

# 25-Hydroxyvitamin D2 and D3 in Serum by LC-MS/MS

## Test Highlights

- Analytical sensitivity is 1 ng/mL.
- High specificity assured by detection of multiple fragments of 25-hydroxyvitamin D2 and D3.
- Required sample volume is 0.15 mL (serum or plasma)
- Accuracy was evaluated with NIST quality control materials (SRM 972).

## Clinical Background

- Vitamin D can be derived from the conversion of 7-dihydrocholesterol to vitamin D3 or from dietary consumption of vitamin D2 (plant origin) and vitamin D3 (animal origin).
- Hydroxylation of vitamins D2 and D3 takes place in the liver, where they are converted to a number of metabolites, including 25-hydroxyvitamin D.
- 25-hydroxyvitamin D represents the main form of vitamin D that is present in circulation and stored in adipose tissues.
- 25-hydroxyvitamin D2 and D3 are precursors of bioactive hormones 1,25-dihydroxyvitamin D2 and D3.
- A small fraction of circulating 25-hydroxyvitamin D is converted by the kidneys to its physiologically active forms: 1,25-dihydroxyvitamin D2 and D3.
- Biosynthesis of 1,25-dihydroxyvitamin D is regulated by the parathyroid hormone (PTH).
- 1,25-dihydroxyvitamin D plays a primary role in the maintenance of calcium homeostasis.
- Mild to modest vitamin D deficiency can be associated with osteoporosis or secondary hyperparathyroidism.
- Severe deficiency may lead to rickets in children and osteomalacia in adults.
- The consequences of vitamin D deficiency (other than bone health) are not fully known, but may include increased susceptibility to infections and muscular discomfort, as well as an increased risk of colon, breast, and prostate cancers.
- Mild 25-hydroxyvitamin D deficiency is common in adults, children, and especially the elderly; severe deficiency is less common.

## Indications for Ordering

- Differential diagnosis of rickets and osteomalacia.
- Monitoring vitamin D replacement therapy.
- Not recommended as initial testing for vitamin D deficiency.

## Interpretation

- Total concentrations of 25-hydroxyvitamin D2 and D3:
  - Deficiency: less than 20 ng/mL
  - Insufficiency: 20–29 ng/mL
  - Optimal level: 30–80 ng/mL
  - Possible toxicity: greater than 150 ng/mL

## Methodology

- 25-hydroxyvitamin D2 and D3 are extracted from the sample and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS).
- The high specificity of tandem mass spectrometry is enhanced by the measurement of two fragments each of 25-hydroxyvitamin D2 and D3 against an internal standard. Use of multiple fragments assures specificity of the analysis for every sample and eliminates potential interferences.

## References

- Holick MF. Vitamin D deficiency. *N Engl J Med*, 2007;357:266–81.
- Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999;69:842–56.
- Vieth R, Ladak Y, Walfish PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. *J Clin Endocrinol Metab*, 2003;88:185–91.

## Test Information

2002348

25-Hydroxyvitamin D2 and D3, Serum by Tandem Mass Spectrometry

For specific collection, transport, and testing information, refer to the ARUP website at [www.aruplab.com](http://www.aruplab.com).

For information on test selection, ordering, and interpretation, refer to ARUP Consult<sup>®</sup> at [www.arupconsult.com](http://www.arupconsult.com).