

**Re-analysis of Data from “*Current status of clinical 25-hydroxyvitamin D measurement:  
An assessment of between-laboratory agreement*”**

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**Abstract:** Publication by Binkley et al. (2010)<sup>1</sup> of a direct comparison between 25(OH)D measurements made in eight laboratories using three measurement techniques has shown that clinicians and patients can expect large uncertainties in laboratory measurements of serum vitamin D. Reconsideration of the published data using bootstrap analysis shows that three of the eight laboratories had systematic calibration errors, and that whereas Diasorin Liaison Total random errors fell within 95% confidence levels of  $\pm 7.7$  nmol/L, the 95% confidence levels for spectrometric methods were proportional at  $\pm 36\%$  for LC-MS/MS and  $\pm 33\%$  for LC-UV. As a result, if clinicians were to apply 95% confidence level error margins, only Liaison measurements would be useful. Further investigation into the causes of the large proportional errors in liquid chromatography methods [LC] is called for, along with more vigilant calibration of both LC and Liaison methods.

## **Background**

As a patient undergoing supplementation to correct 25-hydroxyvitamin D insufficiency, I became concerned when measurements of my serum 25(OH)D from different testing laboratories<sup>2</sup> returned results differing by a factor of two. Binkley et al (2010) showed there were large variations between laboratories, but the conclusions drawn by the authors – that DiaSorin Liaison measurements showed larger statistical variation than did LC-MS/MS<sup>3</sup> - seemed not to conform with my experience. So I undertook a re-analysis of the published data to gain a better understanding of the situation.

## **Methods**

Since I did not have access to the raw data from the original paper, I extracted it manually from Binkley et al. Fig. 3. This probably introduced an error of at least  $\pm 1$  nmol/L, and since some plotted points were not visible, some data were inadvertently omitted. Nevertheless, clear results emerged. Published data from 25 samples were measured as follows: (a) with the University of Wisconsin LC-UV system; (b) three LC-MS/MS systems in laboratories M1, M2, M3; (c) four DiaSorin *Liaison Total* chemiluminescent systems in laboratories L1, L2, L3, L4. Regression studies (Table 1) showed that systems L1, M1 and M2 exhibited significant slope calibration errors when compared to the other systems. Among the systems with little calibration error, very good agreement was achieved between the Liaison systems L2, L3, and L4. Therefore, I used the average of results from these three systems as the bootstrap reference for further analysis.

<b>Laboratory:</b>	<b>L1</b>	<b>L2</b>	<b>L2</b>	<b>L4</b>
<b>Slope:</b>	0.78	0.996	0.991	1.015

<b>Laboratory:</b>	<b>UW</b>	<b>M1</b>	<b>M2</b>	<b>M3</b>
<b>Slope:</b>	1.025	1.162	1.168	1.015

**Table 1.** Linear regression slopes (intercept forced = 0) for results from eight systems using the average of data from L2, L3 and L4 as the bootstrap reference. Systems **L1**, **M1** and **M2** were considered to be mis-calibrated.

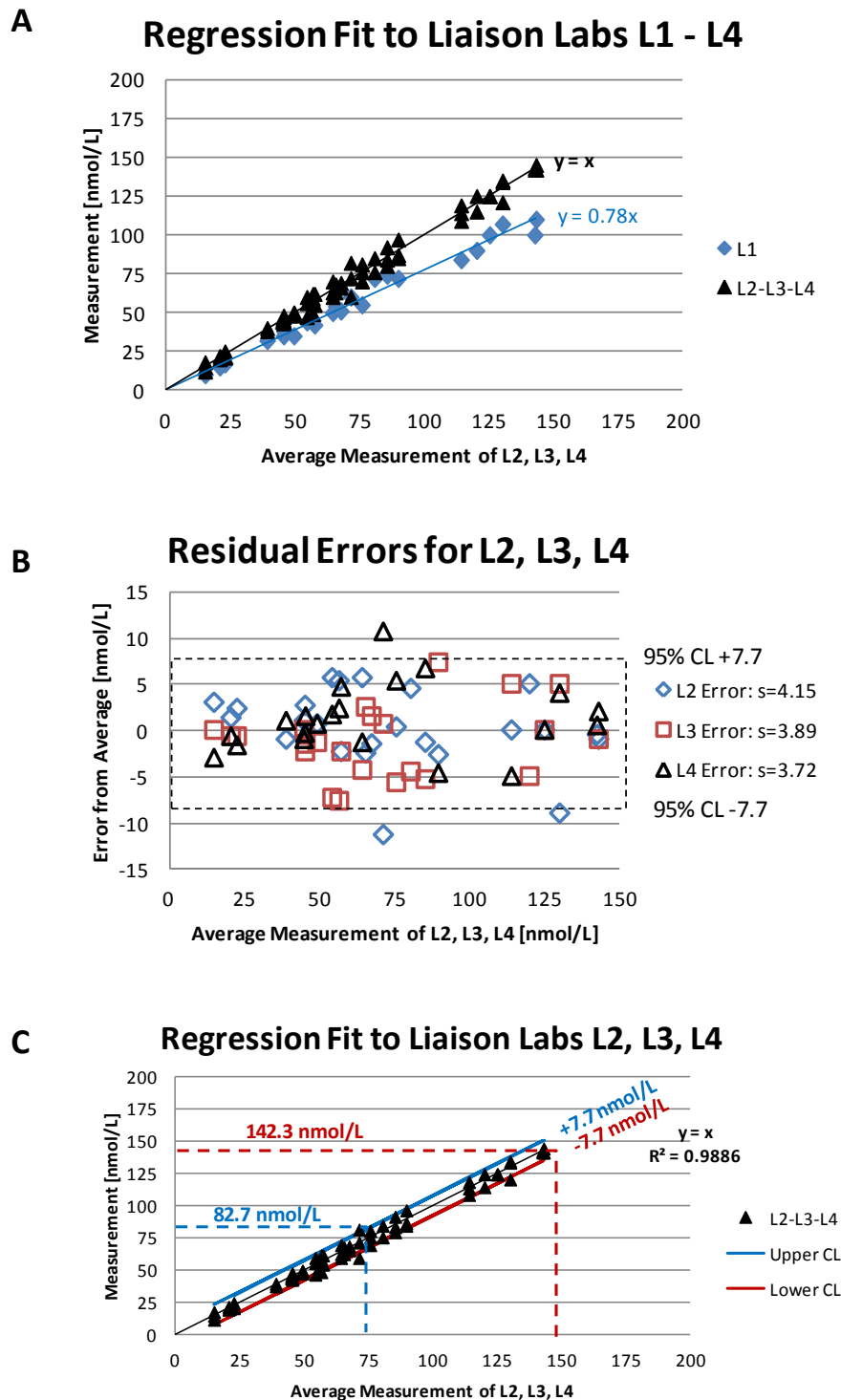
### Random Measurement Errors of Well-Calibrated Systems

Omitting data from the mis-calibrated system L1, we see (Fig. 1A) that Liaison data is tightly grouped around the unity-slope regression line. Plotting the residual error (Fig. 1B) shows a 95% confidence interval of  $\pm 7.7$  nmol/L, statistically independent of measurement value. This translates to a requirement (Fig. 1C) that measurements made by well-calibrated Liaison systems should lie between 82.7 and 142.3 nmol/L (33-57 ng/mL) to ensure 95% confidence that actual values lie between 75 and 150 nmol/L (30-100 ng/mL). This constraint is of little practical significance.

However, for the LC systems, error is proportional to measurement value ( $\pm 33\%$  for LC-UV and  $\pm 36\%$  for LC-MS/MS M3). These proportional random errors are small at low values, they are so large at the cut point of 75 nmol/L (30 ng/mL) there is a 2:1 ratio between upper and lower 95% confidence level values. This causes the measurement window for 95% confidence to disappear entirely in the case of LC-MS/MS M3, the well-calibrated system. Systems with poor calibration (like M1 and M2) further reduce the measurement window in addition to biasing the result above or below the actual value.

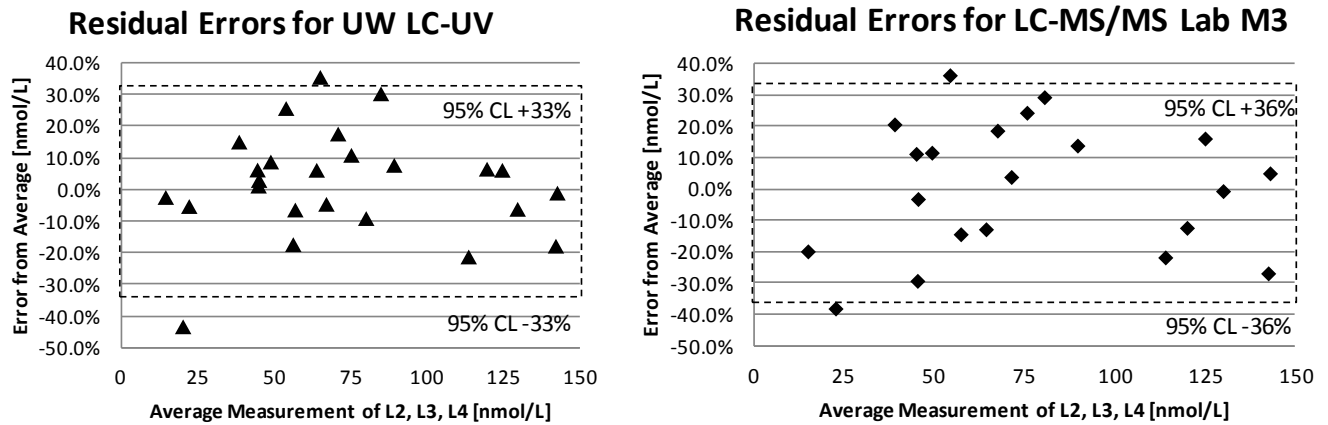
### Conclusion

Although both chemiluminescent and spectroscopic systems are mis-calibrated in today's laboratories, precise calibration is of value only for the Liaison systems which, when properly calibrated, yield accurate measurements. The main problem for liquid chromatographic systems is large proportional random errors. Are these errors fundamental to the analysis of blood plasma, or are they correctible with improved technique? Given the prevalence of LC systems in clinical laboratories, it would be well worth the effort to investigate this further so that the high resolution of LC systems could be used with competitive accuracy.



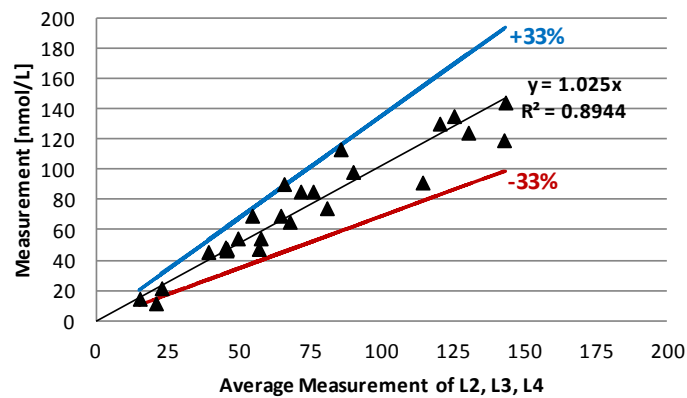
**Fig. 1.** Regression of Liaison Lab results (A) against bootstrap consensus (L2, L3, L4) shows that Lab L1 has a systematic calibration error. A plot of random errors (B) for L2, L3, and L4 shows 95% confidence limits of  $\pm 7.7$  nmol/L for these well-calibrated measurements. Therefore, the acceptance window for well-calibrated Liaison measurements should be 82.7 – 142.3 nmol/L (33.2 – 57.1 ng/mL) for 95% confidence that the true values lie between the recommended limits<sup>4</sup> of 75 and 150 nmol/L (30-100 ng/mL).

**A**



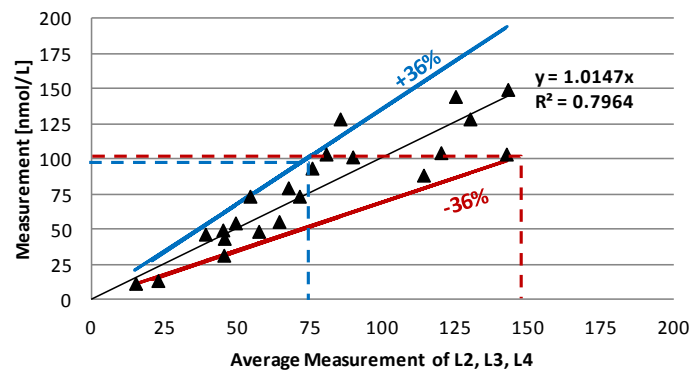
**B**

### Regression Fit to UW LC-UV



**C**

### Regression Fit to LC-MS/MS Lab M3



**Fig. 2.** A plot of random errors (A) for UW LC-UV and LC-MS/MS M3 shows 95% confidence limits of  $\pm 33\%$  and  $\pm 36\%$  respectively. Random error is proportional to measurement value (B, C) such that the acceptance window vanishes entirely (C), allowing 100nmol/L as the only acceptable measurement for 95% confidence that actual values lie between 75 and 150 nmol/L.

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<sup>1</sup> Neil Binkley, Dianne C. Krueger, Sarah Morgan and Donald Wiebe, "Current status of clinical 25-hydroxyvitamin D measurement: An assessment of between-laboratory agreement," *Clinica Chimica Acta*, 411 (2010) 1976 – 1982.

<sup>2</sup> Diasorin Liaison Total from LabCorp. LC-MS/MS from Stanford Hospital and Mayo Clinics.

<sup>3</sup> Binkley et al abstract: "For Liaison, the primary error was likely random, whereas the major LC-MS/MS assay error source was biases likely due to calibration issues."

<sup>4</sup> <http://ods.od.nih.gov/factsheets/vitamind/> [accessed 1/2/2012]: "Emerging evidence links potential adverse effects to...high levels, particularly >150 nmol/L (>60 ng/mL)". Other sources quote higher upper limits. See: <http://www.uvadvantage.org/portals/0/pres/> [Accessed 1/2/2012].