

# Assays for the Determination of 25(OH)D

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# Disclosure

- ∅ I am a consultant to the DiaSorin Corporation.
- ∅ The 25(OH)D test is FDA-approved for the assessment of nutritional vitamin D sufficiency in healthy subjects. It is not intended to be used in the diagnosis of any specific disease and DiaSorin does not support its use in this context.

# 25(OH)D Assays

## ∅ FDA-Cleared:

1. RIA's ( Diasorin, IDS)
2. CLIA's (Diasorin, IDS, not Roche)
3. HPLC (ESA Biosciences)

## Non FDA-Cleared “Home Brew”

1. All LC/MS assays

# Usage of 25(OH)D Assays Worldwide

- ∅ 45 % Diasorin Liaison CLIA
- ∅ 45% LC/MS “homebrew” (many)
- ∅ 10% Other; RIA, CLIA, HPLC ect

## In Vivo Recovery of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> in Human Samples : Pre and Post Vitamin D<sub>2</sub> Supplementation

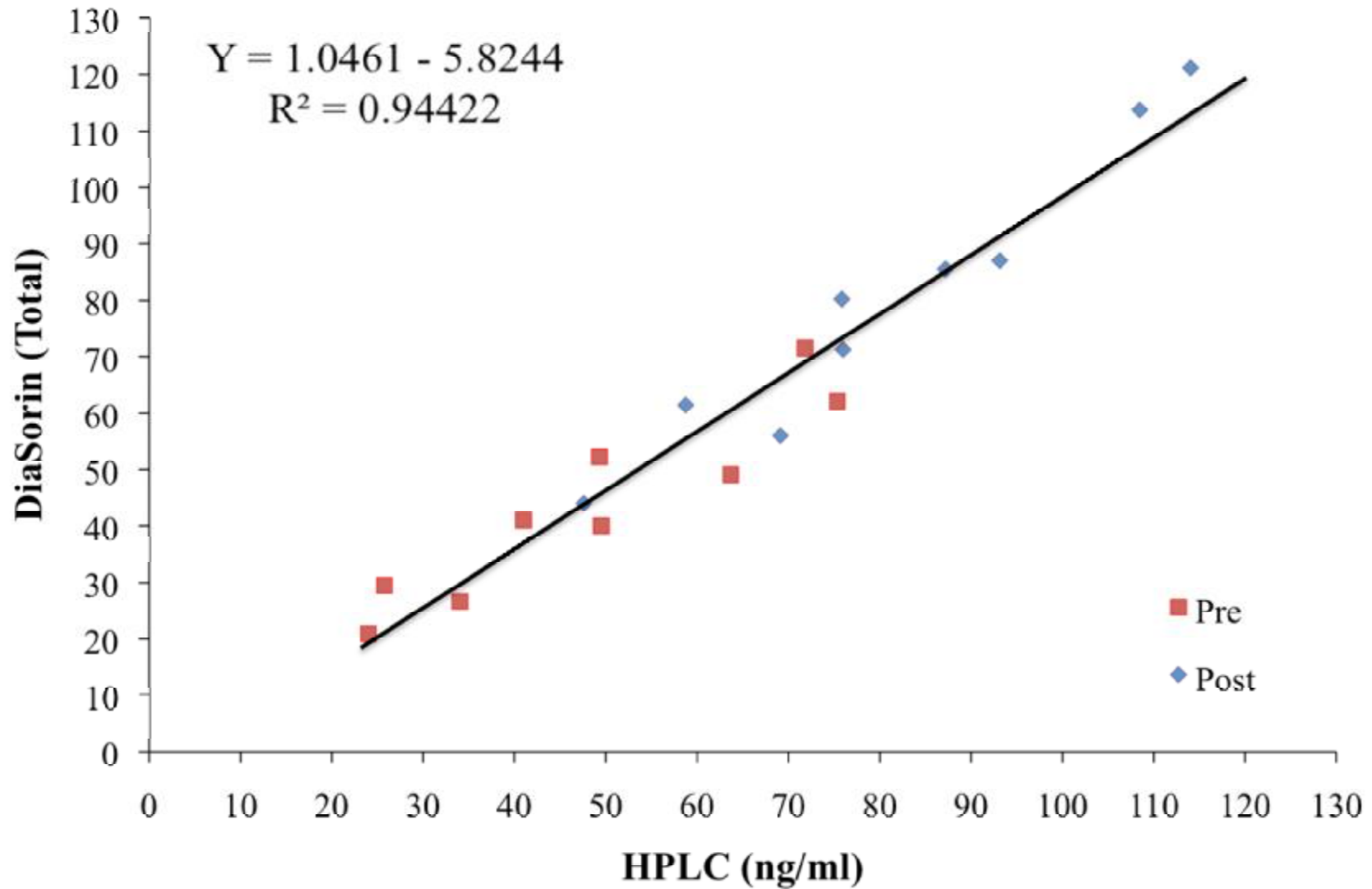


Table 1. Comparison of 25(OH)D concentrations measured by the DiaSorin Liaison and HPLC as a result of various exogenous and endogenous treatments.

Sample ID	DiaSorin Liaison	HPLC
	Total 25(OH)D (ng/ml)	
Baseline	43.7 ± 16.8	48.3 ± 19.0
Vitamin D <sub>2</sub>	81.1 ± 21.9	80.0 ± 23.5
<sup>1</sup> Baseline + 25(OH)D <sub>2</sub>	51.0 ± 16.8 (22.8%)	79.7 ± 19.0 (98.3%)
<sup>1</sup> Baseline + 25(OH)D <sub>3</sub>	63.7 ± 20.4 (62.7%)	80.0 ± 18.5 (99.0%)
Horse Serum	12.7 ± 1.0	4.7 ± 0.2
<sup>2</sup> NIST Level 1 [22-24]	24.4 ± 0.8 (106%)	26.0 ± 1.1 (113%)
<sup>2</sup> NIST Level 2 [12-14]	19.8 ± 0.5 (152%)	15.9 ± 0.7 (122%)
<sup>2</sup> NIST Level 3 [42-46]	27.2 ± 1.0 (61.8%)	48.1 ± 3.0 (109%)

<sup>1</sup>32 ng/ml was added to each of 9 samples. Values in parentheses represent amount of 25(OH)D recovered as a % of mean values.

<sup>2</sup>Values in brackets are expected values provided by NIST. Values in parentheses represent amount of 25(OH)D recovered as a % of mean values.

# NIST Samples

## DiaSorin LIAISON vs HPLC

NIST Control	Expected (ng/ml)	DiaSorin Total (ng/ml)	HPLC (ng/ml)
1 (normal)	26 ± 6	24.4 ± .82	26 ± 1.1
2 (normal +HS)	14 ± 2	19.8 ± .45	15.9 ± .08
3 (normal + 25-OH-D <sub>3</sub> )	~52	37.2 ± .95	48.1 ± 3.0
HS (Sigma)		12.7 ± .95	4.7 ± .21

# NEVER NEVER NEVER

- ∅ NEVER use exogenously “spiked” 25(OH)D<sub>2</sub> OR 25(OH)D<sub>3</sub> to assess or validate ANY direct plasma or serum assay ie Diasorin, Roche or IDS
- ∅ NEVER use anything but human specimens in these direct assays.

# Controls for the 25(OH)D Assays

- ∅ Should perform equally in all assay types as to not introduce more confusion than previously existed.
- ∅ This can ONLY be achieved with endogenous human samples.
- ∅ Such controls are under development by Dr. Linda Thienpont, Laboratory for Analytical Chemistry, University of Ghent.

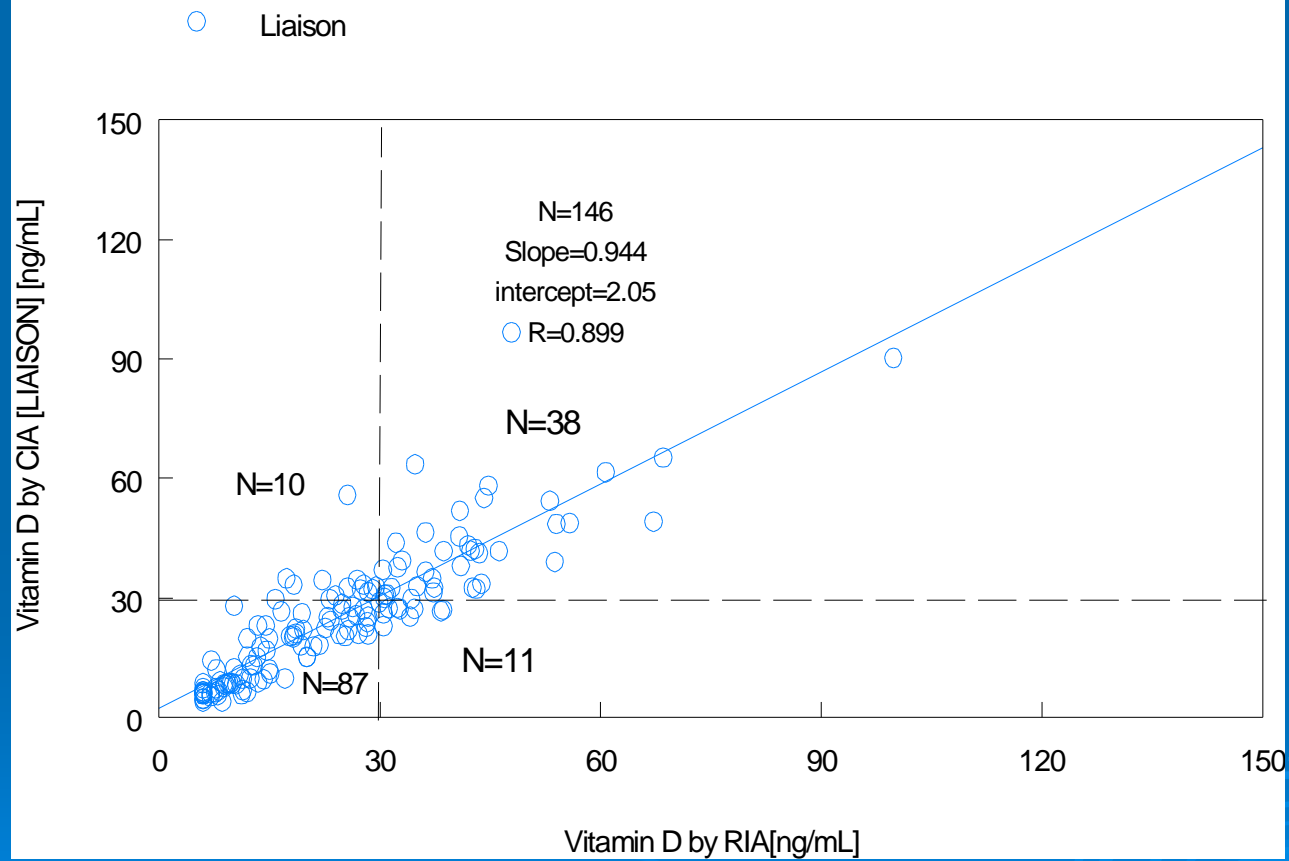
# Clinical Reference Range for 25(OH)D

- ∅ The clinical reference range for 25(OH)D has largely been established with the FDA-cleared DiaSorin tests.
- ∅ A laboratory should only use the clinical reference range for 25(OH)D derived from clinical data using DiaSorin methods for a procedure that has been validated against the DiaSorin methodology.
- ∅ Clinical studies include NHANES III, WHI, and the Harvard-based Health Professionals studies and MANY smaller studies.

## Summary of Deming Regression: Method vs DiaSorin LIAISON® TOTAL-D™

Method	Calibration Material & Method	Slope	Int.	R	N
DiaSorin LIAISON® TOTAL-D™	U.V. quantification	N/A	N/A	N/A	48
DiaSorin RIA	U.V. quantification	1.02	-1.0	0.92	48
Commercial RIA	U.V. quantification	1.10	-3.0	0.92	48
Commercial CLIA	To LC-MS/MS	0.93	2.5	0.88	48
HPLC	U.V. quantification	1.14	0.0	0.84	48
LC-MS/MS Site #1	Unknown	1.06	0.9	0.92	48
LC-MS/MS Site #2	Unknown	1.35	1.3	0.91	48
LC-MS/MS Site #3	Unknown	1.33	0.1	0.92	48
LC-MS/MS Site #4	Commercial Material #1 (multi point)	1.43	-4.3	0.91	48
LC-MS/MS Site #5	Comm. Mater. #1 (single point)	1.11	0.2	0.90	48
	Comm. Mater. #2	1.00	0.6	0.89	
	Comm. Mater. #3	1.17	-0.7	0.91	

# Vitamin D: RIA vs. LIAISON



# FDA Position on Separate 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> Reporting

- ∅ ESA Biosciences applied for a 510K FDA approval in 2007 to market an hplc test to determine 25D<sub>2</sub> and 25D<sub>3</sub> separately.
- ∅ The FDA response was “Previous 25D tests cleared by the FDA have only provided the Total 25D level so unless you can show clinical utility for separate reporting your request is denied”.
- ∅ In 2008 the ESA test was approved for Total 25D testing and the software had to be rewritten to accommodate the stipulation.
- ∅ The same will apply to LC/MS assays.

Thank you

