The Assessment of Circulating 25(OH)D and 1,25$(OH)_2$D: Emergence as Clinically Important Diagnostic Tools

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The science of vitamin D assay technology has progressed significantly over the past 4 decades. The clinical utility of these measurements has moved from esoteric into mainstream clinical diagnosis. This movement has been driven by the realization that vitamin D is involved bodily systems beyond skeletal integrity. The clinical assay techniques for circulating 25(OH)D and 1,25$(OH)_2$D have progressed away from competitive protein binding assay (CPBAs) that utilize serum binding proteins or cellular receptors and tritiated reporters to radioimmunoassay (RIAs) that utilize both I^{125} and chemiluminescent reporters. These advances have allowed direct serum analysis of 25(OH)D in an automated random access format that provides a huge sample throughput. Detection of circulating 25(OH)D can also be achieved utilizing direct high-performance liquid chromatographic (HPLC) or liquid chromatography coupled with mass spectrometry (LC-MS) techniques. These methods are accurate, however, they require expensive equipment and restrict sample throughput in the large clinical laboratory. Claims have been made that these latter methods are superior to the automated RIA formats but actual data have not shown this to be true. Further, there is no advantage of reporting 25$(OH)D_2$ and 25$(OH)D_3$ separately as this has been shown to only confuse physicians. Only total 25(OH)D should be reported for diagnostic purposes. Direct serum detection of 1,25$(OH)_2$D is unlikely to occur for many reasons as a sample pre-purification will always be required. However, a semi-automated chemiluminescent detection system with automated sample preparation is in final development for the determination of circulating 1,25$(OH)_2$D. These advances will allow both 25(OH)D and 1,25$(OH)_2$D to be detected in an accurate, rapid fashion to meet the clinical demands that are emerging.