Introduction

1,25-dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. It stimulates intestinal calcium absorption, increases bone resorption and inhibits parathyroid hormone (PTH) production by direct action on the parathyroid glands. Circulating levels of 1,25D are used in conjunction with 25-hydroxyvitamin D (25D) and PTH in the differential diagnosis of various bone diseases. 1,25D production is impaired in end stage renal disease (ESRD) and levels are often low in these patients. As such 1,25D is an obvious addition to the IDS-iSYS bone and minerals metabolism products line consisting of 25-Hydroxy Vitamin D, Intact PTH, Intact PINP, CTX-I (Crosslaps®), Ostase® BAP and N-MID® Osteocalcin.

Measurement of the 1,25D has always been difficult due to its lipophilic nature, its low circulating concentration and the presence of higher concentration of other vitamin D compounds. Our aim was to develop and commercialise an IVD CE marked automated 1,25D assay which could simplify the determination of 1,25D in serum or plasma, utilising the well established and proven IDS immunoeXtraction technology.

Materials and Methods

Methods

Serum or plasma sample is purified by immunocapsule. During the purification process, the 1,25D antigen binds to the immobilised highly specific anti-1,25D monoclonal antibody. Interfering substances were removed through washing steps. The 1,25-dihydroxyvitamin D in the sample were eluted with ethanol and evaporated. The dried immunopurified sample was reconstituted with Assay Buffer prior to quantitation by the automated IDS-iSYS system. The reconstituted immunopurified samples can be measured immediately or can be tightly capped and stored at 2-8°C or at -15°C or below for up to two days. Two Extraction Control levels are provided to monitor the integrity of samples purification process.

In the chemiluminescence immunoassay, the purified 1,25D sample competes with 1,25D-acridinium (1,25D-ACR) for a limited amount of biotinylated polyclonal anti-1,25D antibody sites. Bound complexes are captured by use of streptavidin-coated magnetic particles. Following the washing step, the bound 1,25D-ACR is measured in a luminometer where the signal generated was inversely proportional to the 1,25D concentration captured by use of streptavidin-coated magnetic particles. Following the washing step, the bound 1,25D-ACR is measured in a luminometer where the signal generated was inversely proportional to the 1,25D concentration captured by use of streptavidin-coated magnetic particles.

The IDS-iSYS 1,25-Dihydroxy Vitamin D reagent cartridge can be stored on board the system for up to seven (7) weeks, with 21 days calibration frequency.

Sample types

1,25D was measured from five (5) different matched immunopurified sample types. The values obtained from serum separator, K2 EDTA plasma, Li Heparin plasma and Na Heparin plasma tubes were expressed as a % bias to the values obtained from the normal serum tubes. Values were similar for all samples with no trends apparent for any tube type (Table 3).

Method comparison

The IDS iSYS 1,25D assay was compared against the IDS 1,25-Dihydroxy Vitamin D (AA-54) immunoassay for the quantitative determination of 1,25D, following CLSI EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples". A total of 121 samples, selected to represent a wide range of 1,25D concentrations [10.7 – 197.5 pmol/L (25.7 – 474.0 pg/mL)], was immunopurified and assayed by each method. Passing Bablok analysis was performed on the comparative data: IDS-iSYS = 1.00 x (IDS RIA) - 2.8. 95% CI. of the slope and intercept were 0.93 to 1.07, and -3.7 to 0.4 respectively, correlation coefficient (r) = 0.95 (P<0.0001) (Fig. 4).

Conclusions

- The IDS-iSYS 1,25-Dihydroxy Vitamin D assay is a complete test system with the proven immunocapsule extraction and automated chemiluminescence immunoassay.
- With excellent precision and good correlation to current methods, the IDS-iSYS 1,25-Dihydroxy Vitamin D assay will be a valuable tool for clinical laboratories to accurate measuring larger number of 1,25D samples in a single working day.
- The IDS-iSYS 1,25-Dihydroxy Vitamin D test system simplifies the 1,25D blood test for clinical laboratories.