

# BLOOD SPOT TEST SPECIFICATIONS

## 25-Hydroxy Vitamin D2/D3

### Clinical Information

Vitamin D3 (cholecalciferol) is a prohormone produced endogenously from the action of ultraviolet light on its precursor, 7-dehydrocholesterol, in the skin, and is also commonly found in over-the-counter vitamin D supplements. Vitamin D2 (ergocalciferol) does not occur naturally, but is manufactured commercially by exposing ergosterol to ultraviolet light, and is the predominant form obtained by prescription in the US. Both are hydroxylated in the liver to form their 25-hydroxy metabolites (25(OH)D2 and 25(OH)D3), the major circulating form of vitamin D in the blood and the commonly accepted measure of vitamin D status. The blood spot assay is performed using liquid chromatography/tandem mass spectrometry (LC-MS/MS), the same method used by the CDC Nutrition Laboratory to obtain accurate 25(OH)D values. Method accuracy is ensured by standardization with the NIST vitamin D standard reference materials. Circulating 25(OH)D3 levels reflect endogenous production as well as vitamin supplementation, whereas 25(OH)D2 levels reflect the results of Vitamin D2 supplementation. Vitamin D2 is believed to be less bioactive than vitamin D3. Vitamin D deficiency is linked with many chronic diseases, including autoimmune diseases, diabetes, cardiovascular disease, and cancer. The reference range is <4 ng/mL for 25(OH)D2 and 20–80 ng/mL for 25(OH)D3.

### References:

Newman MS, Brandon TR, Groves MN, et al. A liquid chromatography/tandem mass spectrometry method for determination of 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3 in dried blood spots: a potential adjunct to diabetes and cardiometabolic risk screening. *J Diabet Sci Technol* 2009;3:156-162.  
Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266-81.  
Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab*. 2004;89:5387-91.  
Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr*. 2006;84:694-7.  
Schleicher RL, Pfeiffer CM. Vitamin D Testing 'How Will We Get it Right?' *AACC Clin Lab News* 2009;35:10-12.

### Assay Method: LC-MS/MS

#### Intra-assay Precision

Intra-assay precision was determined by choosing three samples spanning the reference range, and analyzing them multiple times within the same run. Results are shown below:

Mean 25-hydroxy vitamin D2 (ng/mL)	Coefficient of Variation (C.V. %)
5.0	7.8
7.7	7.6
19.6	9.0

Mean 25-hydroxy vitamin D3 (ng/mL)	Coefficient of Variation (C.V. %)
10.6	8.6
30.4	11.3
50.7	7.7

#### Inter-assay Precision

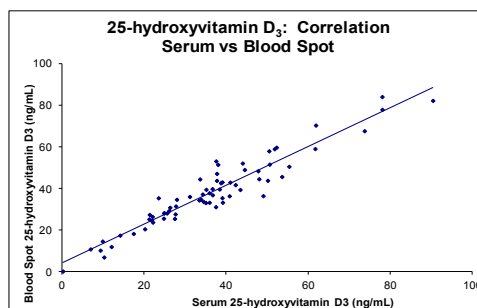
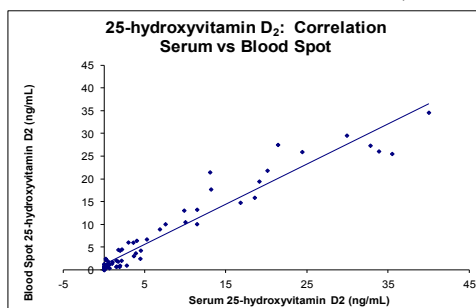
Inter-assay precision was determined by choosing three samples spanning the reference range, and analyzing them multiple times throughout different runs. Results are shown below:

Mean 25-hydroxy vitamin D2 (ng/mL)	Coefficient of Variation (C.V. %)
2.8	19
11.6	11
28.4	9

Mean 25-hydroxy vitamin D3 (ng/mL)	Coefficient of Variation (C.V. %)
7.5	15
23.9	11
54.5	10

#### Accuracy

To test the accuracy of the dried blood spot assay for 25-hydroxy vitamin D, dried blood spot samples collected at the same time as corresponding serum samples were analyzed by linear regression. Resulting correlation data are shown below (25-OH vitamin D2, R = 0.97; 25-OH vitamin D3, R = 0.95):



#### Sensitivity

The lower limits of detection of the 25-hydroxy vitamin D2 and D3 assays are 0.5 and 1.9 ng/mL, respectively.

#### Analyte Stability

The dried blood spot samples are stable for more than 1 month at room temperature.

#### Specimen Collection

Kits for blood spot collection contain a filter paper collection card, finger lancets, an alcohol prep pad, sterile gauze, a band-aid, easy-to-follow instructions, and a mailer to return the sample for analysis.