

## Sample stability

Sample stability is an important issue for DEQAS as samples are sent worldwide at ambient temperature. Solutions of vitamin D and its metabolites are known to be light sensitive and relatively unstable. However, in experiments conducted before the regular dispatch of samples (Fig.1), 25-OHD was shown to be very stable in serum, probably as a result of the tight binding to vitamin D binding protein and the relative opacity of aqueous solutions to UV radiation (ref.2). However the stability studies were performed using a chromatographic method and we cannot guarantee that matrix changes (e.g. a rise in serum pH) that inevitably occur at ambient temperature might not affect the performance of less rigorous methods.

A more recent experiment was conducted using an in-house LC-MS/MS method.

## Stability of 25-OHD at 30 degrees C

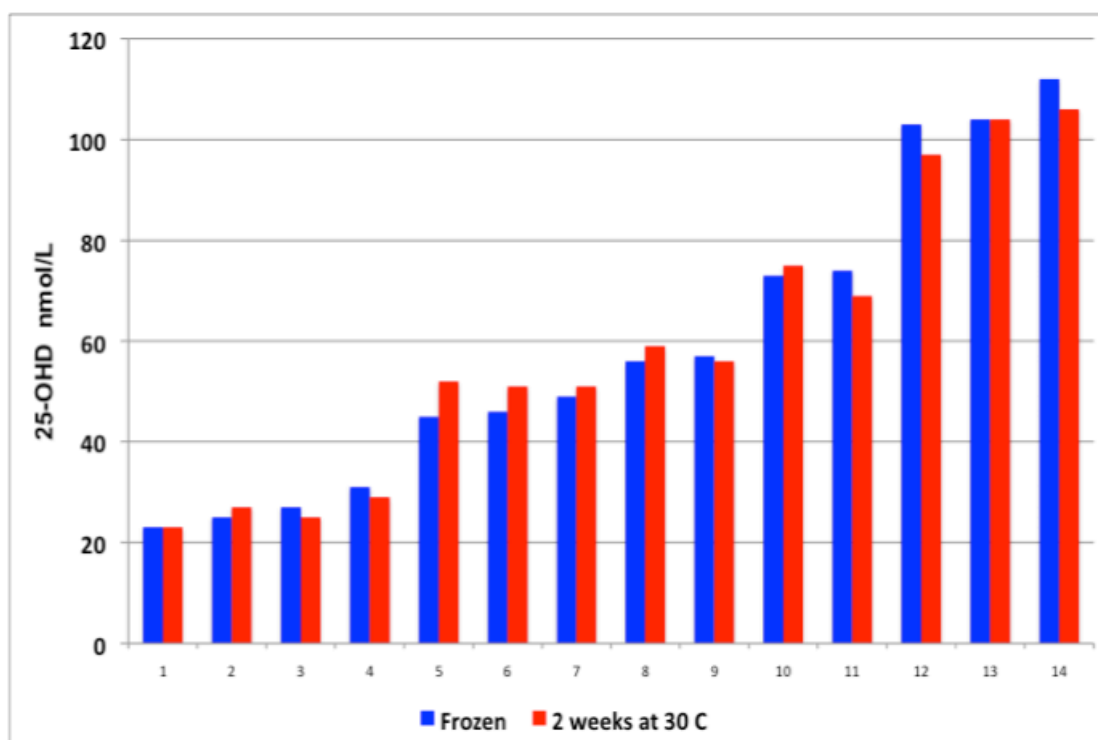


Fig.1 Stability of 25-OHD in samples kept at 30 degrees C for 2 weeks. Measurements made by an in-house extraction / chromatography competitive protein binding assay (adapted from Haddad JG, Chyu KJ.) (Ref.1.)

## Stability of 25-OHD at Room Temperature (RT)

5 DEQAS samples analysed by LC-MS/MS

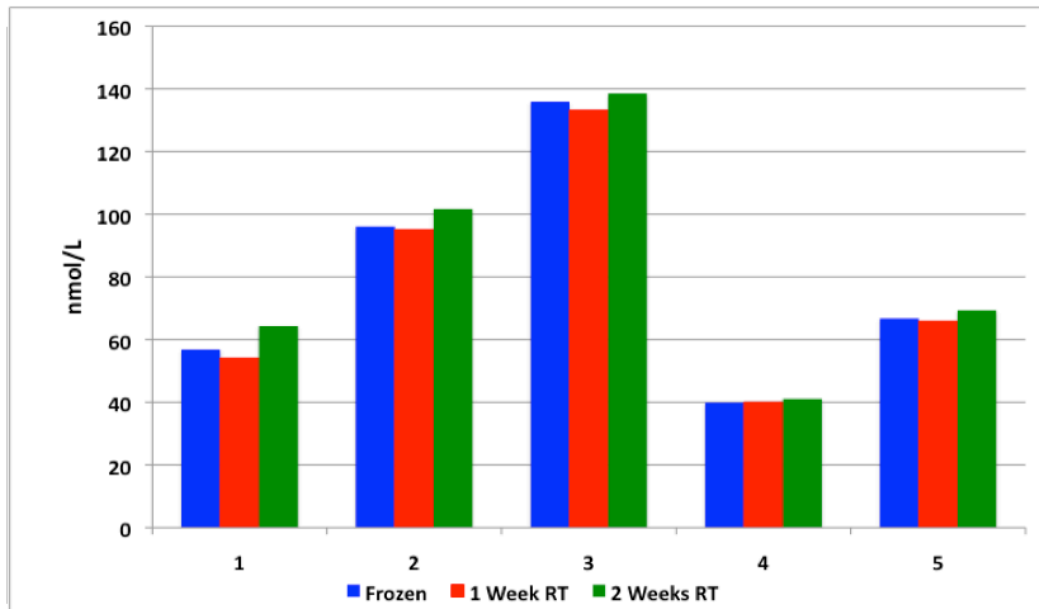


Fig.2 Stability of 25-OHD<sub>3</sub> in DEQAS samples stored at ambient temperature for 1 and 2 weeks.. Measurements made by an in-house LC-MS/MS assay.

### References

1. Haddad JG, Chyu KJ. Competitive protein-binding radioassay for 25-hydroxycholecalciferol. *J Clin Endocrinol Metab* 1971;33:992–5.)
- 2, Hollis B. Measuring 25-hydroxyvitamin D in a clinical environment; challenges and needs. *Am J Clin Nutr* 2008;88(suppl):507S–10S.