NOTES ON INTERPRETING DEQAS REPORTS (25-OHD)

Definitions

Target Value

The value assigned by the NIST Reference Measurement Procedure against which performance is judged (from April 2013).

ALTM (All Laboratory Trimmed mean)
Submitted results are ranked in ascending order and the highest 5% and lowest 5% (10% in total) are removed. The arithmetic mean of the remaining results is the ALTM.

Example: 410 results submitted
10% of 410 = 41
Round to the next even number =42
Remove 21 from the top and 21 from the bottom of the ranked results
Calculate arithmetic mean of remaining 368 results = ALTM

Standard Deviation

A 'robust estimator' of Standard Deviation is calculated after applying weighting factors to the trimmed results (368 in the above example).
A detailed account of this is given in Healey's paper (1).

Bias

The difference between the submitted result (R) and the Target Value (TV), expressed as a percentage of the Target Value.

Example: TV = 46.2 nmol/L
R = 49.5 nmol/L
Bias = [(49.5 – 46.2) / 46.2] x 100 = 7.1%

Assessment of '<' results

Where results are reported as '<', the inequality sign will be ignored and the number used to calculate the bias from the target Value.

Example: **Target Value 15.0**
Participant's result <12.5,
Calculated Bia -16.7% (acceptable)

Participant's result <10
Calculated Bias -33.3 (unacceptable)

Method Mean (MM)

The trimming process is also applied to results from individual method groups (those with 10 or more users) to give a Method Mean, SD, and Bias of results from the Method Mean. These data are given in the report but are not used in the assessment of performance.
Occasionally we calculate MM bias (from the TV) and calculate the average method bias over a distribution cycle. The estimated bias is necessarily based on a relatively small number of results and takes no account of its variability with concentration.

Outliers

Any of the submitted results (410 in the first example) > 3SD from the TV are defined as outliers. These are placed in a separate column at the margins of the frequency distribution histograms (page 1 of the report) and are highlighted (*) in subsequent pages.

Performance Assessment

1. Only the first 4 samples of a distribution are used in the assessment of 25-OHD performance (from April 2013). The 5th sample is not assessed and is reserved for use in investigations that might involve spiking or other manipulations known to cause problems in some non-extraction immunoassays (2).

2. The performance target is the TV ± 25%. In individual distributions, failure to meet the performance target in fewer than 3 (75%) of the 1st 4 samples is regarded as unsatisfactory. Failure to submit fewer than 3 results for the first 4 samples of a distribution is also regarded as unsatisfactory.

3. UK participants who have unsatisfactory performance in three consecutive distributions are regarded as 'persistent poor performers' and will be contacted by the DEQAS Organiser to discuss how the problem is being resolved. DEQAS is obliged to report persistent poor performers to the National Quality Assurance Advisory Panel (NQAAP) for Clinical Chemistry.

4. Participants who achieve acceptable performance in 12 (75%) or more of the 'assessable' samples (usually 16) in the distribution cycle are awarded a certificate to this effect. Successful participants will be able to download the certificate over the internet. **Participants must submit results for all four distributions to get a certificate**.

   Should participants be required to produce evidence of their performance before the certificate is available, we recommend they use the Bias Table (see below) published with the final report. This displays the bias obtained on each sample for the current and previous 5 distributions.

Report layout

(It would be helpful to have a copy of a report in front of you when reading these notes.

First page. This contains all the information needed to assess your performance.

a. A frequency distribution of all results and the distribution of results submitted by users of your method (green/shaded). The ALTM and SDs are marked at the top of each histogram, and the position of your result indicated by a short line against the relevant column.

b. On the right of page 1 are given details of sample number, TV, ALTM, the trimmed mean of results submitted for your method (MM) and the bias of your result from the TV. Also given is the bias of your result from the ALTM and MM although these are not used in performance assessment.

Finally, we record the method we believe you are currently using. If this not correct you should e-mail the Organiser immediately.

Pages 2 - 6

These pages list the methods used, the number of results submitted for each, followed by the Method Means, Standard Deviation (SD) and the Coefficient of Variation (CV%) for each sample.
For those participants more familiar with ng/ml (ug/L), values in these units are given in parentheses.

Page 7 (BiasTable)

This table summarises your data for the samples in the current and 5 previous distributions. The right hand column gives your % Bias from the Target Value.
Performance is regarded as unacceptable if the % Bias is >25.0 %.

Subsequent Pages

These give a complete listing of all results, with the TV, ALTM, SD and CV printed at the bottom of each page. Outliers are marked (*)

NOTE. DEQAS is unusual among proficiency testing schemes in providing this listing and its continued provision is under review. Meanwhile, we suggest that participants do not print these pages, which can be referred to on-line if and when you wish to view them.

Frequently Asked Questions

Q1. **Why does DEQAS use the Target Value and not the Method Mean in assessing performance.**
A1. The Target Value can be regarded as the 'true' value and, irrespective of method, is that which participants should be aiming for. Use of the Method Mean gives no indication of absolute accuracy, just how well the participant is doing compared to others using the same method.

Q2. LC-MS/MS generally seems to give higher results than immunoassays and my results are often positively biased. As a user of LC-MS/MS, why can't my results be judged against the LC-MS/MS Method Mean?

A2. See also **A1**.
Failure to resolve 3-epi-25-OHD_3 and other isobars from 25-OHD_3 is likely to contribute to the positive bias of many LC-MS/MS assays. We believe the fairly generous performance limits (TV± 25%) takes account of this.

Q3. Do I have to return results for every distribution to achieve acceptable performance?
A3. Yes.
A certificate is not awarded unless results are returned for all 4 distributions during a distribution cycle.

Q4. Why doesn't DEQAS include more samples containing 25-OH-D_2?
A4. Most, if not all, the subjects donating blood to DEQAS are not supplemented with vitamin D_2 and therefore do not contain significant amounts of 25-OH-D_2. Due to the problems experienced with spiked samples (2), we rely on special donations from supplemented individuals (usually colleagues) and most DEQAS samples contain only 25-OH-D_3.
Some immunoassays are not co-specific for 25-OH-D_2 and samples containing this metabolite are currently excluded from performance assessment. The policy of excluding samples containing 25-OHD_2 is being reviewed.

Q5. Are vitamin D metabolites stable at ambient temperature?
A5. Yes.

DEQAS and others have demonstrated that vitamin D metabolites in serum are very stable, probably due to their being tightly bound to protein. However, the original DEQAS experiments were done with one particular method and we cannot guarantee that every method is unaffected by the matrix changes (e.g. increasing pH) which inevitably occur in samples stored at ambient temperature. To minimize this possibility, we would recommend that participants freeze DEQAS samples at -20 to -40 degrees Celsius immediately on receipt. Samples for the US are shipped overnight to our agent in Atlanta who, on the day of arrival, forwards them by priority mail.

Q6. Can I view DEQAS reports at any time?
A6. Yes,
an interim report is available before the results submission deadline but can only be viewed after you have submitted your own results.
The interim report gives the ALTM and %Bias from the ALTM but not the NIST assigned target values.
Remember that the reliability of the statistics will increase with the number of results submitted. Interim reports based on a small number of results can be very unreliable

Participants are notified by e-mail when the Final Report is available for downloading.

Archived reports can be viewed and printed at any time after logging in and selecting the month and year from the drop-down.

REFERENCES

1. Healey MJR (1979) Clin Chem **25** (5); 675-677. Outliers in Clinical Chemistry Quality- Control Schemes

2. Carter GD, Jones JC and Berry JL (2007) J Steroid Biochem Mol Biol **103;** 480-482. The anomalous behaviour of exogenous 25-hydroxyvitamin D in competitive binding assays.