Review Article

Vitamin D Controversies in the Laboratory Medicine: A Review of Clinical Guidelines and Recommendations

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Keywords

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Abstract

A narrative review of the main guidelines and recommendations published from 2011 up to date about the status of vitamin D deficiency has been carried out. The objective of this review is to discuss the origin of the controversy about the status of this entity, as well as the evolution of the methodological aspects and clinical situations that require vitamin D screening.

The results obtained indicate that the criteria defining vitamin D status, according to two studies published in 2011, the Institute of Medicine (IOM) recommendations and the Endocrine Society (ES) guidelines, regardless the affected population.

Concerning the methodology used, progress has been made thanks to the Vitamin D Standardization Program (VDSP), although the most recent results from the external Vitamin D External Quality Program Assessment Scheme (DEQAS) indicate that there is still a significant bias among the different immunoassays available.

In relation to the criteria for screening, an agreement is observed in the most recent publications.

Introduction

Vitamin D remains to be a controversial issue for several reasons: the lack of consensus to define vitamin D status [1], the great rise of publications that relate the concentration of 25-hydroxyvitamin D (25-(OH)D) to different pathophysiological situations without enough evidence [2], the analytical variability derived from the various

methodologies [3], and the lack of consensus among scientific societies and governmental health institutions in countries in which the refundability of vitamin D supplements depends on the definition of hypovitaminosis or the quantification of baseline vitamin D levels according to clinical diagnosis [4]. All these reasons are causing an increase in the measurement of 25-(OH) D in clinical laboratories [5,6], in the number of supplemented patients [7], and the need to agree on decision values in reports [4].

In order to explain the current situation, it is necessary to understand how the main vitamin D guidelines and recommendations have evolved. In 1991, the United Kingdom Nutrition Committee (COMA) established for first time that plasma levels of 25-(OH)D below 8 ng/mL were present in children with rickets [8]. However, it was not until 2011 when the main aspects responsible for establishing nutritional recommendations for vitamin D emerged, and, therefore, the reference intervals of plasma concentrations associated to the nutritional status of the population appeared.

Two of these aspects, described by the COMA (updated in 2016) [9] and the Institute of Medicine (IOM) with the study of the population of USA and Canada [10], agree in defining the deficiency status. However, the IOM expands the states to insufficiency, sufficiency, and toxicity.

In 2011, the clinical practice guideline on vitamin D of the Endocrinology Society was published [11], presenting notable differences from the two previous approaches regarding the definition of vitamin D status. The reason for this discrepancy may be that this latest guideline is based on the vitamin D recommendations of the International Osteoporosis Foundation (IOF), established on the basis of randomized clinical trials in the adult population [12].

The controversy generated in the scientific community by this latest guideline was such that, from 2017 to 2019, three international conferences were held in Italy to discuss topics related to the definition of vitamin D status and methodological aspects of the quantification of 25-(OH)D plasma levels [13-15], reaching the conclusion of the need for standardization of the methodology in order to achieve consensus in the definition of vitamin D status.

The Vitamin D Standardization Program (VDSP) was founded in 2010. As a result of the tools developed by the VDSP, currently there are a reference method, standard reference materials (SRMs), quality standards based on biological variability for both reference and routine laboratories, and external quality assurance criteria that programs must meet.

Currently, only two quality assurance programs meet the VDSP requirements, the one of the College of American Pathologists (CAP) and the Vitamin D External Quality Assessment Scheme (DEQAS). In this programs, target values are assigned to each serum sample using the NIST (The National Institute of Standards and Technology) or CDC (Centers for Disease Control and Prevention) Reference Measurement Procedure (RMP), and participants' performance of specific methods for 25-(OH)D and other vitamin D metabolites are assessed [16]. In this regard, a recent DEQAS publication shows the analytical variability of the main current methods for measuring the concentration of 25-(OH)D [17].

The objective of this study is to review the definitions of vitamin D status in the main guidelines and recommendations on the main scientific databases, as well as the current state of the methodology available for its quantification.

Material and Methods

Over the last decade, the number of vitamin D-related publications has dramatically increased. Therefore, we decided to focus on the largest and most relevant guidelines, recommendations, and position statements to define vitamin D status, as well as on recent studies of our interest to analyze methodological quality.

We established a time period from January 2011 to December 2023. In case of more than one review being published by the same scientific entity throughout this period of time, the latest one was considered.

Search strategy

A strategic search was carried out using several electronic databases: Medline/PubMed, Web of Science, and Scopus; and looking for combinations of the following search terms: vitamin D, deficiency, nutrition, references values, dietary references, 25-(OH)D measurement, clinical practice guideline, recommendations, and position statement. Studies not written in English or Spanish were excluded.

Results

A total of 40 issues that establish vitamin D status, 9 clinical guidelines and 31 recommendations of population studies supported by relevant scientific organizations and/or committees have been reviewed.

The main aspects related to the clinical laboratory that determine the vitamin D status since 2011 are summarized in Table 1. It is based on the three main documents published up to date: the clinical practice guideline of the ES of 2011, the recommendations of the IOM of 2011, and the recommendations of the Scientific Advisory Committee on Nutrition (SACN) of 2016. Table 1 shows laboratory advices to establish vitamin D status, as 25-(OH)D cutoff points, reference intervals according to the type of requirements, the need for screening, and the methodology recommended for the measurement of 25-(OH)D [9-11].

Table 1: Comparison of the recommendations of the Endocrine Society, the Institute of Medicine and the Scientific Advisory Committee on Nutrition about the optimal concentration of 25-(OH) vitamin D.

Serum 25-(OH)D cutpoints	**SACN. Vitamin D and Health, 2016 [9]** Serum 25-(OH)D concentration is an indicator of exposure to vitamin D (from skin synthesis and dietary intake). • 25 nmol/L (10 ng/mL) In order to protect musculoskeletal health, it is recommended that serum 25-(OH)D concentration in all individuals in the UK should not fall below 25 nmol/L at any time of the year. • <30 nmol/L (<12 ng/mL) A serum 25-(OH)D concentration <30 nmol/L was associated with: increased risk of rickets, impaired fractional calcium absorption and increased risk of osteomalacia in young and middle-aged adults, and impaired fractional calcium absorption and fracture risk in older adults. A serum concentration of 30 nmol/L was considered to be consistent with the lower end of requirements. • 50 nmol/L (20 ng/mL) It was also concluded that there was a trend for maximal calcium absorption at serum concentration of 50 nmol/L. 50 nmol/L would cover the needs of most individuals in terms of vitamin D and this was used to establish the RDAs intake value for vitamin D. Little causal evidence for additional benefits on BMD, fracture risk or osteomalacia risk at serum 25-(OH)D concentration >50 nmol/L.	A. Catharine Ross et al. Dietary Reference Intakes for Calcium and Vitamin D. IOM, 2011 [10] < 30 nmol/L (< 12 ng/mL) = deficiency 30 – 50 nmol/L (12 – 20 ng/mL) = Inadequacy, but not for all persons > 50 nmol/L (> 20 ng/mL) = Sufficient level > 75 nmol/L (> 30 ng/mL) = not associated with increased benefit The committee noted with some concern that serum 25-(OH)D cut-points defined as indicative of deficiency for vitamin D have not undergone a systematic, evidence-based development process.	**Holick et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an ES Clinical Practice Guideline, 2011 [11]** < 50 nmol/L (< 20 ng/mL) = deficiency 50 – 73 nmol/L (20 – 29 ng/mL) = insufficiency 75 – 250 nmol/L (30 – 100 ng/mL) = Sufficient level

Screening	Not provided	Not evaluated. This committee considers that the evidence surrounding bone health provides a reasonable and supportable basis to allow the vitamin D to be used for DRIs development.	There is not sufficient evidence to recommend screening individuals who are not at risk for deficiency. Candidates for screening: rickets, osteomalacia, osteoporosis, chronic kidney disease, hepatic failure, malabsorption syndromes, hyperparathyroidism, some medications, African-American and Hispanic children and adults, pregnant and lactating women, older adults with history of falls, older adults with history of nontraumatic fractures, obese children and adults, granuloma-forming disorders, some lymphomas.
Assays for 25-(OH)D levels	Quantification of serum 25-(OH)D concentration can vary considerably (15-20%) depending on the type of assay used and across different concentration ranges.	There are differences in assay methodologies used. Reports in the literature for serum 25-(OH) D measures should be carefully interpreted, taking into account the type of assay employed, use of automation, year of analysis, and context of the analysis.	All clinical assays, including 25-(OH)D measurements, are subject to variability. Such variability confounds attempts to define a single "cut point" value as indicating low vitamin D status. For clinical care, all current methodologies seem adequate if they target 25-(OH)D values higher than current cut points.
Rickets and Osteomalacia	Evidence on vitamin D and rickets is mainly observational. Individual and mean serum 25-(OH)D concentrations of children with rickets were < 25 nmol/L (<10 ng/mL) in the majority of studies. Evidence on osteomalacia is limited mainly to case reports in which serum 25-(OH)D concentrations ranged between 4 and 20 nmol/L (1.6-8 ng/mL).	Serum 25-(OH)D levels lower than 27 to 30 nmol/L (10 to 12 ng/mL) are not diagnostic but associated with an increased risk for developing rickets. The risk of rickets increases below a serum 25-(OH)D level of 30 nmol/L (< 12 ng/mL) and is minimal when serum 25-(OH) D levels range between 30 - 50 nmol/L (12-20 ng/mL). Moreover, when calcium intake is inadequate, vitamin D supplementation to the point of serum 25-(OH)D concentrations up to and beyond 75 nmol/L (30 ng/mL) has no effect.	All available evidence suggests that children and adults should maintain a blood level of 25-(OH)D above 20 ng/ml to prevent rickets and osteomalacia, respectively. However, to maximize vitamin D's effect on calcium, bone, and muscle metabolism, the 25-(OH)D blood level should be above 30 ng/ml.

Falls and fractures	Evidence on vitamin D and falls is mixed but, overall, was suggestive of a beneficial effect of vitamin D supplementation in reducing fall risk in adults ≥ 50y with mean baseline serum 25-(OH)D concentrations ranging between < 25 and around 80 nmol/L (<10-32 ng/mL).	Some studies identified specific serum concentrations of 25-(OH) D below which falls, fractures, or bone loss increased; these values ranged from approximately 40 to 80 nmol/L. (16 – 32 ng/mL). Although some studies suggested that serum 25-(OH)D concentrations of approximately 40 nmol/L (16 ng/mL) are sufficient to meet bone health requirements for most people, findings from other studies suggested that levels of 50 nmol/L and higher (> 20 ng/mL) were consistent with bone health.	25-(OH)D between 30 and 40 ng/ml are consistent with the threshold for hip and nonvertebral fracture prevention from a recent meta-analysis of double-blind randomized controlled trials (RCT) with oral vitamin D.
Non-musculoskeletal health outcomes	There are insufficient data to draw conclusions on the relationship between serum 25-(OH)D concentration and non-musculoskeletal health outcomes	Outcomes related to cancer/ neoplasms, cardiovascular disease and hypertension, diabetes and metabolic syndrome, falls and physical performance, immune functioning and autoimmune disorders, infections, neuropsychological functioning, and preeclampsia could not be reliably linked with calcium or vitamin D intake and were often conflicting.	Numerous studies have demonstrated an association of vitamin D deficiency with increased risk of more than a dozen cancers; autoimmune diseases, including both type 1 and type 2 diabetes, rheumatoid arthritis, Crohn's disease, and multiple sclerosis; infectious diseases; and cardiovascular disease. There are, however, very few RCT with a dosing range adequate to provide evidence for the benefit of vitamin D in reducing the risk of these chronic diseases

25-(OH)D: 25-hydroxyvitamin D; BMD: bone mineral density; DRIs: dietary reference intakes; ES: Endocrine Society; IOM: Institute of Medicine; RDAs: recommended dietary allowances; SACN: Scientific Advisory Committee on Nutrition.

It is remarkable that the clinical practice guideline of the ES from 2011 principally disagrees on the definition of vitamin D deficiency and sufficiency, and propose higher cutoff points as reference than those published in population studies carried out in the US, Canada, and UK: for ES the deficiency status is < 50 nmo/L, while for IOM and SACN it is at levels < 25-30 nmol/L, and for ES the sufficiency status is between 75-250 nmol/L, while for IOM and SACN sufficiency is reached at levels 50 nmol/L, with no evidence of benefit above 75 nmol/L. This means that ES differs in the preventive values for bone and musculoskeletal health (rickets, osteomalacia, fractures, and falls), establishing concentrations between 10-20 ng/mL higher than the recommendations of IOM and SACN (falls and fractures: for IOM and SACN prevention is from 20-32 ng/mL, while for ES it is from 30-40 ng/mL; rickets and osteomalacia: for IOM prevention is from 12-20 ng/mL, while for ES it is from 20 ng/mL). Nevertheless, the three main studies agree on the methodological variability for the determination of 25-(OH)D.

The main guidelines and recommendations that have emerged subsequently and up to date are listed in Table 2, consisting of 8 clinical guidelines [24, 29, 32, 36, 37, 41, 49, 54] (3 of them related to bone health [32,41,49]), and 29 studies related to recommendations on vitamin D status [18-54]. Table 2 shows information of interest to clinical laboratories, such as the ranges to define vitamin D status, methodological aspects recommended, the need for population screening, and similarity with the main previous publications.

Depending on the tendency followed, the definition of vitamin D status may differ. In this sense, 13 studies apply the IOM recommendations, 11 studies take into consideration the recommendations of the ES, 8 studies collect information from both aspects, and only 3 studies consider the recommendations of the SACN. Among 2019 and 2023 there has been a trend in taking into consideration from both IOM [10] and ES [11].

Table 2: Status of 25-(OH)D levels according different guidelines, position statement and recommendations, and consistency with ES 2011, IOF 2010, IOM 2011 or SACN 2016.

Clinical guideline/ Position Statement/ Recommendation, year	Status of vitamin D and 25(OH) D concentration	Information about laboratory assay	Measurement of 25 (OH)D as screening test and recommended testing in:	Consistent with:
New Reference Values for Vitamin D, German Nutrition Society, 2012 [18]	Serum 25-(OH)D concentrations of 50 nmol/L (20 ng/mL) or higher are considered an indicator of optimal vitamin D status. Currently, 30 nmol/l (12 ng/mL) is the concentration that is deemed necessary for reliable rickets prophylaxis.	Not provided	Not provided	IOM 2011 IOF 2010
Vitamin D and health in adults in Australia and New Zealand: a position statement, 2012 [19]	Vitamin D adequacy: ≥ 50 nmol/L (≥ 20 ng/mL) at the end of winter (10–20 nmol/L higher at the end of summer). Mild vitamin D deficiency: 30–49 nmol/L (12-19 ng/mL). Moderate vitamin deficiency: 12.5–29 nmol/L (5-11 ng/mL). Severe vitamin D deficiency: < 12.5 nmol/L (< 5 ng/mL).	The bias and imprecision of many automated methods may be problematic at the lower, clinically and analytically important range (< 50 nmol/L) of the assay. Some laboratories are using more precise methods of analysis, such as LC-MS/MS	Screening in groups at high risk for vitamin D deficiency: people with a disability or chronic diseases, fair-skinned people and those at risk of skin cancer who avoid sun exposure, obese people, people working in an enclosed environment. In some high-risk groups (dark-skinned migrants, people in residential care establishments) screening test it is not necessary.	IOM 2011
British Paediatric and Adolescent Bone Group's position statement on vitamin D deficiency [20]	Deficiency: < 25 nmol/L 25-(OH)D (< 10 ng/mL). Insufficiency: 25-50 nmol/L 25-(OH)D (10-20 ng/mL). Sufficiency: > 50 nmol/L 25-(OH) D (> 20 ng/mL).	Not provided	Not provided	IOM 2011
Vitamin D: Still a topical matter in children and adolescents. A position paper by the Committee on Nutrition of the French Society of Paediatrics, 2012 [21]	The normal range was defined by the mean +/- 2 SD of the 25-(OH) D value sampled in a population of healthy subjects, i.e., 25 to 137.5 nmol/L (10-55 ng/mL) for European and North American populations.	The measurement method must be reliable and take into account the 2 fractions: 25-(OH)D2 and 25-(OH) D3. Laboratories must use external quality assurance programs such as the DEQAS international control system.	Not provided	IOM 2011
Recommended intake of calcium and vitamin D: positioning of the Nutrition Committee of the AEP, 2012 [22]	In adults, an indirect correlation between 25(OH)D and PTH levels permit accepting the deficiency cutoff point at 50 nmol/L (20 ng/ml). This level tends to apply to children of any age.	Lack of standardization of measurement methods.	Not provided	IOM 2011

Evaluation of dietary reference values for vitamin D, Health Council of the Netherlands, 2012 [23]	25-(OH)D ≥ 30 nmol/L (≥ 12 ng/mL) all the year for people aged between 4 and 70, including lactating women, and ≥ 50 nmol/L (≥ 20 ng/mL) in subjects above 70 years.	Serum 25-(OH)D concentration is associated with a CV of 15 - 20%, due to variations in analytical methods.	Not provided	IOM 2011
Guideline: Vitamin D supplementation in pregnant women. World Health Organization, 2012 [24]	IOM determined serum levels of 25-(OH)D > 50 nmol/L (> 20 ng/mL) as adequate for pregnant women. However, other experts argue that optimal levels should be >75 nmol/L (> 30 ng/mL).	Not provided	Not provided	IOM 2011 ES 2011
Vitamin D deficiency: Evidence, safety, and recommendations for the Swiss population. Expert report for the FCN, 2012 [25]	Vitamin D deficiency: < 50 nmol/L (< 20 ng/mL) Severe Vitamin D deficiency: < 25 nmol/L (< 10 ng/mL) Vitamin D insufficiency: 25-49 nmol/L (10 to 19 ng/mL) Adequate Vitamin D threshold: ≥ 50 nmol/L (≥ 20 ng/mL) Desirable Vitamin D for fall and fracture reduction: 75-110 nmol/L (30 - 44 ng/mL).	Assay variability for 25-(OH)D measurement d epends on the methodologies used. Efforts to improve assay comparability are important using uniform standards available through the NIST.	Only in individuals at high risk for severe vitamin D deficiency: bone disorders, hyperparathyroidism, older adults with falls or low trauma fractures, obesity, pregnant and lactating women not taking vitamin D supplements, children and adults with a dark skin tone, athletes who primarily exercise indoors, chronic kidney/ hepatic diseases, and malabsorption syndromes.	IOM 2010 IOF 2010 ES 2010
Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. Nordic Council of Ministers [26]	A serum 25-(OH)D concentration of 50 nmol/L (20 ng/mL) is used as an indicator of sufficiency, and a concentration of 30–50 nmol/L (12-20 ng/mL) is considered to indicate insufficient status.	The VDSP has the aim of standardizing serum 25-(OH)D concentration measurements. Results from some immunoassay methods have shown lower 25-(OH) D values as compared to HPLC or LC-MS/MS (standard method proposed). This should be accounted for when interpreting results.	Not provided	IOM 2011

Vitamin D and health in pregnancy, infants, children and adolescents in Australia and New Zealand: a position statement. Australian and New Zealand Bone and Mineral Society; Osteoporosis Australia, 2013 [27]	Severe deficiency 25-(OH)D: <12.5 nmol/L (<5 ng/mL). Moderate deficiency 25-(OH)D: 12.5–29 nmol/L (5-11.6 ng/mL). Mild deficiency 25-(OH)D: 30–49nmol/L (12-19.6 ng/dL). Sufficient 25-(OH)D: ≥ 50 nmol/L (≥ 20 ng/mL). Elevated 25-(OH)D: >250nmol/L (> 100 ng/mL). The recommended level for serum 25-(OH)D in infants, children, adolescents and during pregnancy and lactation is 50 nmol/L (20 ng/mL), and 10–20 nmol/L (4-8 ng/mL) higher at the end of summer.	There is a degree of imprecision in current testing (around 10%). Laboratories offering 25-(OH)D testing are required to participate in external quality assurances programs.	There is inadequate evidence to recommend population-wide screening for vitamin d status in infants, children and adolescents. Only in case of one or more risk factors for low vitamin D: lack of skin exposure to sunlight, dark skin, medical conditions or medication affecting vitamin D metabolism.	IOM 2011
Vitamin D in the Healthy European Paediatric Population, 2013 [28]	Sufficiency 25-(OH)D: > 50 nmol/L (> 20 ng/mL). Severe deficiency 25-(OH)D: < 25 nmol/L (< 10 ng/mL).	There are essential inter-assay differences in commercially available 25-(OH)D tests.	Not provided	IOM 2011 IOF 2010 ES 2011
Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe-recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency, 2013 [29]	Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Suboptimal status 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Adequate status 25-(OH)D: 75 – 125 nmol/L (30-50 ng/mL). High vitamin D supply: 125-250 nmo/L (50-100 ng/mL). Risk for overall health outcomes: > 250 nmol/L (>100 ng/mL). Toxic status: > 500 nmol/L (> 200 ng/mL).	Methods must measure both 25-(OH)D2 and 25-(OH)D3. Intra-assay CV should be < 5%, and inter-assay CV < 10%.	Not provided	IOM 2011
Recommended Vitamin D intake and management of low Vitamin D status in adolescents: a position statement of the Society for Adolescent Health and Medicine, 2013 [30]	Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Insufficient status 25-(OH)D: 50-72.5 nmol/L (20-29 ng/mL). Normal vitamin D status: > 75 nmol/L (> 30 ng/mL). Optimal vitamin D status for adolescents: 75 – 125 nmol/L (30- 50 ng/mL). Toxic status 25-(OH)D: > 500 nmol/L (> 200 ng/mL).	Not provided	Testing in high risk of low vitamin D status: increased skin pigmentation, frequent use of sunscreen, obesity, specific diet, cultural convention associated with body coverage, malabsorption syndromes, amenorrhea, pregnancy or lactation, immobilization, bariatric surgery, chronic kidney/hepatic diseases, specific medication, recurrent fractures or low bone mineral density status.	ES 2011

Recommendations Abstracted from the American Geriatrics Society Consensus Statement on Vitamin D for Prevention of Falls and their consequences, 2014 [31]	A serum 25-(OH)D concentration of 75 nmol/L (30 ng/mL) should be a minimum goal to achieve in older adults, particularly in frail adults.	Not provided	In older adults only in situations of risk: hypercalcemia, individuals taking medications that bind to vitamin D or accelerate the breakdown, obesity, malabsorption syndromes, intake below recommended.	ES 2011
Clinician's Guide to Prevention and Treatment of Osteoporosis, 2014 [32]	Insufficiency: serum 25-(OH)D < 75 nmol/L (< 30 ng/mL).	Not provided	Not provided	IOF 2010
Optimizing Bone Health in Children and Adolescents, 2014 [33]	25-(OH)D reference interval for healthy children and adolescents: ≥ 50 nmol/L (≥ 20 ng/mL). 25-(OH)D reference interval for people at increased risk of fracture: ≥ 75 nmol/L (≥ 30 ng/mL).	Not provided	Evidence is insufficient to recommend universal screening. Screening only in children and adolescents with reduced bone mass and/or recurrent low-impact fractures.	IOM 2011
Dietary reference values for vitamin D. EFSA Panel on Dietetic Products, Nutrition and Allergies, 2016 [34]	For adults, infants and children there is evidence of an increased risk of adverse musculoskeletal health outcomes and adverse pregnancy-related health outcomes at serum 25-(OH)D concentration below 50 nmol/L (20 ng/mL).	The introduction of a NIST standard reference material for vitamin D has been a step forward in providing a reference measurement procedure against which assays could be standardized to avoid variability of results. Free serum 25-(OH)D and plasma/serum 1,25-(OH)2D concentration cannot be used as a biomarker of vitamin D status.	Not provided	IOM 2011 SACN 2016
Global consensus recommendations on prevention and management of nutritional rickets, 2016 [35]	Sufficiency 25-(OH) D: > 50 nmol/L (> 20 ng/mL). Insufficiency 25-(OH)D: 30-50 nmol/L (12-20 ng/mL). Deficiency 25-(OH) D: < 30 nmol/L (< 12 ng/mL). Toxicity 25-(OH)D: > 250 nmol/L (> 100 ng/mL).	The reliability of immunoassays is questioned particularly at low and high concentrations of 25-(OH) D. The reduction of the inter-laboratory variation in 25-(OH)D measurements are observed using HPLC-MS/MS with the application of NIST standard reference materials.	**Not provided**	IOM 2011

Clinical practice guidelines for vitamin D in the United Arab Emirates, 2016 [36]	Deficiency 25-(OH)D: < 50 nmol/l (< 20 ng/mL) . Insufficiency 25-(OH)D: < 75 nmol/L (< 30 ng/mL). Recommended 25-(OH)D level: 75 – 150 nmol/L (30-60 ng/mL).	All clinical assays are subject to significant assay variability. The comparability of 25-(OH) D results seems likely to improve as uniform standards (NIST).	Testing only in pretreatment and in situations of risk: bone disorders, abnormalities of calcium and/or phosphate metabolism, hyperparathyroidism, specific medication, malabsorption syndromes, eating disorders, chronic kidney/hepatic diseases, granulomatous disorders, cancer, cardiovascular diseases, metabolic syndrome, chronic autoimmune diseases, hospital admissions secondary to infectious diseases, institutionalized persons, and those with disabilities.	ES 2011
Vitamin D: supplement use in specific population groups. National Institute for Health and Clinical Excellence. 2014 (Updated 2017) [37]	Deficiency 25-(OH)D: <25 nmol/L (<10 ng/mL).	Not provided	25-(OH)D must be only measured when there are symptoms or very high risk of deficiency.	SACN 2016
Recommended vitamin D levels in the general population. Grupo de Trabajo de Osteoporosis y Metabolismo Mineral de la Sociedad Española de Endocrinología y Nutrición, 2017 [38]	They suggest maintaining serum 25-(OH)D concentrations between 75 and 125 nmol/L (30 – 50 ng/mL) to achieve the health benefits of vitamin D. Elevated 25-(OH)D values >125-150 nmol/L (> 50 – 60 ng/mL) could be associated with risk for cardiovascular death or any other cause of death.	Not provided.	Screening only in individuals with risk factors: bones disorders, chronic kidney/hepatic diseases, malabsorption syndromes, hyperparathyroidism, specific medication, pregnant and lactating women, institutionalized persons, obesity, reduced sun exposure, granulomatous disorders, some lymphomas.	ES 2011

Vitamin D in European children-statement from the European Academy of Paediatrics (EAP), 2017 [39]	Sufficiency 25-(OH)D: >50 nmol/L (20 ng/mL). Deficiency 25-(OH)D: <25 nmol/L (10 ng/mL).	Considerable variability exists among the various assays available and among laboratories.	There is no evidence for routine vitamin D screening in healthy children. Testing in situations at risk for deficiency: bones diseases, darker pigmented skin, reduced sun exposure, chronic liver/kidney disease or with malabsorption, dietary inadequacy, obesity, long-term parenteral nutrition, institutionalized children, and with anticonvulsant medication.	IOM 2011
Assessment criteria for vitamin D deficiency/ insufficiency in Japan: proposal by an expert panel supported by the Research Program of Intractable Diseases, Ministry of Health, Labour and Welfare, Japan, the Japanese Society for Bone and Mineral Research and the Japan Endocrine Society, 2017 [40]	Sufficiency 25-(OH) D: ≥ 75 nmol/L (≥ 30 ng/ mL). Insufficiency 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Deficiency 25-(OH) D: < 50 nmol/L (< 20 ng/mL).	Serum 25-(OH)D level may vary depending on the assay used. Standardization of the assay will be needed.	Not provided	ES 2011
Vitamin D and bone health: A practical clinical guideline for patient management, Royal Osteoporosis Society, 2018 [41]	Deficiency: plasma 25-(OH) D < 25 nmol/L (<10 ng/ mL). Inadequate in some people: plasma 25-(OH)D of 25-50 nmol/L (10 -20 ng/mL) Sufficiency: plasma 25-(OH)D > 50 nmol/L (> 20 ng/mL)	Measurement of plasma 25-(OH)D is the best way for estimating vitamin D status. The assay should have the ability to recognise all forms of 25-(OH)D (D2 or D3) equally. This means that it should use either HPLC-MS/MS. None of the immunoassays offers the ability to recognize all forms of 25-(OH)D.	Universal screening of asymptomatic population is not recommended. They only suggest testing 25-(OH) in patients with musculoskeletal symptoms attributed to vitamin D deficiency, and in situations where malabsorption or poor compliance with medication is suspected.	IOM 2011 SACN 2016

Italian Association of Clinical Endocrinologists (AME) and Italian Chapter of the American Association of Clinical Endocrinologists (AACE) Position Statement: Clinical Management of Vitamin D Deficiency in Adults, 2018 [42]	25-(OH)D concentrations of 50 nmol/L (20 ng/mL) are appropriate in the general population. They recommend maintaining levels above 75 nmol/L (> 30 ng/mL) in situations of risk.	The same method must be used for serial measurement of 25-(OH)D in any patient. The standardization of 25-(OH)D levels by immunoassay methods to LC-MS/MS will provide valid conclusions about the actual health implications of vitamin D deficiency.	Screening of 25-(OH) D is not indicated in healthy people. Testing only 25-(OH) D in: bones disorders, older adults with falls and/or non-traumatic fractures, chronic kidney/ hepatic diseases, cystic fibrosis, malabsorption syndromes, hyperparathyroidism, specific medication, pregnant and lactating women, institutionalized persons, obesity, reduced sun exposure, granulomatous disorders, some lymphomas.	IOM 2011 ES 2011
Vitamin D in pediatric age: consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians, 2018 [43]	Severe deficiency 25-(OH) D: < 25 nmol/l (< 10 ng/ mL). Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/ mL). Insufficiency 25-(OH)D: 50-74 nmol/L (20-29 ng/ mL). Sufficiency 25-(OH) D: > 75 nmol/L (> 30 ng/mL). Hypovitaminosis D: < 75 nmol/L (< 30 ng/mL).	Some methods available for determining 25-(OH)D still present poor accuracy and precision. The isotope dilution- LC-MS/MS is considered the best method for measuring serum 25-(OH)D.	Screening 25-(OH)D in healthy individuals is not recommended. 25-(OH) D evaluation should be limited in children and adolescent with risk factors for vitamin D deficiency, in subjects that require supplementation during the whole year or receiving drugs affecting vitamin D metabolism, dark skin, reduced sunlight exposure, obesity, inadequate diets, chronic kidney/hepatic diseases, malabsorption syndromes, chronic therapies.	ES 2011

Recomendaciones para la valoración bioquímica del estatus de Vitamina D. Comisión de Hormonas de la SEQC-ML, 2019 [44]	25-(OH)D concentrations below 25 nmol/L (12 ng/mL) are inadequate, because they are associated with an important increase in the risk for rickets in children and osteomalacia in adults. 25-(OH)D concentrations around 75 nmol/L (30 ng/mL) are adequate for a good bone health. 25-(OH)D concentrations less than 50 nmol/L (20 ng/mL) are suboptimal.	There is lack of agreement of results with the different methods. Most clinical laboratories use automated immunoassays with CDC Certified Vitamin D Program (VDSP), which show acceptable overall correlation with LC-MS/MS methods used as reference. The external quality program DEQAS has shown a gradual reduction in the CV between laboratories.	Screening without risk factors for 25-(OH) D deficiency is not recommended. Patients that should be screened: bones disorders, chronic kidney/hepatic diseases, malabsorption syndromes, specific medication hyperparathyroidism, abnormalities of calcium and/or phosphate metabolism, unexplained high levels of alkaline phosphatase, suspected toxicity. Basal 25-(OH) D level is not necessary in case of: obesity, dark skin, reduced sunlight exposure, institutionalized persons.	IOM 2011 ES 2011
Recomendaciones de la SEIOMM en la prevención y tratamiento del déficit de vitamina D, 2021 [45]	Serum 25-(OH)D levels between 62.5-125 nmol/L (25 - 50 ng/mL) are recommended to achieve the bone health benefits. In patients with osteoporosis or at risk for fracture, 25-(OH)D between 75 – 125 nmol/L (30 - 50 ng/mL) are recommended. Maximum concentration 25-(OH) D: 125 - 220 nmol/L (50-88 ng/mL).	It is recommended that the laboratory have a quality assurance program certification and the standardization of serum 25-(OH)D determinations to minimize analytical variability.	Screening for 25-(OH) D deficiency in people with risk factors: people with weakness muscle and/or risk of falls, dark skin, reduced sunlight exposure, bone diseases, advanced age and/or institutionalized persons, cognitive deficiency, smoking, obesity, inadequate diets, risk of malnutrition, malabsorption syndromes, renal or hepatic insufficiency, hypo and hyperparathyroidism, bones diseases, pregnant and lacting, medications that interfere with cytochrome P450.	ES 2011
Screening for Vitamin D Deficiency in Adults: US Preventive Services Task Force Recommendation Statement, 2021 [46]	More research is needed to determine the cut-off point that defines vitamin D deficiency and whether that limit varies depending on the patient clinical outcome or by subgroups defined by race, ethnicity or sex.	Evidence suggests that results depend on the testing method and vary among laboratories using the same testing methods.	The current evidence on the benefits of screening for vitamin D deficiency is lacking. Therefore, the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults cannot be determined.	None

Recommendations on the measurement and the clinical use of vitamin D metabolites and vitamin D bin ding protein – A position paper from the IFCC Committee on bone metabolism, 2021 [47]	Differences exist in the definition of vitamin D deficiency, insufficiency, and sufficiency, creating a great deal of controversy. The most critical factor that confounds efforts to develop consensus in clinical and nutritional public health guidelines for interpreting serum 25-(OH)D concentrations is the substantial variability that still exists in many assays that have been used over time to measure 25-(OH)D. The lack of assay standardization is the main source of bias.	The best sample to measure 25-(OH)D is serum. Many immunoassays suffer from dependent deviations and manufacturers should improve these assays. Standardized LC-MS/MS methods are currently the only tools able to measure 25-(OH)D regardless of the nature of the sample. CDC started an international Vitamin D standardization certification program, led to an improvement in the number of standardized 25-(OH)D assays. Limits for total CV and mean bias should be ≤ 10% and ≤ 5%, respectively, for routine clinical laboratories.	Not provided	None
Recomendaciones de uso adecuado de pruebas y suplementos de Vitamina D en población general. Ministerio de Sanidad, 2021 [48]	There is lack of consensus on optimal 25-(OH)D values, but there is a minimum agreement: >50 nmol/L (>20 ng/mL) is recommended and <25 nmol/L (<10 ng/mL) must be avoided at all ages. Consensus results of expert groups (delphi model) are: Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Insufficiency 25-(OH)D: 50-74.75 nmol/L (20-29.9 ng/mL). Optimal 25-(OH)D: 75-125 nmol/L (30-50 ng/mL).	There are different quantification methods available. LC-MS/MS is the gold standard technique.	In asymptomatic healthy adults without risk factors for 25-(OH)D deficiency, there is no proved evidence to test 25-(OH) D levels. Screening is recommended in people with risk factors: bone metabolism alterations, obesity, malabsorption syndromes, and others.	ES 2011
The clinician's guide to prevention and treatment of osteoporosis, 2022 [49]	The current normal range for 25-(OH)D levels is between 75 and 125 nmol/L (30-50 ng/mL). In healthy individuals, serum 25-(OH)D ≥50 nmol/L (≥20 ng/mL) may be sufficient, but in the setting of known or suspected metabolic bone disease ≥75 nmol/L (≥30 ng/mL) is appropriate.	Not provided	Not povided	IOM 2011 ES 2011

Role of vitamin D supplementation in the management of musculoskeletal diseases: update from an European Society of Clinical and Economical Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) working group, 2022 [50]	Deficiency or severe deficiency 25-(OH)D: < 25nmol/L or <30 nmol/L (<10 ng/mL or <12 ng/mL) (depending on the expert society), when the focus was the prevention of rickets/osteomalacia. Insufficiency or deficiency 25-(OH)D: < 50 nmol/L (<20 ng/mL), if the concern was suppression of PTH.	There is an absolute need for a standardized method. LC-MS/ MS methods generally perform better than immunoassays, but all LCMS/MS methods are not equivalent. 24,25-(OH)2D and VMR (vitamin D metabolite ratio) are promising tools to evaluate vitamin D deficiency.	25-(OH)D testing is appropriate in bones diseases, hyperparathyroidism, malabsorption syndromes, medications affecting metabolism of vitamin D, chronic kidney disease, hypophosphatemia and hypo/hypercalcemia, pigmented skin, and isolated elevation of alkaline phosphatase.	IOM 2011 ES 2011
Vitamin D. Fact Sheet for Health Professionals. National Institute of Health, 2022 [51]	Vitamin D deficiency: <30 nmol/L (<12 ng/mL). Inadequate for bone and overall health in healthy individuals: 30 to <50 nmol/L (12 to <20 ng/mL). Adequate for bone and overall health in healthy individuals: ≥50 nmol/L (≥20 ng/mL) . 25-(OH) D linked to toxicity: >125 nmol/L (>50 ng/mL).	Assessing vitamin D status by measuring serum 25-(OH)D concentrations is complicated by the considerable variability of the available assays. The international VDSP has developed procedures for standardizing the laboratory measurement of 25-(OH) D to improve clinical and public health practice.	There isn't any national professional organization that recommends population screening for vitamin D deficiency in asymptomatic patients.	IOM 2011
Definition, Assessment, and Management of Vitamin D Inadequacy: Suggestions, Recommendations, and Warnings from the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS), 2022 [52]	In the general population: Deficiency 25-(OH) D: <25 nmol/L (<10 ng/ mL). Insufficiency 25-(OH)D: < 50 nmol/L (20 ng/ mL). Optimal 25-(OH)D: 50-124.8 nmol/L (20–50 ng/mL). Population at risk or treatment with bone modifying agents: Deficiency 25-(OH)D: <25 nmol/L (<10 ng/ mL). Insufficiency 25-(OH)D: < 74.9 nmol/L (< 30 ng/mL). Optimal 25-(OH)D: 74.9-124.8 nmol/L (30–50 ng/mL).	There is an urgent need for standardization/ harmonization for a correct interpretation of clinical studies and for clinical practice. The assessment of serum 25-(OH)D levels is mostly performed using immunochemiluminescence methods with intra-assay and inter-assay variability of 10-20%. The LC-MS/ MS is considered the most accurate and precise method for research and clinical use.	It is recommended not to perform 25-(OH) D measurement in the general population. Measurement of 25-(OH)D levels is only recommended when it is necessary for the clinical management of the patient.	IOM 2011 ES 2011
Vitamin D – a scoping review for Nordic nutrition recommendations 2023 [53]	There is a growing agreement that : Deficiency 25-(OH)D: <25-30 nmol/L (<10-12 ng/mL). Sufficiency 25-(OH)D: > 50 nmol/L (>20 ng/ mL).	All measurements should be standardized. The LC-MS/MS is considered the most valid method for measurement of Vitamin D metabolites.	Not provided	IOM 2011

Guidelines for preventing and treating vitamin D deficiency: A 2023 Update in Poland, 2023 [54]	Deficiency 25-(OH) D: < 50 nmol/L (< 20 ng/mL). Insufficiency 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Sufficiency 25-(OH) D: 75-125 nmol/L (30-50 ng/mL). Toxicity 25-(OH)D: > 250 nmol/L (> 100 ng/mL).	The measure of 25-(OH)D should be subject to quality assurance by the certifying system DEQAS.	The screening of serum 25-(OH)D is not recommended. In the risk group is strongly recommended: increased demand for physiological reasons, malabsorption syndromes, diseases of liver and bile ducts, respiratory diseases, infectious diseases, systemic connective tissue diseases, skin diseases, diseases of nervous system, decreases production of vitamin D3 in the skin, nutritional features, long-term use of drugs, malignant neoplasms, granulomatous diseases, mental illness, cardiovascular diseases, chronic fatigue syndrome, inpatient treatment, pre and post-transplant.	ES 2011

25-(OH)D: 25-hydroxyvitamin D; 25-(OH)D2: 25-hydroxyvitamin D2; 25-(OH)D3: 25-hydroxyvitamin D3; 24,25-(OH)2D: 24,25-dihydroxyvitamin D; AIDS: acquired immunodeficiency syndrome; CDC: Centers for Disease Control and Prevention; CV: coefficient of variation; DEQAS: vitamin D external quality program assessment scheme; ES: Endocrine Society; IOF: International Osteoporosis Foundation; IOM: Institute of Medicine; HPLC: high pressure liquid chromatography; LC-MS/MS: liquid chromatography-tandem mass spectrometry; NIST: The National Institute of Standards and Technology; PTH: parathyroid hormone; SACN: Scientific Advisory Committee on Nutrition; VDSP: Vitamin D Standardization Program.

There is uniform consensus about the lack of need for general population screening [46]. However, there are differences regarding the target population for vitamin D deficiency screening. There is also an agreement on the need for using standardized methods to measure 25-(OH)D as an indicator of vitamin D status, and on the participation in external quality programs established by the Vitamin D Standardization Program (VDSP).

The main controversial aspects of vitamin D related to clinical laboratory from the three international conferences held among 2017 and 2019 are displayed in Table 3 [13-15]. These conferences highlight the need for standardization of the methodology to determine 25-(OH)D.

Table 3: Laboratory aspects at International Conferences on controversies in vitamin D between 2017 – 2019.

Representative articles from International Conferences (2017-2019) on controversies in vitamin D	**Summary of laboratory aspects**
Controversies in vitamin D: Summary Statement from an International Conference (Pisa, June 2017) [13]	Available guidelines suggest that 25-(OH)D values <12 ng/mL (< 30 nmol/L) are associated with an increased risk of rickets/osteomalacia, whereas 25-(OH)D concentrations between 20 and 50 ng/mL (50 to 125 nmol/L) appear to be safe and sufficient for skeletal health in the healthy general population. It is not clear whether these guidelines should be considered with regards to individuals who have metabolic bone diseases, such as osteoporosis or primary hyperparathyroidism. Need for a standardized determination of 25-(OH)D concentration is crucial for a clearer definition of vitamin D status: deficiency, sufficiency or excess.
Consensus Statement from 2nd International Conference on Controversies in Vitamin D (Siena, September 2018) [14]	Existing data are insufficient to define with certainty low or high vitamin D status thresholds because of the lack of standardized 25-(OH)D measurements. Defining vitamin D status using serum 25-(OH)D concentration with standardized methodology is recommended. Assays should demonstrate standardization or alignment with reference methodology proposed by the VDSP. Laboratories should participate in a 25-(OH)D accuracy program (DEQAS or CAP). Manufacturers should develop assays with ability to accurately measure 25-(OH)D2 and 25-(OH)D3 in various clinical circumstances. The risk for developing rickets/osteomalacia is increased at a 25-(OH)D concentration ≤ 12 ng/mL (30 nmol/L). This threshold may vary depending on other conditions such as calcium and phosphate nutrition, parathyroid hormone (PTH) levels, and season. The 25-(OH)D concentration ranges among normal subjects are between 50 and 125 nmol/L. An upper 25-(OH)D threshold of 125 nmol/L is advisable.
Controversies in Vitamin D: A Statement from the Third International Conference (Gubbio, September 2019) [15]	Severe vitamin D deficiency, defined as <12 ng/mL (30 nmol/L) is seen in approximately 7% of the population worldwide, with variation among countries and populations. The circulating 25-(OH)D concentration is widely accepted as the best marker of vitamin D status, although with little physiologic regulation. There is ongoing debate with regard to whether free 25-(OH)D or the ratio [24,25-(OH)2D]/[25-(OH)D] is a superior marker than total 25-(OH)D. There is consensus that 25-(OH)D levels below 12 ng/mL (30 nmol/L) are clearly deficient and levels above 30 ng/mL (75 nmol/L) are clearly sufficient. There is disagreement on levels between 12 and 30 ng/mL (30 and 75 nmol/L). Some guidelines recommend a threshold value of 20 ng/mL (50 nmol/L), whereas others aim for ≥30 ng/mL (≥ 75 nmol/L). This discussion is largely based on the lack of 25-(OH)D assay standardization.

25-(OH)D: 25-hydroxyvitamin D; 25-(OH)D2: 25-hydroxyvitamin D2; 25-(OH)D3: 25-hydroxyvitamin D3; 24,25-(OH)2D: 24,25-dihydroxyvitamin D; CAP: college of American pathologists; DEQAS: vitamin D external quality program assessment scheme; VDSP: Vitamin D Standardization Program.

Discussion

This review identified and scrutinized, from data of the main guidelines, three major issues related to vitamin D status assessment: the difficulty in defining the desirable levels, which may vary according to underlying conditions, the variability in the assay methodology, and the need of standardization. Indeed, these controversial topics were also considered as major issues in a recent study [55].

Despite global consensus on the need to use standardized methodology to correctly determine vitamin D status in the general population, guidelines and/or recommendations continue to take into consideration studies from the IOM, the ES, or both, when at the time of their publication there was not a standardized methodology.

Another remarkable controversy is the origin of the ES recommendations, based on the IOF recommendations derived from randomized clinical trials in adult population [12] and being a guide for patients with chronic disorders, as clarified one year later by the same working group of the ES [56]. In our opinion, a methodological and population bias appears in the guidelines and recommendations that only take into consideration one of the possible indications: the IOM recommendations are aimed at the general population, while the Endocrine Society guideline is based on the needs of population with chronic pathologies that can affect bone metabolism. Given the different goals of the IOM and the ES clinical practice guideline, it is not surprising that their recommendations differed. This situation, together with the rise of publications with contradictory results from the majority of observational studies, is producing a lack of agreement between clinical laboratories to establish recommendations to measure 25-(OH)D and reference intervals to establish vitamin D status depending on the type of population.

Regarding methodological aspects, clinical laboratories must be aware of their analytical limitations for the correct interpretation of results. Due to the increasing number of samples received by routine clinical laboratories, the use of an automated methodology and, therefore, immunoassays certified by the Center of Disease Control and Prevention (CDC) for vitamin D are necessary [57].

The latest published results from DEQAS [17] indicate that, although the results from immunoassays have reduced the imprecision among methods, a bias continues to appear in low and high values, and non-assessment of the 25-(OH)D2 metabolite may not reflect vitamin D status when supplementation is performed with vitamin D2. To understand these limitations, it is important to participate in an external quality program that meets the VDSP criteria.

After the review of the existing evidence, the current situation would be as follows: it is generally accepted that 25-(OH)D concentrations < 25 nmol/L (<12 ng/mL) are deficient and can affect bone and musculoskeletal health, and that concentrations > 75 nmol/L (> 30 ng/mL) are sufficient for any type of population (age, ethnic group and pathophysiological condition, with or without risk for vitamin D deficiency). The controversy appears in concentrations between 25-75 nmol/l (12-30 ng/mL), in wich the definition of vitamin D status will depend on age and risk factors. This way, concentrations between 25-50 nmol/L (12-20 ng/mL) may be sufficient for some people, but not for the entire healthy population. Therefore, and in accordance with the recommendations of the IOM [10] and the ES [11], concentrations > 50 nmol/L (> 20 ng/mL) are sufficient for a healthy population without risk factors under 60-65 years, and concentrations > 75 nmol/L (> 30 ng/mL) are sufficient for the global population, and necessary in patients with risk factors, regardless of age.

Another important item reviewed is when the determination of the concentration of 25-(OH)D is indicated. There is agreement about not performing screening in population without risk of vitamin D deficiency, being reinforced with the publication on 2021 of the US Preventive Services Task Force, in the latest consensus on vitamin D resulting from the 6th International Conference on Vitamin D and in the recent guideline published by the ES [46, 55, 58]. There is also consensus in measuring 25-(OH)D in symptomatic patients and in those at risk of deficiency. However, there is no accordance in defining risk situations of vitamin D deficiency that do require such determination. The most recent guidelines and recommendations agree on analyzing population with bone disorders (osteoporosis, rickets, osteomalacia, unjustified fractures, alterations in phosphocalcium metabolism, hypo- and hyperparathyroidism, elevated alkaline phosphatase without justification), chronic kidney and liver diseases, malabsorption and medication that interferes at the cytochrome P450 levels, as it is described in Table 2. There is also a recommendation to directly supplement without measuring levels in patients at risk of suffering from deficiency, but without chronic diseases: little sun exposure, institutionalized people, or dark-skinned and obese people [19,31,41,44, 58].

The assessment of vitamin D status becomes relevant especially when the refundability of vitamin D supplements depends on governmental criteria, sometimes diverging from guidelines due to lack of consensus [4]. For this reason, clinical laboratories must make an effort and unify reports to facilitate clinical decision-making: it would be convenient to use the units of the international system of nomenclature (nmol/L), to report not reference range but clinical decision values, and it is crucial for all laboratories to be aware of the performance and limitations of their 25-(OH)D assays to ensure the reliable assessment of vitamin D status.

In conclusion, although there have been advances in methodology, with automatized methods and traceable calibrators by the CDC standards, there is a paralysis in the development of current population studies with standardized methodology to accurately establish the status of vitamin D in both healthy population and population at risk for vitamin D deficiency.

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