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Measurement of Vitamin D metabolites: an international perspective on methodology and clinical interpretation☆

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Abstract

The International Quality Assessment Scheme for Vitamin D metabolites (DEQAS) was introduced in 1989. Initially, the aim was to improve the reliability of 25-hydroxyvitamin D (25-OHD) assays but the scheme was extended in 1997 to include 1,25-dihydroxyvitamin D (1,25$(OH)_2$D). DEQAS has 95 members in 18 countries (January 2003). Five serum samples are distributed quarterly and participants are given up to 6 weeks to return their results for statistical analysis. The majority of participants use commercial kits for both analytes. A performance target was set by an advisory panel in 1997 and, at present, requires participants to get 80% or more of their results within ±30% of the All-Laboratory Trimmed Mean (ALTM). The performance targets are under continual review. In 2003, 59% of participants met the target (cf. 52% in 2000). A questionnaire, distributed in January 2003, requested information on methods and the interpretation of results. Reference ranges varied but there was reasonable agreement on the 25-OHD concentrations below which Vitamin D supplementation was advised. A minority (22%) of respondents was unsure whether Vitamin D_3 or Vitamin D_2 was used to treat patients in their locality. The majority (52%) of assays for 1,25$(OH)_2$D were done 'on demand' and others for apparently spurious reasons. Most respondents thought participation in DEQAS extremely important and the planned introduction of on-line reporting should enhance its value.

Keywords: 25-Hydroxyvitamin D; Quality assessment; DEQAS

1. Introduction

The International External Quality Assessment Scheme for Vitamin D metabolites (DEQAS) was established in 1989 following consistent reports of poor performance for assays of 25-hydroxyvitamin D (25-OHD) [1,2] and 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) [1]. Initially DEQAS covered only 25-OHD but was extended to include 1,25$(OH)_2$D in 1997. The number of participants have grown from approximately 25 in 1989 to 95 in 2003, of whom 37 currently also measure 1,25$(OH)_2$D. The aim of DEQAS was to improve the accuracy and precision of these assays by regularly distributing serum samples to participants throughout the world and thus establishing performance targets. It was initially intended to provide GC–MS values for all samples distributed but this has not been possible. However, the All Laboratory Trimmed Mean (ALTM) [3] was shown to be an appropriate routine target value by comparison with GC–MS [4]. To encourage improved performance, certificates of proficiency are awarded to all participating laboratories whose performance meets the target set by an advisory panel [5]. The initial performance target was set in 1997 and required participants to get 80% or more of their results within ±33% of the ALTM. The target is under continual review, was altered to ±30% in 2000 and is likely to be tightened further over the coming years.

Demand for 25-OHD and 1,25$(OH)_2$D assays has undoubtedly been stimulated by the introduction of commercial kits, which are designed for ease of use in non specialist laboratories. In common with other routinely measured analytes, the reliability of results can only be assessed by participation in an external quality assessment scheme. Indeed, this is often required as part of the laboratory accreditation

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process. Rapid feedback and efficient communication between participants and scheme organisers is considered to be extremely important. To facilitate this, a web-based reporting system has been developed [6]; DEQAS participants sending results via the internet will have immediate access to up-dated statistics. The addition of a 'message board' will facilitate communication between participants, manufacturers and DEQAS. In addition to monitoring assay performance, DEQAS is in a unique position to collate information about the use and interpretation of 25-OHD and 1,25$(OH)_2$D assays. To this end, DEQAS distributed a questionnaire to all participants in January 2003. The results of this questionnaire are reported here together with details of performance over the last 3 years.

2. Materials and methods

2.1. Serum pools

Blood was collected anonymously from polycythaemic patients undergoing therapeutic venesection. The serum was stored at −40 °C. Prior to distribution, pools were thawed and screened for Hepatitis B and C, and HIV. To ensure sterility, the serum was passed through a 0.2 μm biological grade filter. Pools were stored at 4 °C overnight and dispensed as 0.5 ml (25-OHD) or 2 ml (1,25$(OH)_2$D) samples for distribution. Samples were sent to participants at ambient temperature by first class post (UK) and by airmail (outside the UK)and normally arrive within 2 weeks of dispatch. Studies in the organiser's laboratory have shown no significant change in results for either analyte during storage for up to 2 weeks at 30 °C.

2.2. Questionnaire

In January 2003, a questionnaire was distributed with the samples to all participants, designed to obtain information on methods, reference ranges, interpretation of assays, and participants' views on the relevance of DEQAS.

2.3. Results and data management

Participants returned results by fax or post on a form provided with the samples or by using a newly set up website (http://www.deqas.org). At the present time approximately 50% use the latter system. On receipt of results, the ALTM is calculated together with an estimator of standard deviation using an algorithm based on weighted results [3]. Accuracy of individual results is assessed by calculating the % bias from the ALTM. A personalised report is sent to each participant, generated by a PC based program written in Delphi. To enhance interactive dialogue, a web-based reporting system giving instant, on-line feedback has been developed [6] using a dedicated Linux server hosted by a commercial company (Positive Internet Company, UK).

3. Results

3.1. Performance

Fig. 1 shows the percentage of participants meeting the performance target for each of the last three distribution cycles. During this 3-year period, the number of participants increased by about 33%.

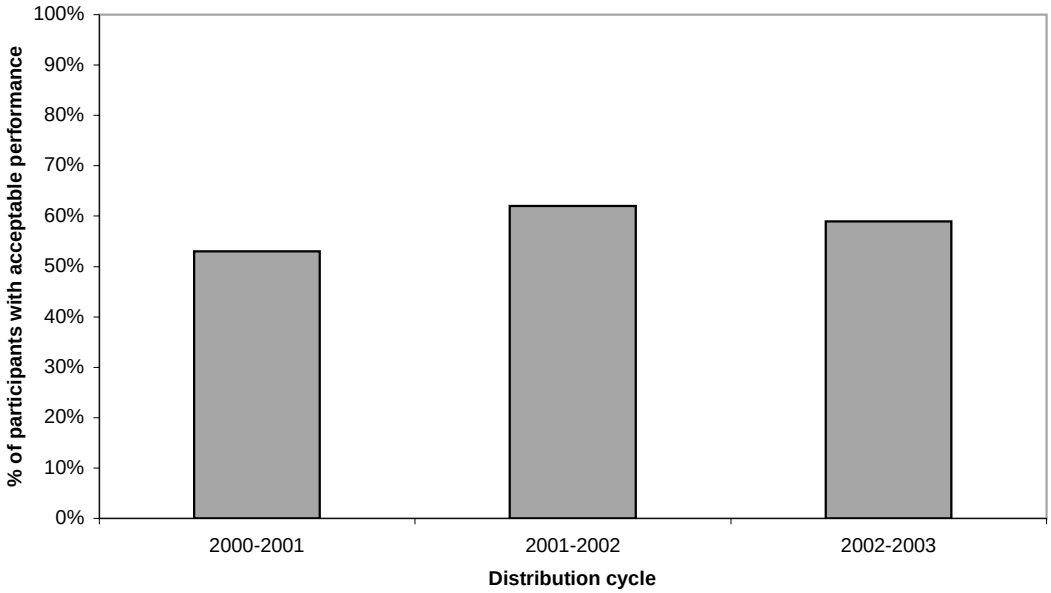

Fig. 1. Percentage of DEQAS participants achieving acceptable performance over the last three distribution cycles.

3.2. Location of participants and methods

Thirteen different countries were represented in 2000 [5] and since then participants from a further five countries (Finland, Iceland, Lebanon, Luxembourg and The Netherlands) have been added. Fig. 2 shows the methods used by those returning results for the January 2003 distribution.

3.3. Reference ranges and intervention criteria

Fifty-five participants returned completed or partially completed questionnaires. Each participant was asked to indicate their reference ranges for both analytes and the concentration of 25-OHD below which Vitamin D supplementation was considered necessary—the 'intervention level'. Fig. 3 shows the 25-OHD reference ranges provided with the median intervention level superimposed. The intervention values varied from 10 to 100 nM (median 40), with the majority of respondents (54%) quoting a level between 30 and 50 nM. Ranges for $1,25(OH)_2D$ quoted by respondents (22 out of 37) varied widely but the lower and upper median concentrations were 43 and 200 pM, respectively.

3.4. Sample workload

Participants were asked to indicate their type of laboratory/institution, workload and, for $1,25(OH)_2D$, the clinical criteria used to justify a request. Fig. 4 shows the approximate number of assays (percentage of total) carried out in each type of institution participating in DEQAS. The majority of 25-OHD assays are performed in government funded hospital laboratories, whereas the majority of $1,25(OH)_2D$ assays are done in private institutions.

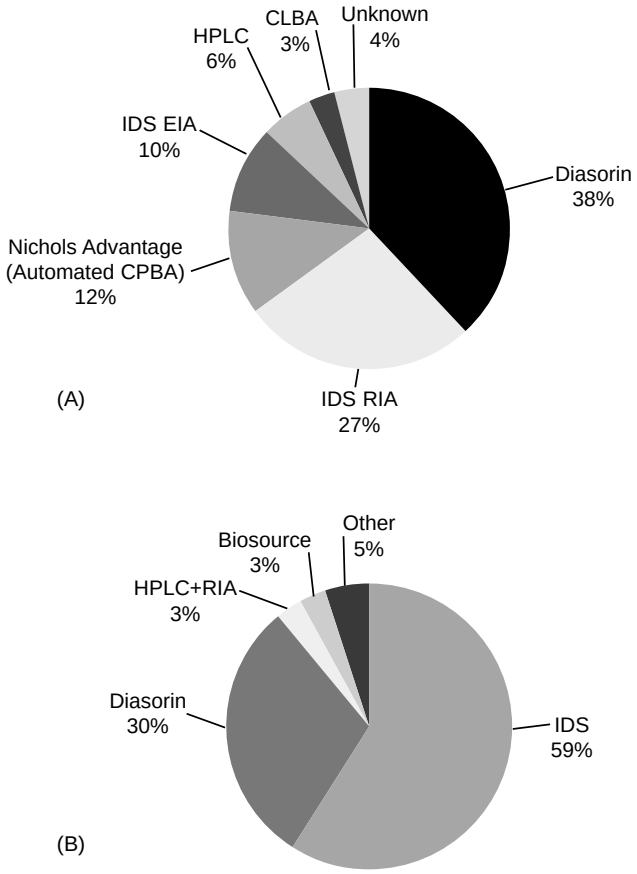

Fig. 2. Methods used by participants for 25-OHD (A) and $1,25(OH)_2D$ (B). CLBA: 'in-house' chromatographic ligand binding assay, EIA: enzyme immuoassay, RIA: radioimmunoassay, CPBA: competitive protein binding assay. Biosource International®, Camarillo, CA 93012; DiaSorin®, Minnisota, USA; IDS®, Tyne & Wear, UK; Nichols Diagnostics®, San Juan Capistrana, CA.

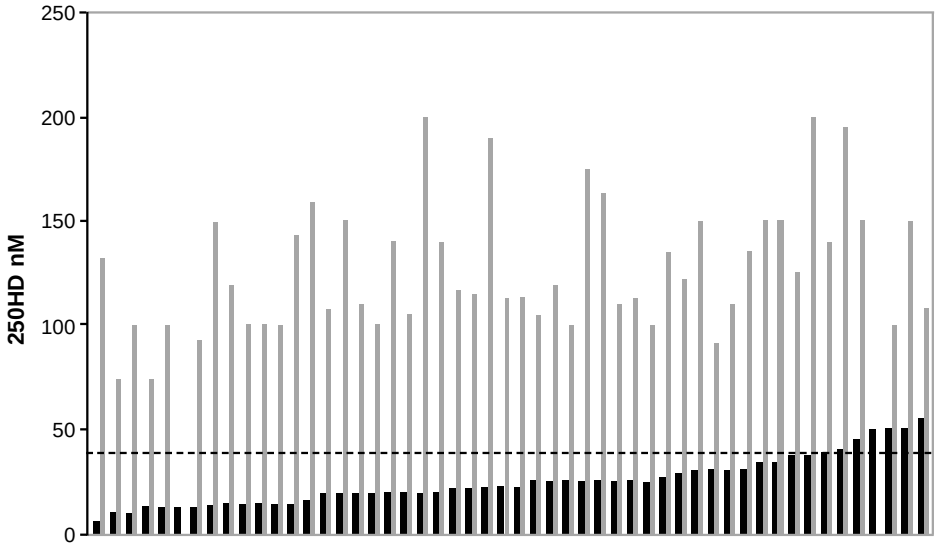

Fig. 3. Lower and upper limits of 25–OHD reference ranges. Each paired column represents data from one questionnaire respondent. The median 'intervention' value (see text) is shown as a dotted line.

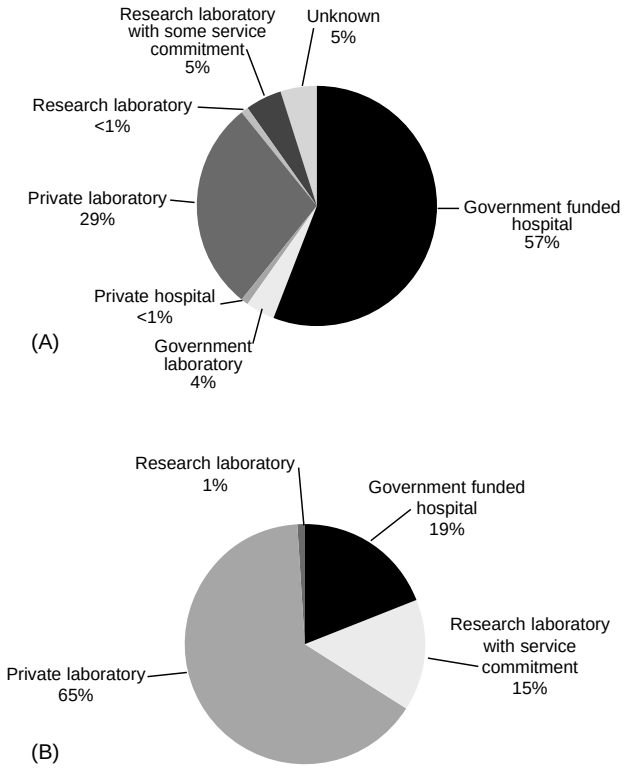

Fig. 4. Percentage of total assays per month for 25-OHD (A) and $1,25(OH)_2D$ (B) performed in different institutions.

3.5. Views on DEQAS

Participants were asked to grade the importance of DEQAS on a scale from 1 (not important) to 10 (extremely important). Seventy-eight percent of respondents gave grades 9 or 10. A significant proportion (65%) felt that accreditation of the scheme by some national or international authority was desirable.

4. Discussion

The availability of commercial kits has undoubtedly led to a worldwide increase in the number of 25-OHD and $1,25(OH)_2D$ assays performed. The apparent simplicity and ease of use of these kits has encouraged the use of these methods in non-specialised laboratories. The need for external as well as internal quality assessment has therefore never been greater. It is perhaps disappointing that in 2003, the fairly undemanding target set by DEQAS has only been achieved by 59% of participants and has fallen slightly since the previous year. Since the demonstration in 1997 [4] that the ALTM was identical to the GC–MS value, a number of new methods have been introduced. This may have contributed to the slight deterioration in overall performance during the last year and there is a need to confirm that the ALTM remains an appropriate target value.

Despite numerous studies [7], there is no universal consensus on what level of 25-OHD indicates a sub-optimal Vitamin D intake. Nevertheless, there was reasonable agreement among the 35 participants who quoted a 25-OHD concentration at which Vitamin D supplementation was considered desirable. The median value of 40 nM is, in the majority of cases, higher than the quoted lower limit of the reference range. These data appear to confirm the prevalence of Vitamin D deficiency in the 'normal' population and that the use of traditionally constructed reference ranges are inappropriate for the interpretation of 25-OHD assays. In the clinical setting, most 25-OHD assays are probably used to diagnose or confirm Vitamin D deficiency. When used for monitoring treated patients, information about the supplement used may be important, as some assays underestimate $25\text{-}OHD_2$. A significant minority of respondents (38%) was unaware of the supplement used in their locality or reported that patients might receive either Vitamin D_2 or Vitamin D_3.

The fact that the majority of 25-OHD assays are performed in government funded hospital laboratories might suggest that there is some clinical justification for these assays. Questionnaire respondents reported a total figure of about 236,000 assays done per year, which suggests that the number of assays per year performed by all DEQAS participants is approaching half a million. If only 10% of these assays revealed Vitamin D deficiency, needing active intervention, it would appear that this screening process is more cost effective than universal supplementation without screening. The continued use of 25-OHD assays as a marker for Vitamin D status would therefore appear to be justified, unlike the majority of $1,25(OH)_2D$ assays performed by our 22 respondents which are done either 'on demand', or for apparently inappropriate clinical reasons. However, the large number of $1,25(OH)_2D$ assays processed by one private laboratory may have skewed our data.

Most existing DEQAS participants thought membership of the scheme was very important. It is hoped that the more rapid dissemination of information made possible by the on-line reporting system will enhance its value. Despite the introduction of commercial kits, the measurement of Vitamin D metabolites remains challenging. Publication of DEQAS performance data is an effective way of demonstrating the validity of results to clinicians, editors of scientific journals and the scientific community in general.

References

[1] M.J.M. Jongen, F.C. Van Ginkel, W.J.F. van der Vijgh, S. Kuiper, J.C. Netelenbos, P. Lips, An international comparison of vitamin D metabolite measurements, Clin. Chem. 30 (1984) 399–403.
[2] E. Mayer, H. Schmidt-Gayk, Inter-laboratory comparison of 25-hydroxyvitamin D determination, Clin. Chem. 30 (1984) 1199–1204.
[3] M.J.R. Healy, Outliers in clinical chemistry quality control schemes, Clin. Chem. 25 (1979) 675–677.
[4] G.D. Carter, J. Nolan, D.J.H. Trafford, H.L.J. Makin, Gas chromatography–mass spectrometry (GC–MS) target values in the international external quality assurance scheme (EQAS) for 25

hydroxyvitamin D (25 OHD), in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone, University of California, Riverside, 1997, pp. 737–738.

[5] G.D. Carter, J. Nolan, D.J.H. Trafford, H.L.J. Makin, Quality assurance of 25-hydroxvitamin D assays: vigilance in the pursuit of excellence, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects, University of California, Riverside, 2000, pp. 793–796.

[6] G.D. Carter, C.R. Carter, Data management in the external quality assessment of assays for vitamin D metabolites (2003) in: Abstract 111 for 12th Workshop on Vitamin D, Maastricht, 6–10 July 2003.

[7] C.J. Bates, G.D. Carter, G.D. Mishra, D. O'Shea, J. Jones, A. Prentice, In a population study, can parathyroid hormone aid the definition of adequate vitamin D status? A study of people aged 65 years and over from the British National Diet and Nutrition Study, Osteoporos. Int. 14 (2003) 152–159.