

Ability of four vitamin D assays to measure vitamin D2

Vitamin D, the sunshine hormone, is essential for healthy bones. Vitamin D is found in two basic forms in nature—vitamin D₃ which is made by skin exposed to sun rays (cholecalciferol), and vitamin D₂ which is made by plants, fungi, and fish (ergocalciferol). Nutritional supplements (treatments) may contain either vitamin D₃ or vitamin D₂-related forms. Here, the vitamin D₃ and vitamin D₂-related forms will be referred to as vitamin D₃, vitamin D₂, and total vitamin D for vitamin D₃+vitamin D₂. Although some studies have shown that vitamin D₂ must be taken at two to three times the dose of vitamin D₃ to achieve the same effect, both are beneficial for bones. Generally, serum vitamin D₂ levels are very low in blood and rise dramatically after supplementation.

Doctors routinely check the blood of patients and those at risk for bone diseases to see if they have sufficient levels of total vitamin D. Total vitamin D concentrations are measured in different ways in clinical laboratories. The gold standard method separates total vitamin D into vitamin D₃ and vitamin D₂ forms, accurately measures the amount of each form, and provides the total vitamin D value (vitamin D₃+vitamin D₂). However, simpler and faster automated assays are more widely used. These automated assays use antibodies or proteins to detect only total vitamin D (vitamin D₃+vitamin D₂). Examples of automated assays include those from manufacturers such as Siemens, Roche, Abbott, and DiaSorin. Automated assays assume that vitamin D₂ and vitamin D₃ are recognized equally by the assay which may not always be the case. In the past, some assays were found to preferentially detect vitamin D₂, whereas others under detected vitamin D₂. It is important to make sure that current assays measure vitamin D₃ and vitamin D₂ equally in order to avoid misdiagnosis.

We compared the total vitamin D results from four current automated total vitamin D assays (from Siemens, Roche, Abbott, and DiaSorin) to the gold standard method results, using blood samples taken from people on vitamin D₂ supplementation. Twenty healthy adults who had never received vitamin D supplements were given oral vitamin D₂ for six months (2400 international units, IU/day). One hundred and forty serum samples were obtained from blood collected before supplementation and once monthly. The tests were run. Vitamin D₂ was almost undetectable in most samples before supplementation, as measured by the gold standard method. Over the first two months vitamin D₂ concentrations went up substantially and vitamin D₃ levels decreased to compensate. (Note that vitamin D₃ concentrations go down because these healthy subjects have a set-point for vitamin D which the body adjusts to.) After two months the concentrations of vitamin D₂ and vitamin D₃ were about the same. We calculated how much the results from the automated assays differed from the gold standard results. This was done using all samples collected over six months and also for all samples collected monthly. The overall differences (or bias) in results for each of the assays compared to the gold standard assay were not significant. In conclusion, although the Siemens and Roche assays showed the least differences, all four automated vitamin D assays were acceptable for use in clinical laboratories to measure total vitamin D (vitamin D₃+vitaminD₂) accurately in patients receiving vitamin D₂ supplements.

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[Performance evaluation of four 25-hydroxyvitamin D assays to measure 25-hydroxyvitamin D2.](#)

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