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**SUBJECT:** New 25-Hydroxyvitamin D Assay

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On Wednesday June 24, 2009, URMCLabs at Strong will be switching to an in-house liquid chromatography-tandem mass spectrometry (LC/MS) assay for 25-hydroxyvitamin D (25-OHD) levels, replacing the current send-out immunoassay (IA). (DiaSorin Liaison II).

#### **Comparison of the two assays**

- Results obtained by the two assays are different.
- The regression equation of over 500 paired data-points is:  $LC/MS = IA \times 0.85 + 7.3$ ; the correlation coefficient ( $r^2$ ) is 0.63.
- LC/MS results will be 15-20% higher, and more so at low concentrations.
- Correlation coefficient ( $r^2$ ) of only 0.63 reflects the scattering of data points around the regression line.
- Quantitative conversion of LC/MS results to equivalent IA results using the regression equation may not be accurate.

#### **Accuracy of the LC/MS assay**

- The new LC/MS method provides a more accurate measurement of 25-OHD
- Direct measurement of 25-OHD<sub>2</sub> and 25-OHD<sub>3</sub> levels and the sum of the two is reported as total 25-OHD level.
- Inaccuracy of the immunoassay is due to:
  - Lack of equal reactivity for 25-OHD<sub>2</sub> and 25-OHD<sub>3</sub>
  - Reactivity toward 25-OHD<sub>2</sub> and 25-OHD<sub>3</sub> PLUS other metabolites.
- The new LC/MS/MS assay is in excellent agreement with Mayo Medical Laboratories (our reference laboratory) and other LC/MS laboratories. The Mayo LC/MS/MS assay is traceable to the original RIA assay that was used to determine many of the published clinical data on 25-OHD (1).
- Infants have significant concentrations of the C-3 epimers of 25-OHD<sub>2</sub> or 25-OHD<sub>3</sub>, which interfere with the LC-/MS/MS assay. Orders for 25-OHD measurements in children less than 1 year will be automatically sent to Mayo Medical Laboratories, which uses a special assay that can accurately detect 25-OHD in the presence of its C-3 epimers.

## Lab report and reference ranges

The lab report on 25-OHD will list three concentration results:

- 25-OHD<sub>2</sub>
- 25-OHD<sub>3</sub>
- Total 25-OHD (sum of 25-OHD<sub>2</sub> and 25-OHD<sub>3</sub>).

The reference ranges for total 25-OHD will be (2):

- <20 ng/ml Deficient
- 21-29 ng/ml Insufficient
- 30-80 ng/ml Optimal
- >80 ng/ml Possible toxicity

The upper limit of the optimal range is not well defined nor is the limit beyond which toxicity (as hypercalcemia) is evident. Most laboratories list the upper limits of the optimal total 25-OHD levels at 80-100 ng/ml. We have chosen 80 ng/ml in order to be consistent with our reference laboratory (Mayo). It is known in the literature that workers who spend a lot of time in the sun can have 25-OHD levels >100 ng/ml and that 25-OHD levels associated with Vitamin D toxicity are almost always > 200 ng/ml. In our Lab, however, levels >80 ng/ml will be flagged as 'Possible Toxicity' because the lowest reported 25-OHD level associated with hypercalcemia is 88 ng/ml (3).

## References:

1. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. *Am J Clin Nutr* 2008; 88(Suppl):507S-10S.
2. Hollick MF. Vitamin D status: Measurement, interpretation, and clinical application. *Ann Epidemiol* 2009; 19:73-78.
3. Rizzoli R, Stoermann C, Ammann P, Bonjour JP. Hypercalcemia and hyperosteolysis in vitamin D intoxication: effects of clodronate therapy. *Bone* 1994; 15:193-8.

If you have any questions, please contact Toxicology Laboratory Director Dr. Tai C. Kwong via the methods listed above.