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Proficiency testing of 25-Hydroxyvitamin D (25-OHD) assays☆

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ABSTRACT

The Vitamin D External Quality Assessment Scheme (DEQAS) has been monitoring 25-OHD assay performance since 1989. The scheme has expanded rapidly in recent years and has 670 participants in 35 countries (July 2009). Five samples of human serum are distributed quarterly and the results analyzed to give an All-Laboratory Trimmed Mean (ALTM) and SD. Each participant has internet access to a preliminary report after submission of results and, following the results deadline, a final report is e-mailed to designated staff in each laboratory. The last 15 years has seen an improvement in mean inter-laboratory imprecision (CV), from 32.0% (1994) to 15.3% (2009) and most major methods are now giving results within plus or minus 7.4% of the ALTM (2009). DEQAS has regularly conducted and reported on a number of investigations into the performance of 25-OHD methods. A gas chromatography–mass spectrometry (GC–MS) reference method for 25-OHD is under development and will be used to assess whether the ALTM remains the most appropriate target for DEQAS samples.

1. Introduction

The International Vitamin D External Quality Assessment Scheme (DEQAS) was established in 1989 after several studies highlighted the poor performance of 25-OHD assays [1–3]. The scheme has expanded rapidly in recent years (Fig. 1) and has 670 participants in 35 countries (July 2009). The relatively large number of samples and frequency of distribution (5 samples quarterly) fulfills requirements in the US of the Clinical Laboratories Improvement Act (CLIA) for endocrinology tests. Although proficiency testing of 25-OHD assays is not compulsory, over 250 US laboratories have voluntarily registered with DEQAS (July 2009) and its importance in monitoring the performance of 25-OHD assays has recently been acknowledged [4].

The primary aim of DEQAS is to monitor the performance of individual laboratories but the data can also be used to assess the performance of the methods used. Help is available to participants and manufacturers in the evaluation and trouble-shooting of new and existing methods. A DEQAS Advisory Panel was established in 1997 and comprises scientists with acknowledged expertise in the vitamin D field and/or proficiency testing schemes. The Panel sets a performance target and participants achieving acceptable performance over a distribution cycle (1 year) receive a certificate. The target, which is reviewed annually, currently requires 80% or more results to be within 30% of the All-laboratory Trimmed Mean [5].

2. Materials and methods

2.1. Sample preparation/transport

Serum is harvested from blood donated with informed consent of patients with haemochromatosis and polycythaemia attending the Oncology outpatient clinic at Charing Cross Hospital (Imperial College Healthcare NHS Trust, London, UK). Permission for DEQAS to use the blood was obtained from the local Ethics Committee and is in accordance with the UK Department of Health guidelines.

Blood is collected into plain polythene bags and left to clot overnight at 4 °C. The serum is transferred to sterile containers and aliquots put aside for viral screening and 25-OHD measurements. The remaining serum is stored at −40 °C. For each distribution, sera are selected to give a range of concentrations which usually includes a low value and, where possible, one above 70 nmol/L. Most DEQAS samples do not contain significant concentrations of 25-OHD_2 but blood is occasionally obtained from subjects taking a vitamin D_2 supplement. Sera are individually screened for hepatitis B and C and HIV before being combined to produce a pool of the required volume.

Pooled serum is passed successively through 0.7 μm glass fibre and 0.2 μm microbiological grade filters before being aliquotted (0.5 ml) into 2.0 ml polypropylene tubes for distribution. Larger volumes are distributed on request, usually to participants who use HPLC or LC–MS/MS methods.

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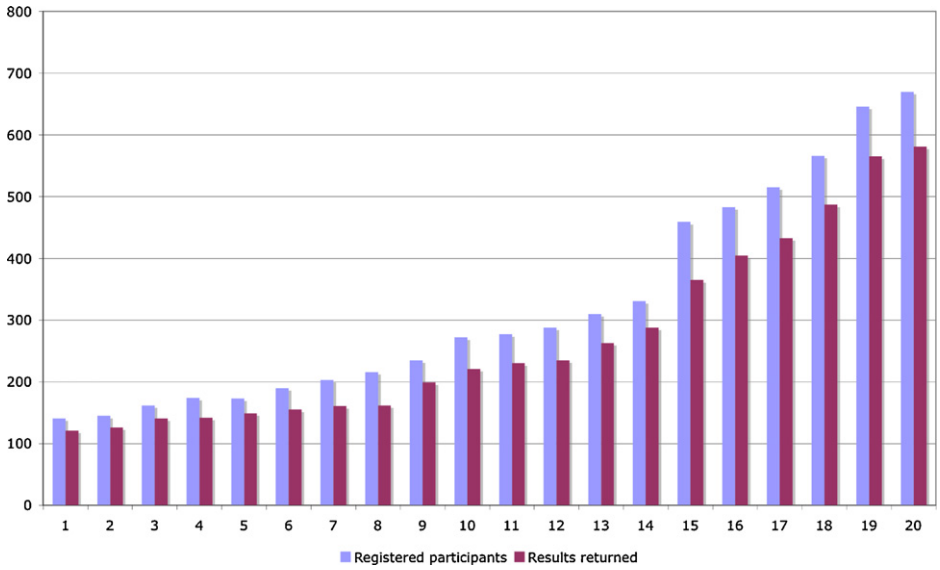

Fig. 1. Participants registered for the 20 distributions October 2004–July 2009 and results submitted*. *Mean return rate 83.5%.

Samples are posted at ambient temperature. Several studies [6,7] have confirmed the stability of vitamin D metabolites in serum and experiments by DEQAS have shown no significant change in 25-OHD concentration during storage for up to 2 weeks at 30 °C. The large number of participants in the US (250+) makes it economically viable to ship them in bulk by airfreight. Samples are sent overnight to a member of the DEQAS Advisory Panel in Atlanta who posts them on by first class or priority mail.

2.2. Submitting results

Participants are allowed approximately 5 weeks to assay the samples and are encouraged to submit their results via the internet, using a form on the DEQAS website. Only results for total 25-OHD are used for statistical analysis, although participants are asked to record the concentrations of 25-OHD_3 and 25-OHD_2 where these have been measured separately. Results can also be e-mailed, faxed or posted.

2.3. Data analysis

Data analysis is performed using a computer software programme on a dedicated server hosted by a commercial company (Positive Internet Company, London, UK). Potential outliers are removed by the method of Healy [5]. Briefly, all returned results are ranked, the top and bottom 5% of results removed and the remaining results used to calculate an All-Laboratory Trimmed Mean (ALTM). An estimator of Standard Deviation is calculated from the trimmed results. The 'accuracy' of each result is judged by calculating its % bias from the ALTM, although it is recognized that the ALTM is only a surrogate for the true value. The Mean, SD and CV% are also calculated for each method.

2.4. Reports

An on-line reporting system allows participants to have instant feedback. After each laboratory has submitted results, data are automatically reprocessed and an interim report can be viewed and downloaded. After they have been reported, results can only be changed by the DEQAS Administrator.

Following the deadline for submitting results, a final report is e-mailed to designated staff in each laboratory. This gives the ALTM and Method Mean (MM) and the % bias of the participant's results for each sample. The report also includes a histogram showing the distribution of all results and those of the participant's own method group.

The reporting system gives on-line access to archived data.

2.5. Inter-laboratory imprecision

Long-term changes in inter-laboratory imprecision were studied by taking the mean CV% of samples distributed during each of the last 15 distribution cycles.

2.6. Method comparisons

The relative accuracy of major methods was assessed from the % bias of each Method Mean from the ALTM. This was done for all 'useable' samples in each of the last two distribution cycles. Samples were excluded where the matrix was altered or they contained significant amounts of 25-OHD_2, which some methods cannot measure quantitatively.

2.7. Investigations

The inclusion of 5 samples in each distribution permits investigations into assay performance, which would be difficult or impossible on a smaller number. Examples of studies undertaken and previously reported by DEQAS have included; recovery of exogenous 25-OHD (July 2005) [8], intra-laboratory imprecision (July 2007) [9], calibration of HPLC and LC–MS/MS assays (January 2008) [10] and the effect of separating gel and EDTA (July 2009) [11].

3. Results

Fig. 1 shows the increase in DEQAS participant numbers over the last 20 distributions, from 141 in October 2004 to 670 in July 2009. Numbers have approximately doubled since January 2008 when the number of registered participants was 331. The proportion of participants submitting results has remained fairly constant, with a mean return rate of 83.5%.

There has been a reduction in inter-laboratory imprecision (Fig. 2) over the last 15 years, from 32% in 1994 to 15.3% in 2009 (April and July). The long-term downward trend stalled in 2002

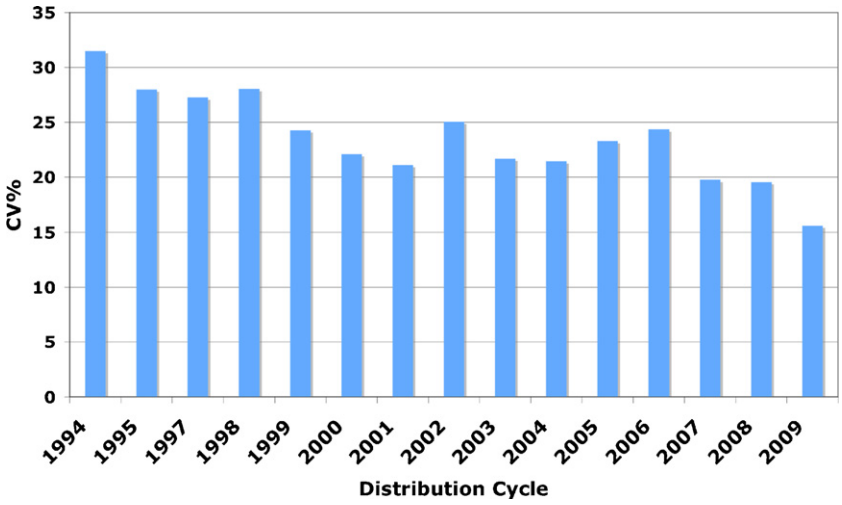

Fig. 2. Imprecision of 25-OHD results (all participants) from 1994 to 2009*. *April and July only.

but appears to have resumed after 2006; the mean CV of the first 2 distributions of the most recent cycle (April 2009–January 2010) is 3.7% lower than for the previous year (April 2008–January 2009).

Fig. 3 reveals that, over the last two distribution cycles, results for the major methods have moved closer. The most noticeable change in bias was shown by the IDS radioimmunoassay which increased from 0.13 to 10.2%. The majority of methods are now positively biased with respect to the ALTM, the exception being the Diasorin radioimmunoassay and Liaison assays which, in the 2008–2009 distribution had a mean bias of −2.1 and −7.4% respectively.

4. Discussion

DEQAS is the only specialist international Proficiency Testing Scheme for 25-OHD. Its rapid expansion over the last few years has probably resulted from an explosion of interest in vitamin D and the addition of 25-OHD assays to the repertoire of many more routine clinical laboratories.

Whilst the overall performance of 25-OHD assays has improved (better agreement between laboratories and methods), 25-OHD remains a difficult analyte to measure.

Automated methods, which have necessarily abandoned conventional solvent extraction and chromatography, may be particularly vulnerable to matrix effects and it is important that samples distributed by proficiency testing schemes should be as close to patient samples as possible. Because DEQAS is organized from a large Teaching Hospital it has access to human blood with a wide range of 25-OHD levels. Samples are generally unadulterated human serum, which avoids potential problems due to dilution with, say, equine serum, or spiking with 25-OHD_3 or 25-OHD_2, a procedure known to give anomalous results in some assays [8]. Blood from most UK patients contain only low levels of 25-OHD_2 and this is known to cause problems for some participants who measure and report 25-OHD_2 and 25-OHD_3 separately [12]. To produce samples containing significant quantities of endogenous 25-OHD_2, DEQAS relies on occasional blood donations from colleagues taking a vitamin D_2 supplement.

Rapid feedback is important in proficiency testing. For this reason, DEQAS participants are encouraged to submit results on-line, after which they have immediate access to updated statistics.

A problem that has hindered attempts to improve inter-laboratory precision is the absence of a reference measurement

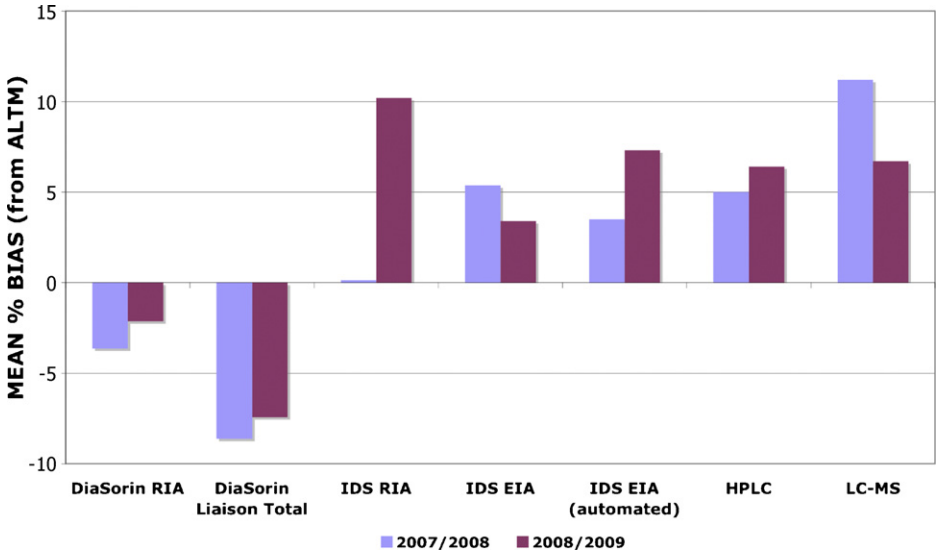

Fig. 3. Relative performance of 25-OHD methods in the last two distribution cycles. RIA: radioimmunoassay, EIA: enzyme immunoassay. DiaSorin, Minnisota, USA, IDS, Tyne & Wear, UK.

procedure (RMP) against which all 25-OHD assays could be standardized. The large positive bias of results obtained with the now defunct Nichols Advantage analyzer probably contributed to the reversal of a long-term decline in inter-laboratory imprecision in 2001, which only resumed after it was withdrawn in 2006 (Fig. 2). The IDS enzyme immunoassay also developed a dose-related positive bias during this period, which disappeared when the kit was recalibrated in 2006.

Recent data (Fig. 3) show that, whilst most methods are now giving similar results, the Diasorin Liaison and IDS radioimmunoassay are giving values which differ by as much as 17.5%. Until a properly validated RMP becomes available there is no way of telling which method, if any, is giving accurate results and talk of a 'gold standard' is misplaced. The ALTM was shown to be a good surrogate for the 'true' value [13] but the scheme is now dominated by the Diasorin Liaison assay which accounts for a third of the results returned. This raises the question of whether the ALTM is still an appropriate target. DEQAS has commissioned the development of a new GC–MS reference method, which will be used check the validity of the ALTM and assign target values to at least some of the distributed samples.

In summary, DEQAS has expanded rapidly in recent years and continues to provide data on aspects of 25-OHD methodology which, by virtue of the large number of contributors, can be more robust than those from smaller studies. The overall performance of 25-OHD assays has improved but there remains an urgent need for a properly validated RMP against which all routine assays could be standardized.

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