25-HYDOXYVITAMIN D ASSAY PERFORMANCE

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DEQAS is the world's largest specialist EQA for vitamin D metabolites and was set up in 1989 after several national and international studies showed considerable variability among results produced by laboratories measuring 25-hydroxyvitamin D (25-OHD). The scheme was extended in 1992 to cover $1,25(OH)_2D$ and DEQAS has recently introduced pilot schemes for $24,25(OH)_2D$ and Free- 25-OHD. The 25-OHD scheme is accuracy based with values assigned to all samples by the NIST Reference Measurement Procedure RMP) enabling participants and manufacturers to judge the accuracy of results against an internationally recognized reference method. Overall accuracy and precision has improved over recent years, not least because of the efforts of the Vitamin D Standardization Program (VDSP) in encouraging immunoassay kit manufacturers to calibrate their methods against RMPs or the NIST SRMs. In April 2016, the mean method bias and CV of most assays was within or close to ± 5% and 10% respectively, the limits adopted by the VDSP. Nevertheless, inter-sample variability of bias remains stubbornly high, particularly in ligand binding assays. This can be due to under-recovery of $25\text{-}OHD_2$ by some methods, non-specific interference from other serum constituents such as lipid (matrix effects) or the cross reactivity of other vitamin D metabolites, particularly $24,25(OH)_2D$. The tight correlation between $24,25(OH)_2D$ and 25-OHD probably contributes to the concentration dependent bias of most 25-OHD immunoassays. A similar correlation between 3-epi-25-OHD and 25-OHD is thought to account for the small but consistent positive bias of LC-MS/MS assays. With the possible exception of the Roche method, the 3-epimer was shown not to cross react in ligand binding assays. A pre-analytical problem became evident in April 2013 when NIST reported the presence of an interferent visible as a shoulder to the 3-epimer peak. This was initially thought to be a plasticiser (DEHP) leaching from the collection bags into the donated blood. However, the structure of DEHP bears no resemblance to vitamin D metabolites and is probably a surrogate for another leached material, the identity of which is not yet known. A comparison of blood collected into plastic bags and glass showed that only one 25-OHD immunoassay was affected. Nevertheless DEQAS decided to purchase serum from a commercial supplier who could supply serum free of leached materials. Large amounts of DEHP are apparently present in samples distributed by at least one other UK based EQA and it is important that scheme organisers ensure that donated blood is free of leached materials which might cause interference in methods used by their participants.