of this assay is 5–140 ng/mL. Reliable test for monitoring vitamin D therapy, regardless of supplementation form
Abbot Architect CIL assay	Abbott Park, Illinois, US	Sheep polyclonal/Acridinium. The cross-reactivity of 25(OH)D is 80%	Chemiluminescence assay using a monoclonal antibody coated onto paramagnetic particles and an acridinium label. The assay is standardized against the NIST SRM 2972	The Architect assay is susceptible to interference from triglyceride at concentrations >500 mg/dL; a triglyceride concentration of 800 mg/dL produces a bias of −10.2%
Roche Elecsys® vitamin D total assay	Roche Diagnostics, Mannheim, Germany	Vitamin D-binding protein microparticles/Ruthenium	Competing protein-binding assay for 25 (OH)D. 25(OH)D conjugate and microparticles are magnetically captured and after washing chemiluminescence is induced	The test can be run on COBAS, ELECSYS, and MODULAR immunoanalyzers
ADVIA Centaur/Centaur XP Vitamin D Assay	Siemens Healthineers, Siemens Healthcare Diagnostics; Erlangen, Germany	After pretreatment with 8-anilino-1-naphalene-sulfonic acid, ethylene glycol and diazolidinyl urea. Incubation with mouse monoclonal anti-vitamin D antibodies/acridinium ester	Paramagnetic particles coated in monoclonal anti-fluorescein antibodies are added. After magnetic capture of the particles and washing, chemiluminescence is induced from the acridinium ester	Reduced risk of confounding assessment with minimal cross-reactivity with 3-epi-25(OH)D https://www.siemens-healthineers.com/es/clinical-specialities/bone-metabolism/advia-centaur-vitamin-d-total-assay
Orgentec 25(OH) ELISA	Diagnostika GmbH, Mainz, Germany	Competitive ELISA. Serum sample extraction of 25(OH)D2/D3 is done automatically inside the Random Access Analyzer	Serum samples are mixed with tracer reagent and the $25(OH)D_2/D_3$ is delivered from the vitamin D-binding protein. The extraction procedure is followed by analysis	This assay is aligned to the NIST SRM 2972. The measurement range of this assay is 5–170 ng/mL (information of the manufacturer)
Beckman Coulter's Access 25 (OH) Vitamin D Total Immunoassay System	Beckman Coulter (Danaher Corporation), Brea, CA, US	ELISA. Serum or plasma $25(OH)D_3$ and $25(OH)D_2$	A98856 - Access 25(OH) Vitamin D Total Kit for UniCel DxI System. Time to have results 40 min. Sample size 30 µL	https://www.beckmancoulter.com/en/products/immunoassay/access-vitamin-d#/especificaciones

Table 1 (continued)

Automated systems	Marketed	Antibody/label	Characteristics/changes	Comments
BioPlex® 2200 25(OH) D total assay	Bio-Rad Laboratories, Inc., Hercules, CA, US	Serum or plasma total 25(OH)D [25 $(OH)D_2$ and 25$(OH)D_3$]	Multiplex flow competitive immunoassay for the determination of total 25(OH)D. The assay is standardized (calibrated) using internal standards, which are traceable to the University of Ghent's ID-LC/MS/MS 25 (OH) vitamin D Reference Method Procedure	The RMP uses certified standardized reference materials (SRM 2972a) from the NIST
Tosoh India 25(OH)D IR CLIA	Tosoh India Pvt, Ltd. (Formerly Lilac Medicare Pvt. Ltd.), Mumbai, India	Total 25(OH)D [25$(OH)D_2$ and 25(OH) D_3]	CLIA is a based test system intended for the quantitative measurement of total concentrations of 25$(OH)D_2$ and 25 $(OH)D_3$ in human serum or plasma samples	https://www.tosohindia.com/our-products/reagents/clia-reagents/bone-metabolism/25-oh-vitamin-d-ir-clia
VIDAS® 25 OH Vitamin D Total Assay	Biomerieux; Paris, France	Total 25(OH)D in human serum or plasma	Enzyme-Linked Fluorescent Assay technique. Single test format and calibration and control only once a month	The total 25(OH)D assay is very well correlated to the LC-MS reference method. https://www.biomerieux-diagnostics.com/vidasr-25-oh-vitamin-d-total
Fujirebio Lumipulse® G non-competitive assay for 25(OH)-vitamin D	A reformulated version of the Abbott Architect 25(OH)D assay. Fujirebio, Miraca Group, Tokyo, Japan	Chemiluminescence enzyme immunoassay for determination of 25 (OH)D and other hydroxyl vitamin D metabolites	Non-competitive automated immunoassay assay for 25(OH)-vitamin D	A reformulated version of the Abbott Architect 25(OH)D assay. https://www.fujirebio.com/en/news-events/lumipulser-g-25oh-vitamin-d-assay-has-been-approved-by-the-vitamin-d-standardization
ABCam vitamin D ELISA kit (ab213966)	ABCam, Cambridge, UK	Determination of 25$(OH)D_3$ and 25 $(OH)D_2$ in human plasma and serum	Total 25(OH) vitamin D ELISA kit, assay time 90 min.	Antibody ab213966 is not species specific. https://www.abcam.com/25oh-vitamin-d-elisa-kit-ab213966.html

Information regarding the assays are provided as per the manufacturers' and scientific articles

25(OH)D 25-hydroxyvitamin D, *CIL* chemiluminescence, *CLEIA* chemiluminescence enzyme immunoassay analysis, *CPBA* competitive binding assay, *DBP* vitamin D-binding protein, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assays, *NIST SRM* US National Institute of Standards and Technology (Gaithersburg, Maryland) Standard Reference Material, *RIA* radioimmunoassay

may produce erroneous results in presence of vitamin D_2 and vitamin D_3 epimers (e.g., in children or pregnant women). However, the chromatographic methods of vitamin D measurements are not always available or are time consuming; therefore, more simple and practical techniques have been developed during recent years. In fact, there is no analytical technology that combines the high detection capability of LC-MS/MS and the rapid automated properties of immunoassay methods.

Comparison between immunoassay vs. chromatographic methods

In the first decades of their use, variable immunoassay performance was in large part due to a lack of standardization and reliable calibrators. This issue was addressed in 2008, with the release of the first standard reference material from the NIST for 25(OH)D (SRM972), which contained four samples with different concentrations of $25(OH)D_2$ and $25(OH)D_3$ and one pool also contains 3-epi-$25(OH)D_3$ [47]. Later, this standard was modified and now contains also target values for $24,25(OH)_2D$ (SRM972a). Furthermore, reference methods for the measurement of 25(OH)D were developed at the University of Ghent and the Center for Disease Control (CDC) [48]. With this standardization effort, it was hoped that the analytical performance of 25(OH)D methods would improve, allowing a better comparability of results. For the purpose of this review, we analyzed the performance of the most frequently used 25(OH)D methods in the DEQAS program between 2014 and 2018. Surprisingly, the number of laboratories that used LC-MS/MS remained constantly high (2014 vs 2018: 150 vs 142), whereas the number of immunoassay users decreased by 25% (2014 vs 2018: 748 vs 581) (Table 2). This reduction was mainly driven by a decreased utilization of the two dominant automated immunoassays in this program from IDS and DiaSorin. RIAs are only employed by very few laboratories and the number is continuously shrinking. Average inaccuracy for LC-MS/MS methods was constantly low for both levels tested (<12%). Between-laboratory variability was 13.5% in 2014 with a constant decrease until 2018 (10.8%). In contrast, automated immunoassays showed highly variable performances with average inaccuracies from 2.4% to 28.4% at target concentrations between 20 and 40 nmol/L (1 nmol/L = 0.4 ng/mL) and from −5.3% to +20% at target concentrations between 50 and 70 nmol/L (Table 2). Four years later, the situation had not substantially changed. While some assays continued to have a rather small average bias of <1 nmol/L, others deviated by 9 nmol/L at both levels. By far, the most frequently used method is the DiaSorin Liaison assay. For this assay, inaccuracy varied between −6.4 and 5.2 nmol/L for both levels and across the entire period. IDS ISYS and SIEMENS ADVIA demonstrated a significantly greater bias from 1.8 to 11.8 nmol/L and from −0.4 to 9.0, respectively (Table 2). In recent years, several manufacturers modified their assays and released new versions. While this helped some manufacturers to improve their performance in the DEQAS program, others remained unchanged. For accurate measurements of 25(OH)D, LC-MS/MS remains the gold standard and offers additional benefits through the possibility to determine simultaneously $25(OH)D_2$, $25(OH)D_3$, $24,25(OH)_2D$, and other metabolites [49–53]. This fact has recently been recognized by External Quality Assessment (EQA) providers, such as DEQAS [54]. In their current program, target values for 24,25(OH)2D are also included [55]. Automated immunoassays with <10% bias can be used safely in clinical practice, whereas methods with >10–15% of systematic bias are rather critical. Wise et al. reported an inter-laboratory assessment of the measurement of total 25(OH)D in 15 different laboratories in order to compare immunoassays with LC-MS/MS results [56]. Among the investigated kits, only half of the immunoassay methods reached a coefficient of variation <10% and only 3 of the 8 immunoassays achieved a bias <5% [56].

The variable performance of immunoassays has several reasons, including matrix effects, poor antibody specificity, cross-reactivity with other 25(OH)D metabolites, and limited release of vitamin D from carrier proteins. In particular, $25(OH)D_2$ and heterophilic antibodies are common causes for erratic results in daily practice. Most of the interferences that disturb immunoassays do not influence LC-MS/MS methods, as they remove proteins and lipids completely prior to analysis and distinguish common metabolites with high specificity. In spite of these limitations, chromatographic methods and immunoassay methods have regression slopes close or near to 1.0 with intercepts [57, 58]. In fact, current automated assays have an acceptable overall correlation with LC-MS/MS methods. Thus Passing–Bablok regression analyses for the most popular immunoassays have been reported for the assay from Abbott, DiaSorin, IDS, and Roche with mean bias <3% as compared to LC-MS/MS [36]. Studies that compared immunoassays with LC-MS/MS are summarized in Table 3 [24, 59–71].

Suggested methods for the measurement of different vitamin D metabolites

Until recently, 25(OH)D was the only vitamin D metabolite of interest to explore vitamin D status and metabolism. Unfortunately, the determination of this vitamin D metabolite, as well as the levels that need to be achieved in healthy or ill individuals are quite problematic and remain an important matter of debate [21, 22]. Recently, other vitamin D metabolites, like 24,25(OH)2D, “bioavailable” or

Table 2 Performance comparison in the DEQAS program of commonly used vitamin D assays.

Method	Manufacturer/instrument	*N* 2014/2018	Bias/imprecision	Level 1 (20–40 nM/L)		Level 2 (50–70 nM/L)	
				2014	2018	2014	2018
LC-MS/MS	Various	150/142	Bias (nM/%)	+3.1/10.6	+1.9/8.2	+6.0/12.6	+3.0/6.7
			Imprecision (%)	13.5	12.7	11.6	10.8
Automated immunoassays	Abbott Architect (old)	75/8	Bias (nM/%)	+4.2/14.4	+0.4/1.7	+2.8/5.9	−1.9/4.2
			Imprecision (%)	11.4	4.7	9.0	5.0
	Abbott Architect (new)	—/68	Bias (nM/%)	—	+0.3/1.3	—	−1.3/2.9
			Imprecision (%)	—	7.9	—	4.3
	IDS ISYS (old)	125/43	Bias (nM/%)	+4.5/15.4	+11.8/50.6	+9.5/20.0	+5.6/12.5
			Imprecision (%)	16.6	26.9	12.7	8.2
	IDS ISYS (new)	—/16	Bias (nM/%)	—	+2.8/12.0	—	−1.1/2.44
			Imprecision (%)	—	14.8	—	12.6
	Roche Total 25(OH)D	137/143	Bias (nM/%)	+1.8/6.2	+5.3/22.7	+0.1/0.21	+0.6/1.3
			Imprecision (%)	17.9	14.0	12.4	9.5
	Roche Vitamin D Total II	—/35	Bias (nM/%)	—	+3.9/16.7	—	+0.2/0.33
			Imprecision (%)	—	16.5	—	11.9
	Siemens ADVIA Centaur	58/55	Bias (nM/%)	+8.3/28.4	+7.1/30.5	+9.0/18.9	+9.0/20.1
			Imprecision (%)	17.9	14.0	12.4	9.5
	DiaSorin Liaison Total	281/198	Bias (nM/%)	+0.7/2.4	+2.3/9.9	−2.5/5.3	+3.2/7.1
			Imprecision (%)	10.4	11.7	9.8	8.2
Manual immunoassays	IDS EIA	57/10	Bias (nM/%)	+6.8/23.3	+5.8/12.2	+5.8/12.2	+2.3/5.1
			Imprecision (%)	13.3	15.3	13.8	18.2
	DiaSorin RIA	10/—	Bias (nM/%)	+6.0/20.6	—	+4.9/10.3	—
			Imprecision (%)	17.4	—	24.7	—
	IDS RIA	5/5	Bias (nM/%)	+8.2/28.0	+11.4/48.9	+12.5/26.3	+12.9/28.7
			Imprecision (%)	5.1	18.1	6.5	47.4

25(OH)D 25-hydroxyvitamin D, *DEQAS* Vitamin D External Quality Assurance Scheme, *EIA* enzyme immunoassay, *IDS* Immunodiagnostic Systems, *RIA* radioimmunoassay

"free" 25(OH)D, vitamin D itself, and 1,25(OH)2D, have emerged as potential new players to better understand the important vitamin D pathway [72, 73].

In the following lines, we will provide a brief overview on the issues regarding 25(OH)D assays and standardization and we will evoke the different metabolites as potential markers of choice to explore vitamin D metabolism.

25(OH)D and standardization program

The first commercially available RIA for 25(OH)D determination was based on a method described by Hollis et al. in 1993 [2], and traditional 25(OH)D cut-offs in use today for vitamin D deficiency (either 20 or 30 ng/ml (50 or 75 nmol/l)) have been defined on the basis of studies that predominantly used this assay [4, 74, 75]. Today, most of the major IVD companies propose a method for 25(OH)D determination. These methods use a competition design, except the one from Fujirebio on the Lumipulse, which is a non-competitive (sandwich) method [76]. If HPLC methods have been described a long time ago, they have been superseded by LC-MS/MS ones, which have the great advantage to present an increased sensitivity and selectivity. Three Reference Measurement Procedure (RMP) have been described and accepted, which allow the exact quantification of 25(OH)D in serum samples.

In these past years, the International Federation for Clinical Chemistry and Laboratory Medicine has made great efforts to promote standardization of laboratory assays [77], which is important to achieve comparable results across different methods and manufacturers. In 2010, the Vitamin D Standardization Program was established to improve the standardization of 25(OH)D assays and a method is considered as standardized if the coefficient of variation is <10% and the bias <5% [78, 79]. The list of these standardized methods can be found on the CDC website (http://www.cdc.gov/labstandards/pdf/hs/CDC_Certified_Vitamin_D_Procedures.pdf). Based on these criteria, a retrospective study demonstrated that the standardization of original measurements of serum 25(OH)D had a significant impact on estimates of vitamin D status, with a higher means overall and in age- and sex-specific analyses in standardized levels, demonstrating that the standardization of assays for 25(OH)D measurement should become common practice [80]. In 2019, 19 methods, coming either from IVD companies or clinical laboratories, were

Table 3 Comparison between automated immunoassays (AIAs) and liquid chromatography mass spectrometry (LC-MS/MS) or gas chromatography (GC) or reference standards.

Authors (ref)	Site of study	Population	Reference (control) method	Assay methods; company	Measured analytes	Comparator (reference sample)	Detected bias	Comments
Abou El Hassan et al. [59]	Toronto, Canada	$n = 137$ participants	LC-MS/MS	Bio-Rad BioPLex 2200; DiaSorin LIAISON	25(OH)D	—	The total imprecision was 9.4%, 6.9%, and 4.5% at concentrations of 39.4, 70.6, and 242.8 nmol/L, respectively. There was a strong association for samples containing 25(OH)D_2 alone ($n = 5$; R(2) = 0.999), 25(OH)D_3 alone ($n = 119$; R(2) = 0.935) or both (n = 13; R(2) = 0.919)	In samples tested by all three methods ($n = 56$), the correlation between the BioPlex and the LIAISON (R(2) = 0.853) was poorer than that of the BioPlex and LC-MS/MS (R(2) = 0.942). The BioPlex assay is suitable for the measurement of total serum 25(OH)D
Al-Haddad et al. [60]	Bahrain	18 children aged 6–12 years, who received a confirmed diagnosis of type 1 diabetes mellitus	Ultra-performance (UP) LC-MS/MS	Chemiluminescence microparticle immunoassay (CMIA)	Total 25(OH)D_2 and 25(OH)D_3	—	The mean total of 25(OH)D levels assessed by UPLC-MS was 49.7 ± 18.8 vs 44.60 ± 13.20 by CMIA ($p = 0.004$)	The CMIA overestimated insufficient values and underestimated deficiency, when compared to UPLC-MS
Annema et al. [61]	Zurich, Switzerland	88 healthy subjects with vitamin D insufficiency <50 nmol/L who were randomized to receive a single 100,000 IU dose of vitamin D_3 ($n = 48$) or placebo ($n = 40$)	LC-MS/MS	Architect 25(OH) assay (Abbott); Roche assay	25(OH)D	NIST SRM 2972	The Architect 25(OH)D assay showed an intra- and inter-assay imprecision of <5% and a good correlation with LC-MS/MS in both vitamin D-insufficient and supplemented subjects, with a negative mean bias of 17.4% and 8.9%, respectively	Compared to Roche assay, Abbott assay underestimated 25(OH)D results in insufficient subjects (<50 nmol/L; mean negative bias of 17.1%). A moderate agreement in classification of vitamin D-insufficient and -supplemented individuals into different vitamin D states between Architect 25(OH)D method and LC-MS/MS
Berry et al. [62]	Norwich, UK	Participants of Northern Finland Birth Cohort 1966	LC-MS/MS	200 samples were assayed using DiaSorin RIA	25(OH)D	—	Concentrations measured by LC-MS were much higher than those measured by DiaSorin RIA, with a mean difference of 12.9 ng/ml	—
Cavalier et al. [63]	Liège, Belgium	250 serum samples in healthy Caucasians and Africans, osteoporotic, hemodialyzed, and intensive care patients, and third trimester pregnant women	LC-MS/MS	Lumipulse G	25(OH)D_2 and C3-epimer recovery	—	Concordance with LC-MS/MS, which was generally higher than competitors, that became excellent in healthy Caucasian individuals ($y = 1.00x - 1.35$ ng/mL)	Measurement uncertainty, with a probability of 95%. No cross-reactivity was observed with C3-epimer
Dowling et al. [64]	Ireland	—	LC-MS/MS without derivatizing agent	Chemiluminescence microparticle immunoassay	25(OH)D	—	The immunoassay overestimation of 25(OH)D as compared with LS-MS was on both 25(OH)D and 24R,25(OH)$_2D_3$ concentrations	The immunoassay overestimation of 25(OH)D measurements relative to LC-MS was related on both 25(OH)D and 24R,25(OH)$_2D_3$ concentrations

Table 3 (continued)

Authors (ref)	Site of study	Population	Reference (control) method	Assay methods; company	Measured analytes	Comparator (reference sample)	Detected bias	Comments
Gorman et al. [65]	Perth, Western Australia	10 adults given an IM injection of 600,000 IU vitamin D_3	LC-MS/MS	Abbot Architect i2000 immunoassay	25(OH)D	—	Serum 25(OH)D levels increased at 3, 4, and 24 weeks postinjection, peaking at 4 weeks [mean ± SEM of 126 ± 7.9 nM (immunoassay) and 100 ± 5.5 nM (LC-MS/MS)]. Serum 24,25 $(OH)_2D_3$ levels increased at 3 and 4 weeks postinjection	—
Ihara et al. [66]	Japan	20 serum samples (pilot study) and 110 additional samples were assessed by six automated 25(OH)D immunoassays available in Japan	LC-MS/MS could completely separate 25(OH)D_2, 25(OH) D_3, and 3-epi-25(OH) D_3	All 6 AIAs produced results greater than ±3·SD. In the pilot study, four of the six AIAs had an average percentage bias (CI larger than ±5%)	Total 25 (OH)	The observed values including total 25(OH)D in SRM 972a were all within ±1·SD of the assigned values	The bias converged from −6.5% to 3.2% after adjustment by LC-MS. 25(OH)D concentrations in AIAs all adjusted to LC-MS converged within ±5% from consensus values	However, some AIAs showed negative or positive bias from the consensus values
Lee et al. [67]	Korea	—	Rapid LC MS/MA 25 (OH)D_2 and 25(OH) D_3 in human serum	LC-MS/MS compared to two automated immunoassays (RIAISON and ADVIA)	25(OH)D_2 and 25(OH) D_3	SRM 972a	Compared to the LC-MS, the mean biases of the RIAISON and ADVIA were +2.4 and +7.9 ng/mL, respectively	The agreement of the LC-MS with the RIAISON was better than that with the ADVIA
Li et al. [68]	Hunan, China	Serum samples of 59 healthy subjects. A dosage of 200,000 IU vitamin D_2 was given after first sampling	LC-MS/MS	Siemens ADVIA Centaur Vitamin D Total (Centaur) and Roche Elecsys Vitamin D Total (Elecsys)	Serum 25 (OH)D_2, 25 (OH)D_3	—	The results showed poor agreement between the immunoassays and LC-MS/MS. The percentage of 25(OH)D_2 cross-reactivity was 45.3% for Centaur and 41.2% for Elecsys and there was no significant difference between Centaur and Elecsys	Centaur and Elecsys perform unsatisfactorily in measuring 25(OH)D levels, especially for 25 (OH)D_2 cross-reactivity. Therefore, clinicians need to be aware of the underestimation of vitamin D status when using these immunoassays
Moreau et al. [24]	—	150 serum samples ranging from 7 to 92 ng/mL were analyzed by all the methods	LC-MS/MS	VIDAS® 25(OH)D Total assay	25(OH) D total	Standardized to NIST reference materials	The VIDAS® 25(OH)D total assay showed excellent correlation to the LC-MS/MS results. The limit of quantification was determined at 8.1 ng/mL	25(OH)D_2 was >80%. At concentrations of 10.5, 26, and 65.1 ng/mL, within-run CVs were 7.9%, 3.6%, and 1.7%, respectively
Nikooyeh et al. [69]	Tehran, Iran	275 serum samples	HPLC	DIAsource—enzyme immunoassay (EIA), DIAsource—radioimmunoassay (RIA), Roche—electrochemiluminescence (ECL), DiaSorin—CLIA, and HPLC, as the reference method	25(OH)D	—	Serum 25(OH)D assay results from all systems correlated with those from HPLC, particularly ECL showed a positive bias (+3.8 nmol/L), whereas CLIA had a negative bias (−11.9 nmol/L)	Both EIA and RIA showed a more or less similar positive bias (8.0 and 8.1 nmol/L, respectively)
	Japan	—				—		

Table 3 (continued)

Authors (ref)	Site of study	Population	Reference (control) method	Assay methods; company	Measured analytes	Comparator (reference sample)	Detected bias	Comments
Satoh et al. [96]			LC-MS/MS simultaneous measurements of multiple analytes	LC-MS/MS compared to conventional RIA	$25(OH)D_3$, 3-epi-25 $(OH)D_3$, 25 $(OH)D_2$, and 24,25(OH) $2D_3$		The values obtained by the RIA method exhibited a mean bias of about 8.35 ng/mL	The LC-MS/MS simultaneous allows the assay of the 4 vitamin D metabolites $25(OH)D_3$, 3-epi-$25(OH)D_3$, $25(OH)D_2$, and $24,25(OH)_2D_3$
Spanaus et al. [71]	Zurich, Switzerland	93 serum samples	LC-MS/MS	Immunoassays from DiaSorin and from Immunodiagnostic Systems (IDS), both fully automated extraction and measurement of $1,25(OH)_2D$	$1,25(OH)_2D$	20 Vitamin D DEQAS samples	Total imprecision was 5.2% or less for the DiaSorin test but reached 20.1% for the IDS iSYS test. $1,25(OH)_2$ vitamin D concentrations measured with the DiaSorin assay showed a strong correlation with $1,25(OH)_2D$ levels measured by LC-MS/MS and a good agreement with method-specific means of DEQAS samples	The DiaSorin test is a valuable analytical option for the determination of $1,25(OH)_2D$. The IDS iSYS test overestimated serum $1,25(OH)_2D$ concentrations, particularly at higher levels

Comparison of liquid chromatography mass spectrometry (LC-MS/MS) or gas chromatography (GC) or reference standards in terms of the characteristics of the automated immunoassays (AIAs), measured analytes, comparator, and measured bias

AIA automated immunoassay, *CI* confidence interval, *CLIA* chemiluminescent immunoassay, *DEQUAS* External Quality Assessment Scheme, *ECL* electrochemiluminescence, *EIA* enzyme immunoassay, *HPLC* high-performance liquid chromatography, *PTH* parathyroid hormone, *ref* reference, *SRM* Standard Reference Material

considered as standardized against the RMP, but still several assays do not comply with the Vitamin D Standardization Program requirement [81]. However, the proportion of the 40 samples that met the bias criterion (<5%) is quite different from one method to the other and ranged from 23% to 85%, with LC-MS/MS methods presenting better results than immunoassays [82, 83].

24,25(OH)$_2$D determination and the vitamin D metabolite ratio (VMR)

24,25(OH)$_2$D can only be measured by LC-MS/MS, which allows simultaneous quantification of 25(OH)D and allow calculating the 25(OH)D/24,25(OH)$_2$D ratio, also known as VMR. The VMR has recently been proposed to better reflect vitamin D deficiency [84]. Indeed, CYP24A1, the enzyme allowing the degradation of 25(OH)D and 1,25(OH)$_2$D into 24,25(OH)$_2$D and 1,24,25(OH)$_3$D sees its expression increased when there is an increased binding and activation of the VDR in response to 1,25(OH)$_2$D [85]. Hence 24,25(OH)$_2$D concentration may thus reflect VDR activity, which is not really the case of 25(OH)D. It has recently been demonstrated that lower 24,25(OH)$_2$D concentration and lower VMR were associated with increased hip fracture risk in community-living older men and women, whereas 25(OH)D was not associated with hip fracture risk. Another interest of 24,25(OH)$_2$D and VMR is that, although concentrations of 25(OH)D and 24,25(OH)$_2$D strongly correlate with each other and are both lower in black Americans than in whites, blacks and whites have equivalent median VMR values [86]. In CKD patients, it has been shown that 24,25(OH)$_2$D was better associated with parathyroid hormone (PTH) than 25(OH)D or 1,25(OH)$_2$D [87]. On the other hand, it is clearly now demonstrated that biallelic mutations in CYP24A1 led to idiopathic infantile hypercalcemia [88]. Many heterozygous mutations of CYPA24A1 have recently been described [89]. If they are associated with a less dramatic phenotype than homozygous mutations, patients suffering from these mutations often present with hypercalcemia, suppressed PTH, and renal stones [90]. A VMR ratio >50 or even 80 should lead to a genetic research of a CYP24A1 mutation. Again, this measurement should be standardized. Fortunately, one candidate RMP has been published [91] and NIST SRM 2972a proposes four standards with certified values [92]. DEQAS data report that about ten laboratories provide 24,24(OH)$_2$D results. These data show quite a large variability, which can partially be attributed not only to the low concentration of the analyte but also to the lack of ongoing standardization program. This latter will be (probably) even more important than the 25-OHD itself, since small variations in 24,25(OH)$_2$D have a dramatic impact on the VMR.

Free 25(OH)D

As a steroid hormone, 25(OH)D circulates bound to carrier proteins, mainly DBP (>85%), albumin (10–15%), and as a free form (<1%). In many cells, most of the uptake of vitamin D is achieved through the megalin system, that captures the VTD-DBP complex, but the free 25(OH)D can also passively enters the cells. This free fraction can be estimated through an equation that takes into consideration the total amount of 25(OH)D, the DBP, and albumin concentrations and their respective affinity for 25(OH)D [93]. This requires an accurate measurement of DBP and it has been shown that only polyclonal antibodies used to measure DBP could correctly detect the different DBP alleles. Free 25(OH)D can also be measured by a commercial ELISA, but there is, up to now, no reference method for this measurement [94]. Even if free 25(OH)D has been proposed as a new way to define vitamin D deficiency, especially in African-American, more studies are needed to answer these assay questions and to characterize associations in diverse populations and conditions.

25(OH)-C3 epimer

Vitamin D can be metabolized through C3 epimerization, a minor pathway. The epimer share identical structure but a different stereochemical configuration and its physiological importance remains unknown. It is found in a significantly high percentage (up to 60%) of neonates and children of age up to 1 year and in adults at a much lower concentration (6.1% of the total 25(OH)D, ranging from 0% to 47%) [95]. The epimer is not detected by the immunoassays but can falsely be measured by LC-MS/MS methods that are not able to separate it from 25(OH)D. Recently, Satoh et al. [96] described a method that simultaneously measures serum 25(OH)D$_3$, 3-epi-25(OH)D$_3$, 25(OH)D$_2$, and 24,25(OH)$_2$D$_3$ levels. Serum samples are first subjected to LC-MS/MS, then ionized by electrospray ionization, and finally detected by selected reaction monitoring [96]. This type of accurate approach allows to neutralize overestimated results due to the 3-epi-vitamin D metabolites observed with LC-MS/MS.

Dried blood spots (DBSs)

Although serum and plasma are standard for measuring the circulating 25(OH)D concentration, DBSs are increasingly exploited in large-scale epidemiological studies [97, 98]. There is good agreement between measurements of 25(OH)D from DBS and plasma, which makes DBS an accurate and robust method that can be used to screen 25(OH)D concentrations [99–102]. Also, the use of DBS for measuring 25(OH)D offers several advantages over serum or plasma. Collection of blood spots is minimally invasive.

Only a small volume is required for the extraction and quantification of 25(OH)D [103]. In addition, DBSs require no refrigeration and are stable in the dark at room temperature, are suitable for long-term storage, and can be easily transported. This creates many opportunities in medical research; for example, DBS routinely collected on Guthrie cards at birth can be used to determine how neonatal vitamin D status affects health outcomes later in life.

$1,25(OH)_2D$

From clinical perspective, $1,25(OH)_2D$ determination should be limited to the differential diagnosis of inborn and acquired disorders in 25(OH)D metabolism and $1,25(OH)_2D$ recognition [4]. These include disorders like sarcoidosis and other granulomatous disorders, pseudovitamin D deficiency and vitamin D-resistant rickets, hypophosphatemic rickets, hyperparathyroidism, 24-hydroxylase deficiency, and tumor-induced osteomalacia. Even if 1,25(OH)D levels are decreased in renal failure, its measurement is not recommended in that context by the Kidney Disease Improving Global Outcomes guidelines [104]. $1,25(OH)_2D$ determination is challenging because it circulates in the picomolar range. If tedious RIAs were the main methods available for its measurement, automated immunoassays are now proposed by IDS on the iSYS or DiaSorin on the Liaison. Among these, the DiaSorin test provides a valuable analytical approach to estimate $1,25(OH)_2D$ levels, with a total imprecision of 5.2% comparison with LC-MS/MS (Table 3) [71]. If LC-MS/MS methods have already been described, there is, up to now, no reference method available for $1,25(OH)_2D$

Alternatives to blood tests

Although effective for correcting hypovitaminosis D, universal supplementation remains, however, controversial [105, 106]. Vitamin D intoxication is extremely rare often due to ingestion of extremely excessive quantities in the range of 50,000–1 million IUs of vitamin D daily for months to years and thus is of little concern. However, patients with granulomatous disorders or 24-hydroxylase deficiency need to be monitored carefully for their vitamin D intake because of their increased sensitivity to vitamin D. Although some have suggested that there is lack of evidence for vitamin D supplementation to be cost-effective [107], many health organizations including the Endocrine Society considers vitamin D deficiency and insufficiency to be a global health problem and increased food fortification programs and supplementation are warranted [4, 22]. There is a misperception about the need to measure 25(OH)D before instituting supplementation as the Endocrine Society and other medical societies do not recommend broad screening before implementing vitamin D supplementation and treatment. Screening should be made in children and adults with specific risk factors as outlined in Endocrine Society Practice Guidelines on Vitamin D [22]. There is also no evidence that based on baseline blood levels of 25(OH)D that the vitamin D dose needs to be adjusted even though it has been suggested that it is required [108]. The reason for this is that there are at least 4 different 25 hydroxylases in the liver that have different binding affinity for the vitamin D receptor. Patients with blood level of 25(OH)D of 10 ng/mL, for example, will quickly raise their blood level of 25 (OH)D into the range of 15–20 ng/mL. However, once a level of approximately 20 ng/mL is achieved it has been reported that for every 100 IUs of vitamin D ingested the blood level will raise by approximately 0.6–1 ng/mL. Patients who are obese require 2–3 times more vitamin D [19]. Unfortunately, these misconceptions have led to marked increases in the ordering of serum 25(OH)D levels, especially in primary care.

On evaluating results from previous studies on alternatives to blood tests, we identified a final number of 12 studies in human beings that were designed and/or tested a questionnaire for the identification of people with hypovitaminosis D (Table 4) [109–120]. The studies tested the performance of already existing dietary questionnaires [111, 112, 114] or general physical questionnaires [116, 117, 120] or isolated questions [113, 115] to detect hypovitaminosis D. Almost all of them used conventional linear statistical methods, and only one used artificial neuronal network methods (i.e., artificial intelligence) [118]. Whatever the model tested, results showed rather good sensitivity to hypovitaminosis D (range, 46–91%) [115, 118] but only modest specificity (range, 35–89%) [116, 118], and each time only a single metrological quality was observed (either sensitive or specific), except with the Vitamin D Status Predictor tool [118]. The latter 16-item questionnaire was able to identify vitamin D insufficiency ≤75 nmol/L with a diagnostic efficacy >96% in community-dwelling older adults [118] and was also effective in hospitalized geriatric inpatients [121]. It can be used as a heteroquestionnaire but is also suitable as a self-administered questionnaire [122]. Other dedicated questionnaires are also available, such as the one developed from the results of the SUVIMAX study and applicable to the general middle-aged population [119].

These findings open new perspectives for the screening for hypovitaminosis D. Such inexpensive and non-invasive tools may undoubtedly help clinicians in decisions to supplement their patients without routinely resorting to an expensive blood test. Consistently, their potential interest in clinical practice was highlighted in recent French guidelines for vitamin D supplementation [123]. Further investigations are needed to determine the feasibility, cost-effectiveness, and clinical utility of such tools in routine practice to

Table 4 Main characteristics of previous studies having designed and/or tested a questionnaire for the identification of people with hypovitaminosis D.

Author (ref)	Population	Location	Hypovitaminosis D	Questionnaire	Metrological properties of questionnaire
Bolek-Berquist et al. [109]	184 healthy young adults Age: 18–40 years	Wisconsin, USA	Serum 25(OH)D <75 nmol/L	General physical questionnaire, including suntan, use sunscreen, and ingestion of serving of milks	Sensitivity 79% Specificity 78%
Gagnon et al. [110]	153 healthy young women Age: 18–41 years	Quebec, Canada	Serum 25(OH)D ≤50 nmol/L	General physical questionnaire, including body mass index, parathyroid hormone, oral contraceptive use, and travels	40% of the variance in serum 25OHD explained by the questionnaire
Formiga et al. [111]	312 older community-dwellers Age: ≥85 years	Spain	Serum 25(OH)D ≤62.4 nmol/L	MNA	Poor MNA score associated with hypovitaminosis D: OR = 1.07 [95% CI: 1.00–1.14]
Hacker-Thompson et al. [112]	122 post-menopausal women Age: ≥85 years	California, USA	Not applicable	Two dietary questionnaires (BVDQ, Block HHHQ98)	No correlation between serum 25OHD and scores on the questionnaires
Annweiler et al. [113]	751 community-dwelling older women Age: ≥75 years	France	Serum 25(OH)D <75 nmol/L	Single question on sun exposure	Sensitivity 52% Specificity 35%
Tsagari et al. [114]	101 patients with hip fracture and 85 community-dwellers without hip fracture Age: ≥65 years	Greece	Serum 25(OH)D <50 nmol/L	MNA	Sensitivity 90.9% Specificity 53.6%
Chevallereau et al. [115]	261 geriatric in- and outpatients Age: ≥65 years	France	Serum 25(OH)D ≤75 nmol/L	Single question on dietary habits	Sensitivity 46% Specificity 74%
Nabak et al. [116]	609 post-menopausal women Age: 60–75 years	Wisconsin, USA	Serum 25(OH)D <50 nmol/L	General physical questionnaire, including skin color, suntan, use sunscreen, sun exposure, and weight	Sensitivity 89% Specificity 35%
Hamdan et al. [117]	261 geriatric in- and outpatients Age: ≥65 years	France	Serum 25(OH)D ≤75 nmol/L	General physical questionnaire, including ethnicity, sadness, body mass index, and history of vertebral fractures	Identification of 3 clinical profiles with 100% hypovitaminosis D
Annweiler et al. [118]	1924 non–vitamin D-supplemented community-dwelling older adults Age: ≥65 years	France	Serum 25(OH)D ≤25 nmol/L Serum 25(OH)D ≤50 nmol/L Serum 25(OH)D ≤75 nmol/L	Nonlinear algorithm combining age, gender, BMI, undernutrition, number of drugs used per day, polymorbidity, history of falls, fear of falling, use walking aids, history of vertebral fractures, use anti-osteoporotic drugs, use glasses, cognitive disorders, sad mood, use psychoactive drugs, living alone	For ≤25 nmol/L: Sensitivity 65%; Specificity 89%; Diagnostic efficacy 82.5% For ≤50 nmol/L: Sensitivity 87%; Specificity 70%; Diagnostic efficacy 81.5% For ≤75 nmol/L: Sensitivity 98%; Specificity 81%; Diagnostic efficacy 96.3%
Deschasaux et al. [119]	1557 non–vitamin D-supplemented middle-aged adults Age: 45–65 years	France	Serum 25(OH)D ≤50 nmol/L	General physical questionnaire, including gender, BMI, physical activity, latitude, season, usual sun exposure, Fitzpatrick phenotype	Sensitivity 67% Specificity 63%
Chevallereau et al. [120]	1991 community-dwelling older adults Age: ≥65 years	France	Serum 25(OH)D ≤50 nmol/L	General physical questionnaire, including polymorbidity, obesity, gait disorders, and sadness	Identification of 5 clinical profiles with different prevalence of hypovitaminosis D ranging between 61% and 87%

25(OH)D 25-hydroxyvitamin D, *BMI* body mass index, *BVDQ* Brief Vitamin D Questionnaire, *Block HHHQ98* Block Health History and Habits Questionnaire 1998, *MNA* Mini Nutritional Assessment, *ref* reference, *USA* United States of America

promote appropriate medical decisions. Their efficiency for replacing monitoring blood tests after the initiation of vitamin D supplements should also be questioned.

Conclusion

There has been significant progress in the development of assays for 25(OH)D and its metabolites. LC-MS/MS continues to serve as the gold standard to quantitatively determine one of the clinically relevant vitamin D metabolites in the circulation. The accurate measurement of total 25(OH)D has made a significant contribution for the clinical management of vitamin D deficiency in patients who are at high risk [22]. This assay, however, should not be used for screening purposes [22]. Whether the free level of 25(OH)D or 24, 24(OH)D will offer any further insight into vitamin D status remains to be determined and is not recommended based on the literature at this time. The assay for 1,25(OH)$_2$D should only be used for inherited and acquired disorders in vitamin D metabolism and recognition and is of no value in determining vitamin D status. The use of a variety of standardized surveys may be of value in alerting health-care professionals about the vitamin D status of their patients.

Author contributions BA and AC designed the study. BA, EC, HPB, FRP-L, MTL-B, GRP-R, PC, CA, SDC, SZ, MH and AF performed the literature search, screening potentially eligible studies, extracting and analyzing data, interpreting results, and contributed to the writing of the article. BA, FRP-L, MTL-B, GRP-R, PC, CA, SZ and MH prepared the tables. MFH contributed to the abstract and wrote the conclusion. BA, AC and MFH critically revised the article for important intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest EC has consulted for DiaSorin, IDS, Fujirebio, and bioMérieux. MFH is a consultant for Quest Diagnostics Inc. MH collaborated in research projects of DiaSorin, Fujirebio, and Roche and received honoraria for scientific presentations from DiaSorin, SIEMENS, and Roche. The other co-authors declare no potential conflict of interest.

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References

1. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord. 2017;18:153–65.
2. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. Clin Chem. 1993;39:529–33.
3. Ersfeld DL, Rao DS, Body JJ, Sackrison JL Jr, Miller AB, Parikh N, et al. Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. Clin Biochem. 2004;37:867–74.
4. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.
5. Roux C, Bischoff-Ferrari HA, Papapoulos SE, de Papp AE, West JA, Bouillon R. New insights into the role of vitamin D and calcium in osteoporosis management: an expert roundtable discussion. Curr Med Res Opin. 2008;24:1363–70.
6. Muscogiuri G, Altieri B, Annweiler C, Balercia G, Pal HB, Boucher BJ, et al. Vitamin D and chronic diseases: the current state of the art. Arch Toxicol. 2017;91:97–107.
7. Muscogiuri G, Barrea L, Altieri B, Di Somma C, Bhattoa H, Laudisio D, et al. Calcium and vitamin D supplementation. Myths and realities with regard to cardiovascular risk. Curr Vasc Pharm. 2019;17:610–7.
8. Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R, et al. Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. Diabetes Care. 2015;38:521–8.
9. Grant WB, Moukayed M. Vitamin D3 from ultraviolet-B exposure or oral intake in relation to cancer incidence and mortality. Curr Nutr Rep. 2019;8:203–11.
10. Altieri B, Grant WB, Della Casa S, Orio F, Pontecorvi A, Colao A, et al. Vitamin D and pancreas: the role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. Crit Rev Food Sci Nutr. 2017;57:3472–88.
11. Altieri B, Muscogiuri G, Barrea L, Mathieu C, Vallone CV, Mascitelli L, et al. Does vitamin D play a role in autoimmune endocrine disorders? A proof of concept. Rev Endocr Metab Disord. 2017;18:335–46.
12. Muscogiuri G, Altieri B, Penna-Martinez M, Badenhoop K. Focus on vitamin D and the adrenal gland. Horm Metab Res. 2015;47:239–46.
13. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem. 2012;58:531–42.
14. Enko D, Fridrich L, Rezanka E, Stolba R, Ernst J, Wendler I, et al. 25-hydroxy-Vitamin D status: limitations in comparison and clinical interpretation of serum-levels across different assay methods. Clin Lab. 2014;60:1541–50.
15. Bikle DD. Vitamin D assays. Front Horm Res. 2018;50:14–30.
16. Rahme M, Al-Shaar L, Singh R, Baddoura R, Halaby G, Arabi A, et al. Limitations of platform assays to measure serum 25OHD level impact on guidelines and practice decision making. Metabolism. 2018;89:1–7.
17. Binkley N, Krueger DC, Morgan S, Wiebe D. Current status of clinical 25-hydroxyvitamin D measurement: an assessment of between-laboratory agreement. Clin Chim Acta. 2010;411:1976–82.
18. Stokes CS, Lammert F, Volmer DA. Analytical methods for quantification of vitamin D and implications for research and clinical practice. Anticancer Res. 2018;38:1137–44.
19. Tirabassi G, Salvio G, Altieri B, Ronchi CL, Della Casa S, Pontecorvi A, et al. Adrenal disorders: is there any role for vitamin D? Rev Endocr Metab Disord. 2017;18:355–62.
20. Muscogiuri G, Altieri B, de Angelis C, Palomba S, Pivonello R, Colao A, et al. Shedding new light on female fertility: the role of vitamin D. Rev Endocr Metab Disord. 2017;18:273–83.
21. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53–8.
22. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and

treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab. 2012;97:1153–8.
23. Hollis BW. Editorial: The determination of circulating 25-hydroxyvitamin D: no easy task. J Clin Endocrinol Metab. 2004;89:3149–51.
24. Moreau E, Bacher S, Mery S, Le Goff C, Piga N, Vogeser M, et al. Performance characteristics of the VIDAS(R) 25-OH Vitamin D Total assay - comparison with four immunoassays and two liquid chromatography-tandem mass spectrometry methods in a multicentric study. Clin Chem Lab Med. 2016;54:45–53.
25. Koivula MK, Matinlassi N, Laitinen P, Risteli J. Four automated 25-OH total vitamin D immunoassays and commercial liquid chromatography tandem-mass spectrometry in Finnish population. Clin Lab. 2013;59:397–405.
26. Moon HW, Cho JH, Hur M, Song J, Oh GY, Park CM, et al. Comparison of four current 25-hydroxyvitamin D assays. Clin Biochem. 2012;45:326–30.
27. Hsu SA, Soldo J, Gupta M. Evaluation of two automated immunoassays for 25-OH vitamin D: comparison against LC-MS/MS. J Steroid Biochem Mol Biol. 2013;136:139–45.
28. Holmes EW, Garbincius J, McKenna KM. Analytical variability among methods for the measurement of 25-hydroxyvitamin D: still adding to the noise. Am J Clin Pathol. 2013;140:550–60.
29. Martins-Costa P, Martins H, Bravo F, Cruz M, Reis J, Oliveira JC. Comparison of automated methods for measurement of 25-hydroxyvitamin D. Clin Lab. 2013;59:885–91.
30. Klapkova E, Cepova J, Pechova M, Dunovska K, Kotaska K, Prusa R. A comparison of four methods (immunochemistry and HPLC) for determination of 25-(OH)-vitamin D in postmenopausal women. Clin Lab. 2017;63:385–8.
31. Wyness SP, Straseski JA. Performance characteristics of six automated 25-hydroxyvitamin D assays: mind your 3s and 2s. Clin Biochem. 2015;48:1089–96.
32. Atef SH. Vitamin D assays in clinical laboratory: past, present and future challenges. J Steroid Biochem Mol Biol. 2018;175:136–7.
33. Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the International Vitamin D External Quality Assessment Scheme. Clin Chem. 2004;50:2195–7.
34. 510(K) FDA submission. Substantial equivalence determination. Decision summary. Assay only template. https://www.accessdata.fda.gov/cdrh_docs/reviews/K071480.pdf (2012).
35. Fuleihan Gel H, Bouillon R, Clarke B, Chakhtoura M, Cooper C, McClung M, et al. Serum 25-hydroxyvitamin D levels: variability, knowledge gaps, and the concept of a desirable range. J Bone Min Res. 2015;30:1119–33.
36. Farrell CJ, Herrmann M. Determination of vitamin D and its metabolites. Best Pract Res Clin Endocrinol Metab. 2013;27:675–88.
37. Lim YK, Park AJ, Kweon OJ, Choi JH. Performance evaluation and measurement uncertainty determination of the new version of the Abbott ARCHITECT 25-OH vitamin D 5P02 assay. Am J Clin Pathol. 2019;151:209–16.
38. Madenci OC, Orcun A, Yildiz Z, Sirmali R, Tuncbilek N, Yucel N, et al. Evaluation of new Beckman Coulter 25(OH) Vitamin D assay and potential improvement of clinical interpretation. Biochem Med (Zagreb). 2017;27:332–41.
39. Gardien P, Moineau MP, Kerspern H, Bordron A, Carre JL. [Comparative study of parathormone and vitamin D measurements by three automats: ADVIA Centaur XP(R) (Siemens), ISYS(R) (IDS) and Liaison(R) (Diasorin)]. Ann Biol Clin (Paris). 2014;72:443–52.
40. Denimal D, Roux S, Duvillard L. Evaluation of the new restandardized 25-hydroxyvitamin D assay on the iSYS platform. Clin Biochem. 2018;52:156–60.
41. Asif M, Groboske SE, Leung EKY, Yeo KJ, van Wijk XMR. Evaluation of a new generation automated assay for 25-hydroxy vitamin D based on competitive protein binding. J Appl Lab Med. 2019;4:247–53.
42. Bonelli FH, Hollis BW. Detection of 1,25-dihydroxyvitamin D in human serum using receptor assisted chemiluminescent hormone assay technology. In: Feldman DP, Pike JW, Bouillon R, Giovannucci E, Goltzman D, Hewison M, editors. Vitamin D. 4th edn. London: Academic Press; 2018. p. 903–7.
43. Garnett E, Li J, Rajapakshe D, Tam E, Meng QH, Devaraj S. Efficacy of two vitamin D immunoassays to detect 25-OH vitamin D2 and D3. Pract Lab Med. 2019;17:e00130.
44. de Koning L, Al-Turkmani MR, Berg AH, Shkreta A, Law T, Kellogg MD. Variation in clinical vitamin D status by DiaSorin Liaison and LC-MS/MS in the presence of elevated 25-OH vitamin D2. Clin Chim Acta. 2013;415:54–8.
45. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, et al. Hydroxyvitamin D assays: An historical perspective from DEQAS. J Steroid Biochem Mol Biol. 2018;177:30–5.
46. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr. 2008;88:507S–10S.
47. Phinney KW, Tai SS, Bedner M, Camara JE, Chia RRC, Sander LC, et al. Development of an improved standard reference material for vitamin D metabolites in human serum. Anal Chem. 2017;89:4907–13.
48. Stepman HC, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate reference measurement procedures for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope-dilution liquid chromatography-tandem mass spectrometry. Clin Chem. 2011;57:441–8.
49. Volmer DA, Mendes LR, Stokes CS. Analysis of vitamin D metabolic markers by mass spectrometry: current techniques, limitations of the "gold standard" method, and anticipated future directions. Mass Spectrom Rev. 2015;34:2–23.
50. Herrmann M, Farrell CL, Pusceddu I, Fabregat-Cabello N, Cavalier E. Assessment of vitamin D status - a changing landscape. Clin Chem Lab Med. 2017;55:3–26.
51. Cavalier E, Souberbielle JC. Vitamin D and its metabolites: from now and beyond. EJIFCC. 2018;29:105–10.
52. Zelzer SG, W; Herrmann, M. Measurement of vitamin D metabolites by mass spectrometry, an analytical challenge. J Lab Precis Med. 2018. https://doi.org/10.21037/jlpm.2018.11.06.
53. Garg U. 25-Hydroxyvitamin D testing: immunoassays versus tandem mass spectrometry. Clin Lab Med. 2018;38:439–53.
54. Burdette CQ, Camara JE, Nalin F, Pritchett J, Sander LC, Carter GD, et al. Establishing an accuracy basis for the Vitamin D External Quality Assessment Scheme (DEQAS). J AOAC Int. 2017;100:1277–87.
55. Carter GD, Ahmed F, Berry J, Cavalier E, Durazo-Arvizu R, Gunter E, et al. External quality assessment of 24, 25-dihydroxyvitamin D3 (24, 25(OH)2D3) assays. J Steroid Biochem Mol Biol. 2019;187:130–3.
56. Wise SA, Phinney KW, Tai SS, Camara JE, Myers GL, Durazo-Arvizu R, et al. Baseline assessment of 25-hydroxyvitamin D assay performance: a Vitamin D Standardization Program (VDSP) Interlaboratory Comparison Study. J AOAC Int. 2017;100:1244–52.
57. Cavalier E, Lukas P, Crine Y, Peeters S, Carlisi A, Le Goff C, et al. Evaluation of automated immunoassays for 25(OH)-vitamin D determination in different critical populations before and after standardization of the assays. Clin Chim Acta. 2014;431:60–5.
58. Cavalier E, Lukas P, Bekaert AC, Carlisi A, Le Goff C, Delanaye P, et al. Analytical and clinical validation of the new Abbot Architect 25(OH)D assay: fit for purpose? Clin Chem Lab Med. 2017;55:378–84.
59. Abou El Hassan M, Lin DCC, Earle T, Spencer M, Blasutig IM. Analytical evaluation of the BioPlex(R) 2200 25-OH vitamin D total assay. Clin Biochem. 2016;49:723–5.

60. Al-Haddad FA, Rajab MH, Al-Qallaf SM, Musaiger AO, Hart KH. Assessment of vitamin D levels in newly diagnosed children with type 1 diabetes mellitus comparing two methods of measurement: a facility's experience in the Middle Eastern country of Bahrain. Diabetes Metab Syndr Obes. 2016;9:11–6.
61. Annema W, Nowak A, von Eckardstein A, Saleh L. Evaluation of the new restandardized Abbott Architect 25-OH Vitamin D assay in vitamin D-insufficient and vitamin D-supplemented individuals. J Clin Lab Anal. 2018;32:e22328.
62. Berry DJ, Dutton J, Fraser WD, Jarvelin MR, Hypponen E. Harmonization study between LC-MS/MS and diasorin RIA for measurement of 25-hydroxyvitamin D concentrations in a large population survey. J Clin Lab Anal. 2017. https://doi.org/10.1002/jcla.22049.
63. Cavalier E, Lukas P, Bekaert AC, Peeters S, Le Goff C, Yayo E, et al. Analytical and clinical evaluation of the new Fujirebio Lumipulse(R)G non-competitive assay for 25(OH)-vitamin D and three immunoassays for 25(OH)D in healthy subjects, osteoporotic patients, third trimester pregnant women, healthy African subjects, hemodialyzed and intensive care patients. Clin Chem Lab Med. 2016;54:1347–55.
64. Dowling KG, Hull G, Sundvall J, Lamberg-Allardt C, Cashman KD. Improved accuracy of an tandem liquid chromatography-mass spectrometry method measuring 24R,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D metabolites in serum using unspiked controls and its application to determining cross-reactivity of a chemiluminescent microparticle immunoassay. J Chromatogr A. 2017;1497:102–9.
65. Gorman S, Zafirau MZ, Lim EM, Clarke MW, Dhamrait G, Fleury N, et al. High-dose intramuscular vitamin D provides long-lasting moderate increases in serum 25-hydroxvitamin D levels and shorter-term changes in plasma calcium. J AOAC Int. 2017;100:1337–44.
66. Ihara H, Kiuchi S, Ishige T, Nishimura M, Matsushita K, Satoh M, et al. Surveillance evaluation of the standardization of assay values for serum total 25-hydroxyvitamin D concentration in Japan. Ann Clin Biochem. 2018;55:647–56.
67. Lee S, Kim JH, Kim SA, Sun YS, Lee A, Park SJ, et al. A rapid and simple liquid-chromatography-tandem mass spectrometry method for measuring 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum: comparison with two automated immunoassays. Ann Clin Lab Sci. 2016;46:645–53.
68. Li L, Zeng Q, Yuan J, Xie Z. Performance evaluation of two immunoassays for 25-hydroxyvitamin D. J Clin Biochem Nutr. 2016;58:186–92.
69. Nikooyeh B, Samiee SM, Farzami MR, Alavimajd H, Zahedirad M, Kalayi A, et al. Harmonization of serum 25-hydroxycalciferol assay results from high-performance liquid chromatography, enzyme immunoassay, radioimmunoassay, and immunochemiluminescence systems: a multicenter study. J Clin Lab Anal. 2017. https://doi.org/10.1002/jcla.22117.
70. Scharla SH, Lempert UG. Evaluation of an automated competitive protein-binding assay for 25-hydroxyvitamin D. Clin Lab. 2016;62:1781–6.
71. Spanaus K, von Eckardstein A. Evaluation of two fully automated immunoassay based tests for the measurement of 1alpha,25-dihydroxyvitamin D in human serum and comparison with LC-MS/MS. Clin Chem Lab Med. 2017;55:1305–14.
72. Galior K, Ketha H, Grebe S, Singh RJ. 10 years of 25-hydroxyvitamin-D testing by LC-MS/MS-trends in vitamin-D deficiency and sufficiency. Bone Rep. 2018;8:268–73.
73. Dirks NF, Ackermans MT, Lips P, de Jongh RT, Vervloet MG, de Jonge R, et al. The when, what & how of measuring vitamin D metabolism in clinical medicine. Nutrients. 2018;10:E482.
74. Perez-Lopez FR. Vitamin D: the secosteroid hormone and human reproduction. Gynecol Endocrinol. 2007;23:13–24.
75. Perez-Lopez FR. Vitamin D and its implications for musculoskeletal health in women: an update. Maturitas. 2007;58:117–37.
76. Omi K, Ando T, Sakyu T, Shirakawa T, Uchida Y, Oka A, et al. Noncompetitive immunoassay detection system for haptens on the basis of antimetatype antibodies. Clin Chem. 2015;61:627–35.
77. The International Federation of Clinical Chemistry and Laboratory Medicine. Bone Metabolism (C-BM). http://www.ifcc.org/ifcc-scientific-division/sd-committees/c-bm/ (2019).
78. Phinney KW, Sempos CT, Tai SS, Camara JE, Wise SA, Eckfeldt JH, et al. Baseline assessment of 25-hydroxyvitamin D reference material and proficiency testing/external quality assurance material commutability: a vitamin D standardization program study. J AOAC Int. 2017;100:1288–93.
79. Sempos CT, Betz JM, Camara JE, Carter GD, Cavalier E, Clarke MW, et al. General steps to standardize the laboratory measurement of serum total 25-hydroxyvitamin D. J AOAC Int. 2017;100:1230–3.
80. Rabenberg M, Scheidt-Nave C, Busch MA, Thamm M, Rieckmann N, Durazo-Arvizu RA, et al. Implications of standardization of serum 25-hydroxyvitamin D data for the evaluation of vitamin D status in Germany, including a temporal analysis. BMC Public Health. 2018;18:845.
81. Bjerg LN, Halgreen JR, Hansen SH, Morris HA, Jorgensen NR. An evaluation of total 25-hydroxyvitamin D assay standardization: where are we today? J Steroid Biochem Mol Biol. 2019;190:224–33.
82. Erdman P, Palmer-Toy DE, Horowitz G, Hoofnagle A. Accuracy-based vitamin D survey: six years of quality improvement guided by proficiency testing. Arch Pathol Lab Med. 2019;143:1531–8.
83. Elsenberg E, Ten Boekel E, Huijgen H, Heijboer AC. Standardization of automated 25-hydroxyvitamin D assays: how successful is it? Clin Biochem. 2017;50:1126–30.
84. Ginsberg C, Katz R, de Boer IH, Kestenbaum BR, Chonchol M, Shlipak MG, et al. The 24,25 to 25-hydroxyvitamin D ratio and fracture risk in older adults: the cardiovascular health study. Bone. 2018;107:124–30.
85. Tryfonidou MA, Oosterlaken-Dijksterhuis MA, Mol JA, van den Ingh TS, van den Brom WE, Hazewinkel HA. 24-Hydroxylase: potential key regulator in hypervitaminosis D3 in growing dogs. Am J Physiol Endocrinol Metab. 2003;284:E505–13.
86. Berg AH, Powe CE, Evans MK, Wenger J, Ortiz G, Zonderman AB, et al. 24,25-Dihydroxyvitamin d3 and vitamin D status of community-dwelling black and white Americans. Clin Chem. 2015;61:877–84.
87. Bosworth CR, Levin G, Robinson-Cohen C, Hoofnagle AN, Ruzinski J, Young B, et al. The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease. Kidney Int. 2012;82:693–700.
88. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. N Engl J Med. 2011;365:410–21.
89. Molin A, Baudoin R, Kaufmann M, Souberbielle JC, Ryckewaert A, Vantyghem MC, et al. CYP24A1 mutations in a cohort of hypercalcemic patients: evidence for a recessive trait. J Clin Endocrinol Metab. 2015;100:E1343–52.
90. Ferraro PM, Minucci A, Primiano A, De Paolis E, Gervasoni J, Persichilli S, et al. A novel CYP24A1 genotype associated to a clinical picture of hypercalcemia, nephrolithiasis and low bone mass. Urolithiasis. 2017;45:291–4.
91. Tai SS, Nelson MA. Candidate reference measurement procedure for the determination of (24R),25-dihydroxyvitamin D3 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem. 2015;87:7964–70.
92. Wise SA, Tai SS, Burdette CQ, Camara JE, Bedner M, Lippa KA, et al. Role of the National Institute of Standards and

Technology (NIST) in support of the vitamin D initiative of the National Institutes of Health, Office of Dietary Supplements. J AOAC Int. 2017;100:1260–76.
93. Peris P, Filella X, Monegal A, Guanabens N, Foj L, Bonet M, et al. Concordance between direct and indirect measurements of free 25-OH vitamin D. Clin Chim Acta. 2017;475:169–71.
94. Malmstroem S, Rejnmark L, Imboden JB, Shoback DM, Bikle DD. Current assays to determine free 25-hydroxyvitamin D in serum. J AOAC Int. 2017;100:1323–7.
95. Bailey D, Veljkovic K, Yazdanpanah M, Adeli K. Analytical measurement and clinical relevance of vitamin D(3) C3-epimer. Clin Biochem. 2013;46:190–6.
96. Satoh M, Ishige T, Ogawa S, Nishimura M, Matsushita K, Higashi T, et al. Development and validation of the simultaneous measurement of four vitamin D metabolites in serum by LC-MS/MS for clinical laboratory applications. Anal Bioanal Chem. 2016;408:7617–27.
97. Kvaskoff D, Ko P, Simila HA, Eyles DW. Distribution of 25-hydroxyvitamin D3 in dried blood spots and implications for its quantitation by tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2012;901:47–52.
98. Mathew EM, Moorkoth S, Rane PD, Lewis L, Rao P. Cost-effective HPLC-UV method for quantification of vitamin D2 and D3 in dried blood spot: a potential adjunct to newborn screening for prophylaxis of intractable paediatric seizures. Chem Pharm Bull (Tokyo). 2019;67:88–95.
99. Heath AK, Williamson EJ, Ebeling PR, Kvaskoff D, Eyles DW, English DR. Measurements of 25-hydroxyvitamin D concentrations in archived dried blood spots are reliable and accurately reflect those in plasma. J Clin Endocrinol Metab. 2014;99:3319–24.
100. Makowski AJ, Rathmacher JA, Horst RL, Sempos CT. Simplified 25-hydroxyvitamin D standardization and optimization in dried blood spots by LC-MS/MS. J AOAC Int. 2017;100:1328–36.
101. Jensen BP, Saraf R, Ma J, Berry S, Grant CC, Camargo CA Jr, et al. Quantitation of 25-hydroxyvitamin D in dried blood spots by 2D LC-MS/MS without derivatization and correlation with serum in adult and pediatric studies. Clin Chim Acta. 2018;481:61–8.
102. Man PW, Heijboer AC, van der Meer IM, Lin W, Numans ME, Lips P, et al. Agreement between measurement of 25-hydroxyvitamin D3 in dried blood spot samples and serum in a Chinese population in the Netherlands. J Steroid Biochem Mol Biol. 2019;195:105472.
103. Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T, et al. A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots. Clin Chim Acta. 2009;403:145–51.
104. Kidney Disease: Improving Global Outcomes CKD-MBD Update Working Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl (2011). 2017;7:1–59.
105. Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, et al. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D-health outcome relationships reflect adverse effects? Dermatoendocrinology. 2016;8:e1187349.
106. Annweiler C, Duval G, Launay CP. Estimating vitamin D status and the choice of supplementation dose. JAMA Intern Med. 2016;176:865.
107. Weaver CM, Bischoff-Ferrari HA, Shanahan CJ. Cost-benefit analysis of calcium and vitamin D supplements. Arch Osteoporos. 2019;14:50.
108. van der Velde RY, Brouwers JR, Geusens PP, Lems WF, van den Bergh JP. Calcium and vitamin D supplementation: state of the art for daily practice. Food Nutr Res. 2014;58 https://doi.org/10.3402/fnr.v58.21796.
109. Bolek-Berquist J, Elliott ME, Gangnon RE, Gemar D, Engelke J, Lawrence SJ, et al. Use of a questionnaire to assess vitamin D status in young adults. Public Health Nutr. 2009;12:236–43.
110. Gagnon C, Baillargeon JP, Desmarais G, Fink GD. Prevalence and predictors of vitamin D insufficiency in women of reproductive age living in northern latitude. Eur J Endocrinol. 2010;163:819–24.
111. Formiga F, Ferrer A, Almeda J, San Jose A, Gil A, Pujol R. Utility of geriatric assessment tools to identify 85-years old subjects with vitamin D deficiency. J Nutr Health Aging. 2011;15:110–4.
112. Hacker-Thompson A, Schloetter M, Sellmeyer DE. Validation of a dietary vitamin D questionnaire using multiple diet records and the block 98 health habits and history questionnaire in healthy postmenopausal women in northern California. J Acad Nutr Diet. 2012;112:419–23.
113. Annweiler C, Schott AM, Beauchet O. Proposal and validation of a quick question to rate the influence of sun exposure in geriatric epidemiological studies on vitamin D. Int J Vitam Nutr Res. 2012;82:412–6.
114. Tsagari A, Toulis KA, Makras P, Skagias K, Galanos A, Lyritis G. Performance of the mini nutritional assessment score in the detection of vitamin D status in an elderly Greek population. Horm Metab Res. 2012;44:896–9.
115. Chevallereau G, Gleyses X, Roussel L, Hamdan S, Beauchet O, Annweiler C. Proposal and validation of a quick question to rate the influence of diet in geriatric epidemiological studies on vitamin d. Int J Vitam Nutr Res. 2013;83:254–8.
116. Nabak AC, Johnson RE, Keuler NS, Hansen KE. Can a questionnaire predict vitamin D status in postmenopausal women? Public Health Nutr. 2014;17:739–46.
117. Hamdan S, Roussel L, Gleyses X, Chevallereau G, Schott AM, Beauchet O, et al. Detection of hypovitaminosis D in older adults: a classification tree analysis. J Am Geriatr Soc. 2014;62:1193–5.
118. Annweiler C, Kabeshova A, Legeay M, Fantino B, Beauchet O. Derivation and validation of a clinical diagnostic tool for the identification of older community-dwellers with hypovitaminosis D. J Am Med Dir Assoc. 2015;16:536 e8–19.
119. Deschasaux M, Souberbielle JC, Andreeva VA, Sutton A, Charnaux N, Kesse-Guyot E, et al. Quick and easy screening for vitamin D insufficiency in adults: a scoring system to be implemented in daily clinical practice. Medicine (Baltimore). 2016;95:e2783.
120. Chevallereau G, Legeay M, Duval GT, Karras SN, Fantino B, Annweiler C. Profiling older community-dwellers with hypovitaminosis D: a classification tree analysis. Int J Vitam Nutr Res. 2019;1–5.
121. Annweiler C, Riou J, Alessandri A, Gicquel D, Henni S, Feart C, et al. Clinical identification of geriatric patients with hypovitaminosis D: the 'Vitamin D Status Predictor for Geriatrics' Study. Nutrients. 2017;9:E658.
122. Annweiler C, Kabeshova A, Callens A, Paty ML, Duval GT, Holick MF. Self-administered Vitamin D status predictor: older adults are able to use a self-questionnaire for evaluating their vitamin D status. PLoS ONE. 2017;12:e0186578.
123. Annweiler C, Legrand E, Souberbielle JC. Vitamin D in adults: update on testing and supplementation. Geriatr Psychol Neuropsychiatr Vieil. 2018;16:7–22.