

1 **Accuracy Evaluation of Three Routine 25-hydroxyvitamin D Assays by Comparing**
2 **with LC-MS/MS**

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9 **Background:** Vitamin D deficiency has been associated with osteopenia, increased risk
10 of death from cardiovascular disease, cancer and etc. Since the US Endocrine Society
11 guideline defines vitamin D deficiency as 25(OH)D less than 20 ng/ml (50nmol/l), the
12 accurate measurement of 25(OH)D in clinical laboratory is essential in medical health
13 care. The purpose of this study was to evaluate accuracy of three routine 25-
14 hydroxyvitamin D immunoassays by comparing with liquid chromatography/tandem
15 mass spectrometry (LC-MS/MS). We focused on the influence of vitamin D-binding
16 protein (DBP) and differences between 25(OH)D assays and LC-MS/MS result in three
17 different groups.

18 **Methods:** Serum samples of 48 healthy individuals, 50 pregnant women, 50 intensive
19 care unit patients were collected. Samples were analyzed with three automated assays
20 (Centaur, Elecsys, and Architect), and an LC-MS/MS. The concentrations of DBP were
21 measured by commercial enzyme-linked immunosorbent assay (R&D Systems).

22 **Results:** Mean 25(OH)D values were 20.1 ng/mL in healthy individuals, 20.6 ng/mL in
23 pregnant women, and 11.9 ng/mL in ICU patients. All three 25(OH)D assays showed
24 significant biases compared with LC-MS/MS method. The mean absolute biases
25 [(routine assay result) - (LC-MS/MS result)] were -2.56 (Centaur), -5.66 (Elecsys), and
26 -6.21 (Architect) ng/mL, with the mean relative % biases ranged from -83.1% to -
27 114.8%. The degree of negative biases were augmented as the increment of 25(OH)D
28 concentrations. DBP concentrations were significantly higher in samples of pregnant
29 women and lower in samples of ICU patients than in healthy individuals. However, the
30 degree of negative biases in three immunoassays was not affected by the DBP levels.

31 **Conclusions:** All assays showed considerable negative biases, but there were

32 differences in the degree of biases. DBP concentrations of pregnant women and ICU
33 patients were significantly different from those of healthy individuals. However, DBP
34 level in each group was not related to the degree of biases in immunoassay. Therefore,
35 when we interpret results of 25(OH)D concentrations, careful consideration is necessary.