

A model answer for the Spring 2010 WMTC exam question:

“Critically evaluate the methods in general use for the measurement of the concentrations of PTH and vitamin D”.

Note: this question is not asking for a description of vitamin D metabolism or calcium homeostasis. Candidates should not spend significant amounts of time on this aspect.

Introduction:

PTH and vitamin D are two tests which have largely moved from specialist to routine clinical chemistry laboratories in the last ten years. Their measurement is of great value in the assessment of disorders of calcium metabolism. Together they illustrate problems generic to immunoassay and HPLC methods. Individually they illustrate specific analytical problems that laboratories should be aware of.

1. PTH methods

All of the methods in general use in UK laboratories are immunoassays of one form or another. PTH is an 84 amino acid peptide molecule, which is progressively cleaved from the N-termini after secretion. Historically, 1st generation immunoassays used antibodies specific to epitopes at the C terminus, and somewhere towards the middle of the molecule. As the extent of processing became clearer, the location of this N-terminal epitope was moved to include the N-terminus itself. As N-terminal PTH fragments accumulate in patients with falling GFR, “intact” assays measure apparently lower concentrations. There is still debate over the possible physiological function of these, but currently it is “whole molecule” or “intact” assays that are believed to be of most clinical use. This illustrates the problem of comparing different immunoassays with different specificities, and the historical clinical data obtained using them.

Whilst plate ELISAs or RIA methods are still in use, by far the most common assays in UK labs (from EQA return data) are automated immunometric assays on large, automated platforms such as the Siemens Centaur, or the Immulite 2000. Similar platforms are available from Roche and Beckman/Coulter. These have short turn-around times (approx 1hr to first result), typically require only a single member of (relatively lower skilled) staff to operate. They can process several thousand samples during a working day, and use primary barcoded tubes. They are often able to use EDTA plasma as a sample type which increases the stability of the sample in-vitro and allows for direct analysis of samples taken from primary care).

As well as problems specific to PTH, the assays also illustrate traditional problems with immunoassays:

Imprecision: often in the order of 10 % within the middle of the reference range (~10-70 ng/ml), higher at lower concentrations if attempting to look at suppressed levels.

Lower limit of detection: typically 5 ng/ml, which may not be sufficient in all clinical circumstances.

Specificity – see discussion on epitopes as discussed above, but also cross-reactivity with PTHrP in patients with solid tumours.

Interferences – including high-dose hook effects, heterophilic antibodies and cross-reactivity with cinacalcet.

Variability in performance lot-to-lot in reagents supplied.

Lack of standardised reference materials

Lack of validity of the manufacturers' reference ranges (often based on patient populations in the sunnier parts of the USA).

Relatively expensive reagents

2. Vitamin D

Vitamin D analysis presents a more complex challenge for analysis than PTH. The steroid molecules exist in multiple different forms (parent, d2, d3; OH and 2-OH; many apparently inactive by products including 24,25). It is also bound to DBP, and little exists in “free” form. The clinically relevant concentrations are those of 25OHD deficient (approx XXX) or insufficient (XXX).

Historically, both immunoassay (including DBP assay) and HPLC have been used in routine clinical laboratories to measure vitamin D.

The immunoassays face particular problems of cross-reactivity from the sterically similar molecules, and the relatively low concentrations. An extraction step was often included to improve assay performance, at the cost of slower throughput. Radioisotopic labels (such as ¹²⁵I) were often used to boost sensitivity to the required level.

Given these challenges, HPLC with either UV or MS/MS detection has long been viewed as a better assay choice. Technical improvements in equipment over the last five years have seen a big increase in the number of UK labs employing HPLC-MS/MS methods.

These are now typically sensitive enough and offer good specificity to the vitamin D molecules of interest (d2 and d3 forms).

However, in comparison to immunoassays to PTH, they require high capital investment, more and higher skilled staff, and often require modifications to existing laboratory buildings (for piped gases etc), as well as specialist support and preventative maintenance from the suppliers. Very recent developments in liquid handling have made many of the assays semi-automated, but typically they

remain more labour intensive and turn-around times slower. The main advantage is improved assay performance and dramatically lower running costs.

There may also be a requirement for higher sample volumes, and assays may only be suitable for plain serum samples (often a problem for paediatrics).

Conclusion:

Overall, both methods illustrate problems with traditionally accepted laboratory methods, as well as providing specific challenges themselves. For PTH, the availability of well-described and understood automated immunoassays has significantly aided clinicians in making rapid diagnoses of calcium metabolism disorders. For vitamin D, new developments in HPLC-MS/MS equipment and sample processing suggest that laboratories will be able to offer more sensitive and specific assays at lower cost in the near future.