



Vitamin D testing with the **LIAISON[®] 25 OH Vitamin D TOTAL kit**

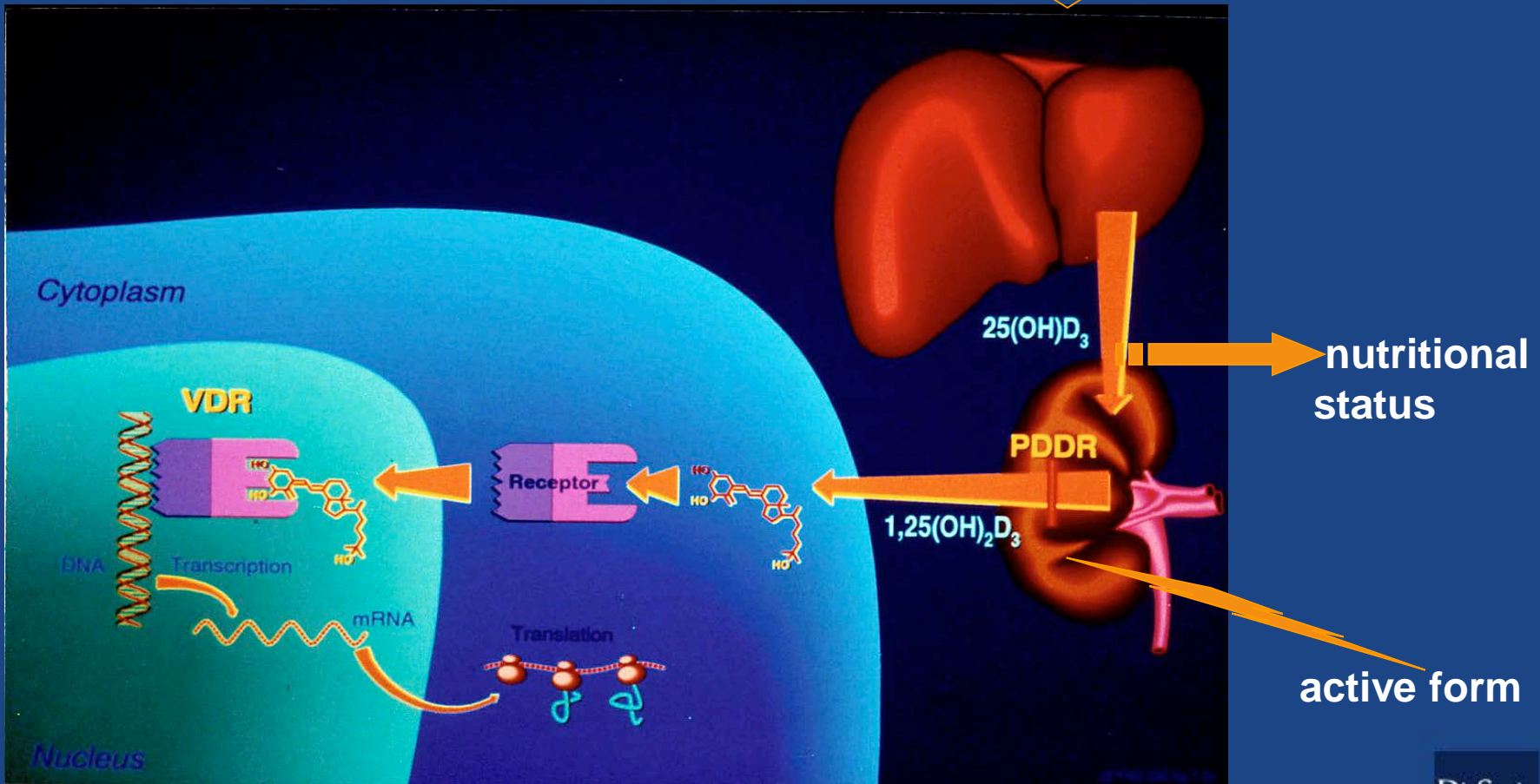
The Diagnostic Specialist

DiaSorin

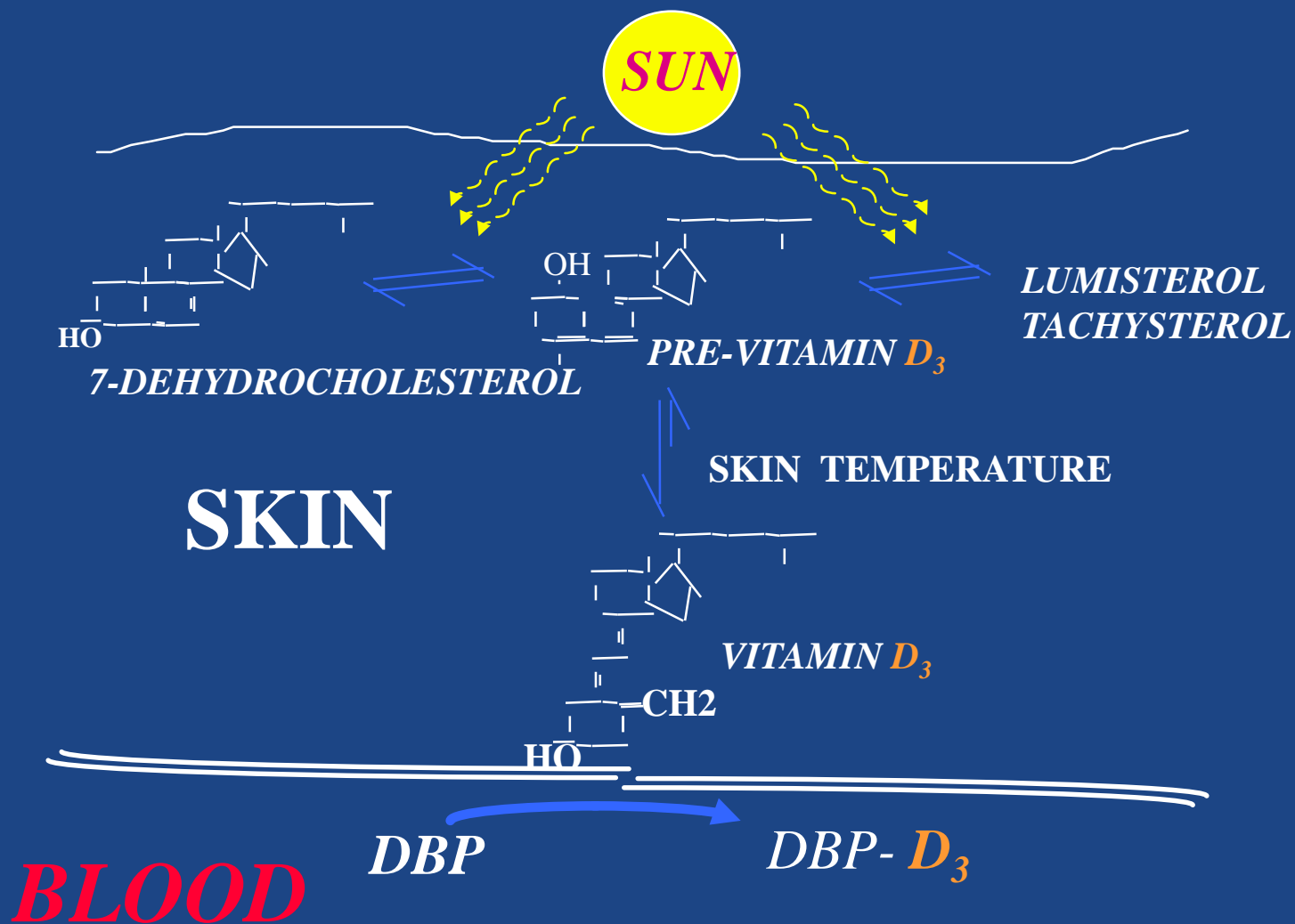
Margherita Banci

Corporate Marketing Manager

Physiology



Skin synthesis of Vitamin D



Vitamin D metabolites

Vitamin D₃ (cholecalciferol)
synthesized in skin
animal origin
contained in supplements

Vitamin D₂ (ergocalciferol)
vegetable origin
contained in supplements

Vit D₂ and Vit D₃
are hydroxylated
to 25 OH Vitamin D
in the liver

TOGETHER Vit D₂ and Vit D₃ ARE the BEST
indicator of vitamin D nutritional status



1,25 dihydroxyvitamin D

The most potent Vitamin D metabolite
its synthesis is stimulated by PTH in the kidney

- regulates bone mineral metabolism
- important role in
 - cancer
 - inflammation
 - and immunity

Zerwekh JE, et al. 1985. J Clin Endocrinol Metab 60:615.
Hendy GN, et al. 2006. Kidney Int 69:218.
Eqils O, et al. 2006. Clin Exp Immunol 143:58.
Lips P. 2006 Prog Biophys Mol Biol 92:4.



Biological Actions of Vitamin D

Intestine

Enhances the intestinal absorption of calcium and phosphate

Bone

Promotes bone remodeling (both formation and resorption)

Kidney

Proximal tubular calcium reabsorption



Vitamin D determination, target patients

In general:

All, when limited exposure to the sun is supposed

All, when malabsorption syndromes are supposed

All, when malnutrition is supposed

All, when limited too low supplementation is supposed

- Children for the correct development of bones to prevent rickets
- People aged >50 yrs for the osteoporosis management
- Homebound Elderly Persons for a better survival

Vitamin D

A number of studies have identified
widespread

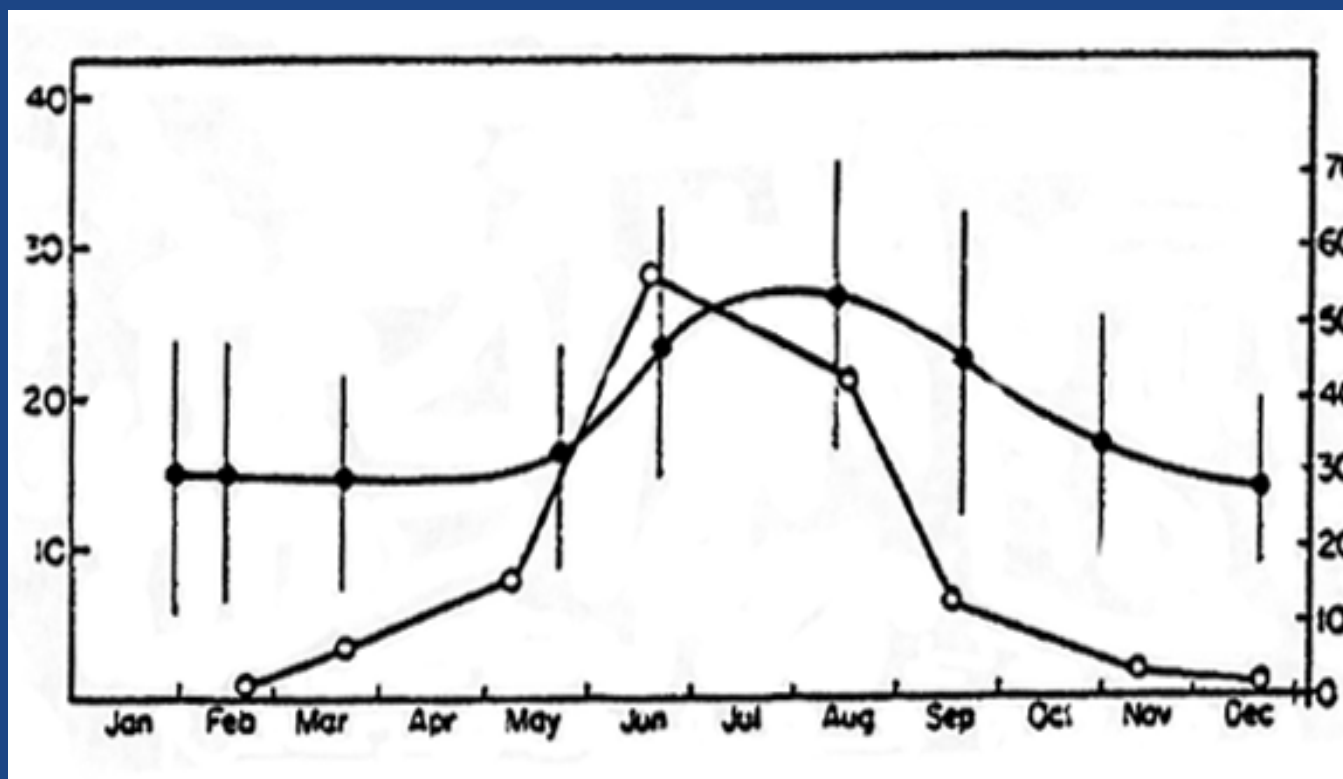
Vitamin D insufficiency

in apparently healthy populations world-wide

Benucci A, et al.1993. J Nucl Biol Med 37:77-82.
Gannage-Yared M-H, et al. 2000. J Bone Miner Res 15:1856-62.
Du X, Greenfield et al. 2001 Am J Clin Nutr 74:494-500.
Bassir M, et al.2001 Acta Paediatr. 90:577-9.
Lamberg-Allardt CJE, et al. 2001. J Bone Miner Res 16:2066-73.
Rucker D, et al. 2002. CMAJ 166:1517-24.

25 OH Vitamin D, measurement issues

Seasonal Variation of 25 OH D levels

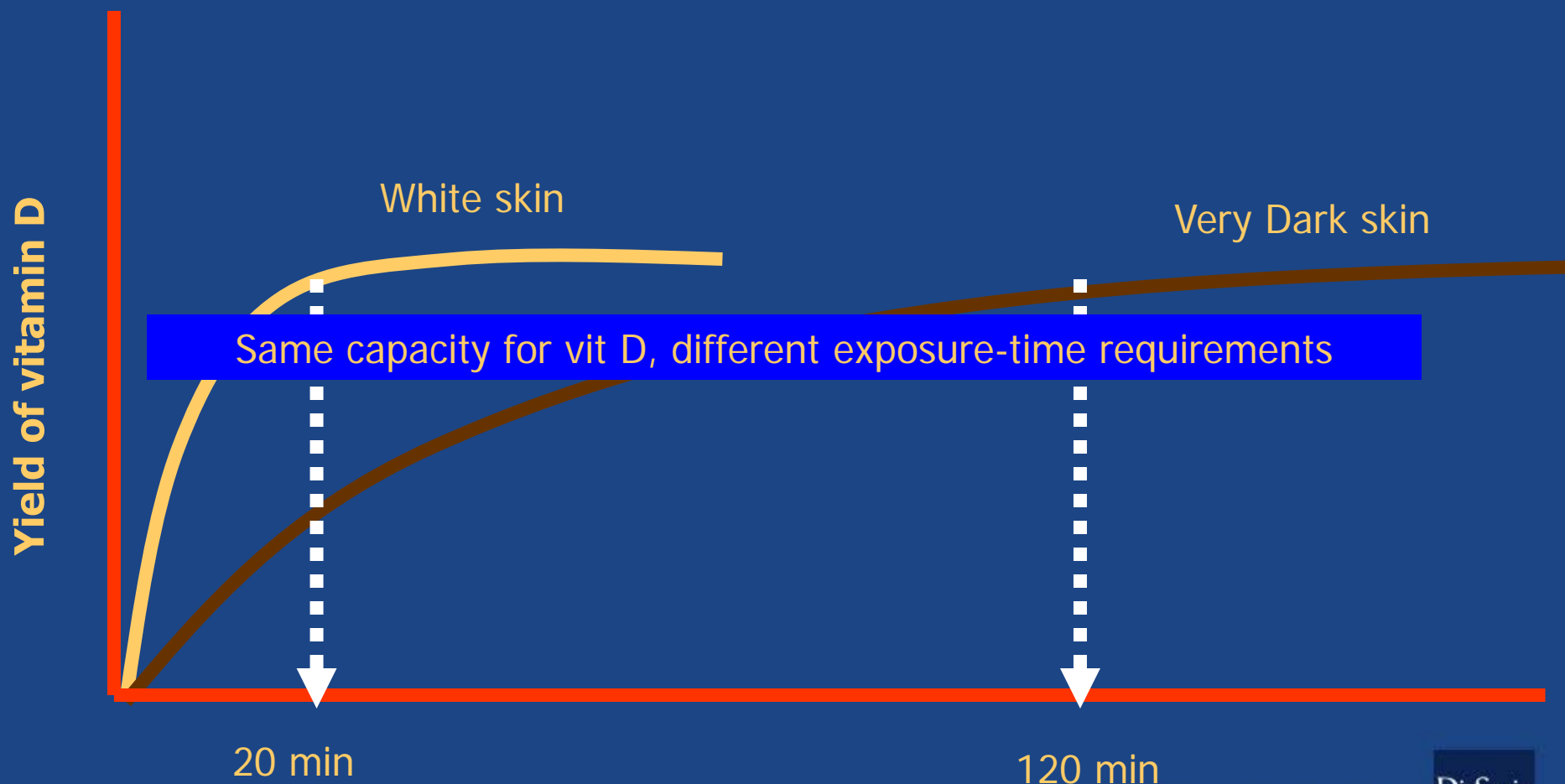


Year, 1978



25 OH Vitamin D, measurement issues

Exposure time and skin colour effects on 25 OH Vitamin D production

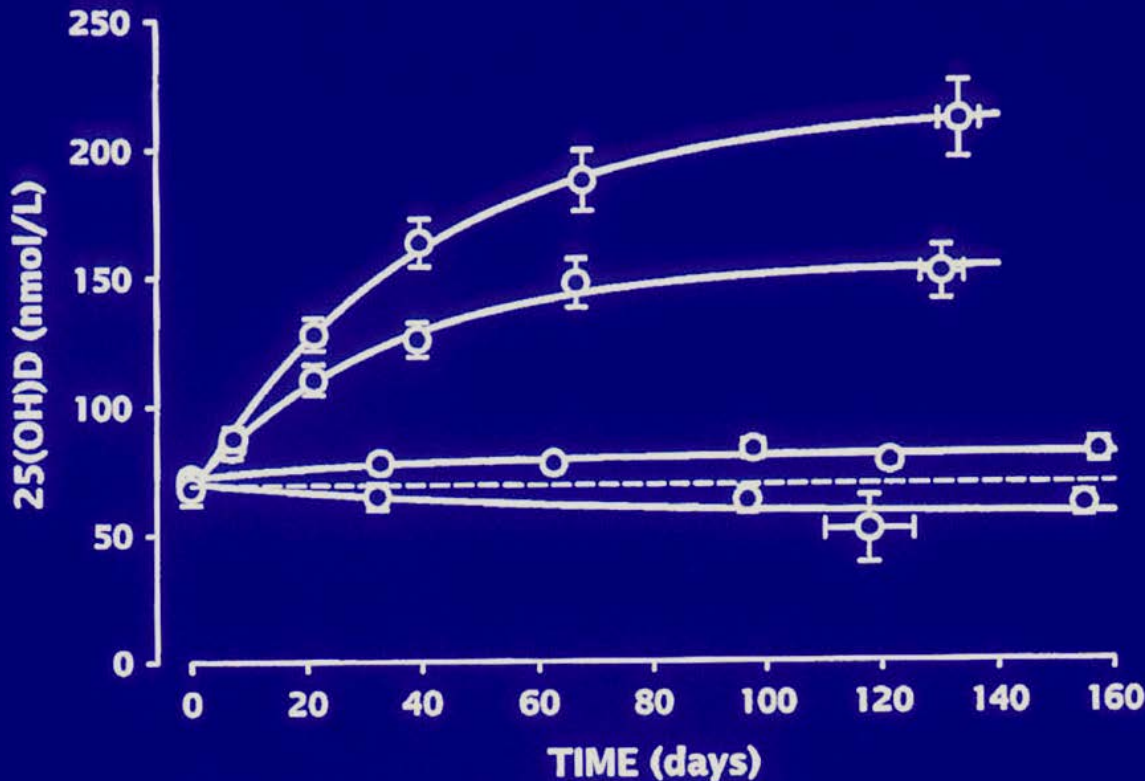


20 min

120 min

25 OH Vitamin D, measurement issues

Circulating 25(OH)D as a Function of Oral Vitamin D Intake



10,000 IU/d

5,000 IU/d

1,000 IU/d

400 IU/d

Recent literature has suggested the following ranges:

Deficiency:	<10 ng/mL	(0 - 25 nmol/L)
Insufficiency:	10 - 30 ng/mL	(25 - 75 nmol/L)
Sufficiency:	30 - 100 ng/mL	(75 - 250 nmol/L)
Toxicity:	> 100 ng/mL	(> 250 nmol/L)



Vitamin D Supplementation

either D₃ and D₂ therapies are considered

A.I. for Adults is 400-600 IU/day

Studies Show Elderly May Need More

700 IU/day (Dawson-Hughes et al., 1995)

Protection against bone loss

800 IU/day (Chapuy et al., 1987)

Reduced biochemical indexes of secondary hyperparathyroidism

800 IU/day (Chapuy et al., 1992)

Reduced risk of hip fracture

Vitamin D itself is rarely measured

The two metabolites measured are:

25 Hydroxyvitamin D (25 OH D)

gives an indication of Vitamin D stores obtained by sun exposure, diet, supplementation defines Vitamin D

deficiency

insufficiency

sufficiency

toxicity

Optimal levels >
30 ng/mL

Dawson-Hughes B, et al. 2005. Osteoporosis Int. 16:713-6.

1, 25 Dihydroxyvitamin D (1, 25(OH)₂D)



**25 OH Vitamin D
UV Quantitation following HPLC**

**25 OH Vitamin D
LC-MS Quantitation**

**25 OH Vitamin D
Radioimmunoassay (RIA)**



25 OH Vitamin D Radioimmunoassay (RIA)

**DiaSorin RIA kit to test 25 OH Vitamin D
stays as the golden standard for clinical
evaluation of deficiency and insufficiency**

LIAISON® 25 OH Vitamin D TOTAL



direct, competitive chemiluminescence immunoassay in serum or EDTA plasma.

- 1st incubation, 10'
25 OH Vitamin D dissociated from its binding protein binds to the specific antibody on the solid phase.
- 2nd incubation, 10'
Tracer 25 OH vitamin D linked to an isoluminol derivative added.
- Washing
- Starter reagents
- Flash chemiluminescent reaction



The light signal measured in relative light units is inversely proportional to the level of the analyte

LIAISON[®] 25 OH Vitamin D TOTAL

On-line extraction procedure with ethanol
Highly specific antibody to identify 25 OH Vitamin D
Fully correlated to the DiaSorin RIA method
Specificity: 100% 25 OH Vitamin D₂ and 25 OH Vitamin D₃

Functional sensitivity: < 4 ng/mL

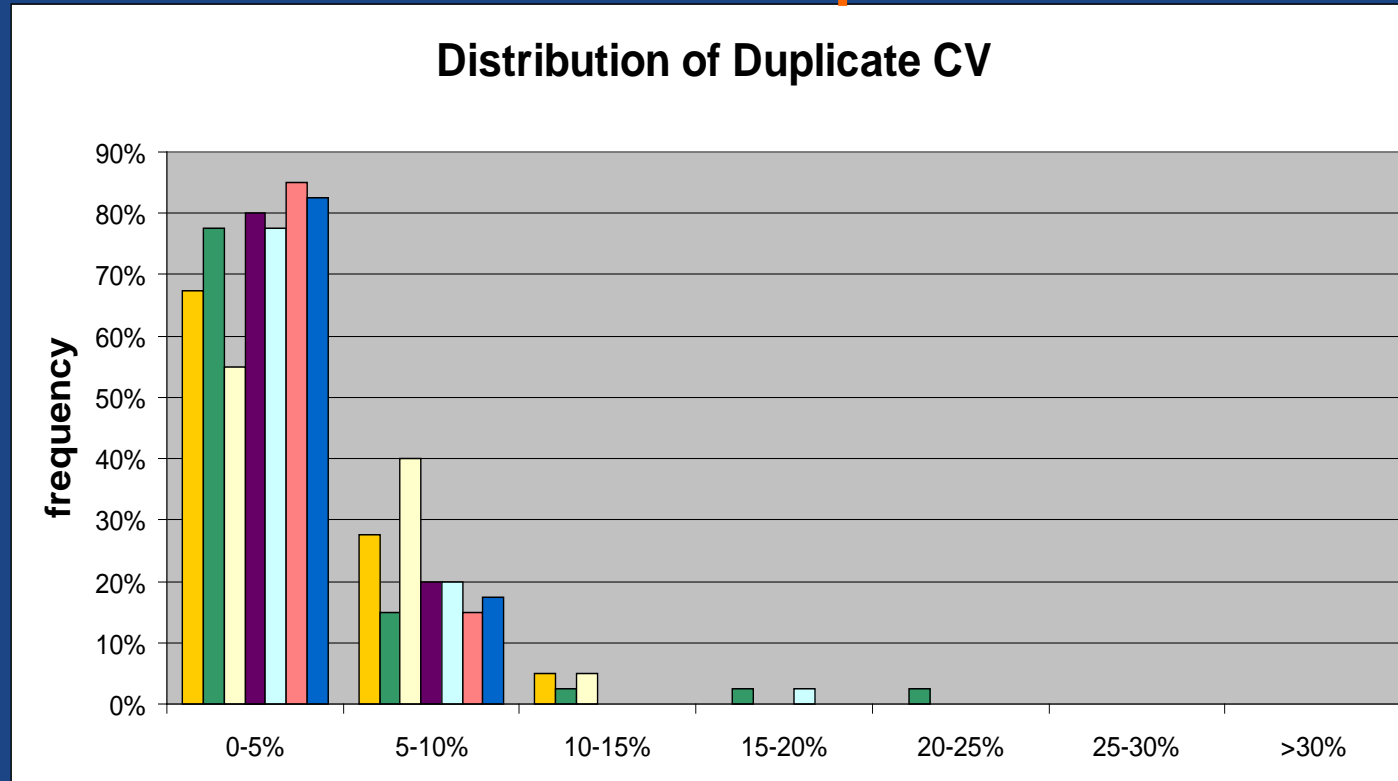
Dynamic range: 4 – 150 ng/mL

Two step incubation time

Time to first result: 35'

Throughput: > 100 tests/hour

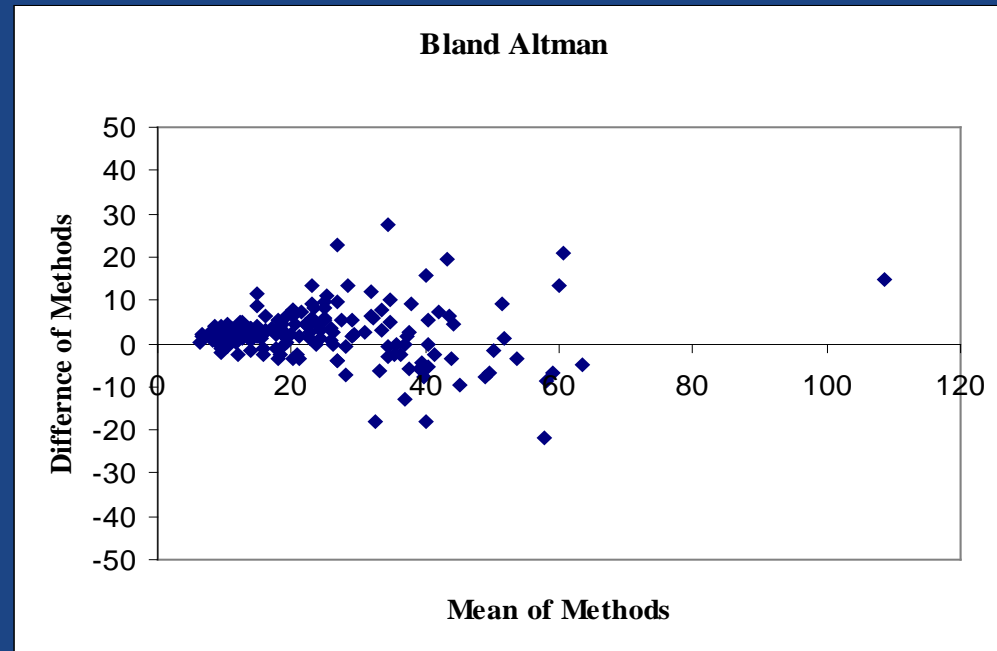
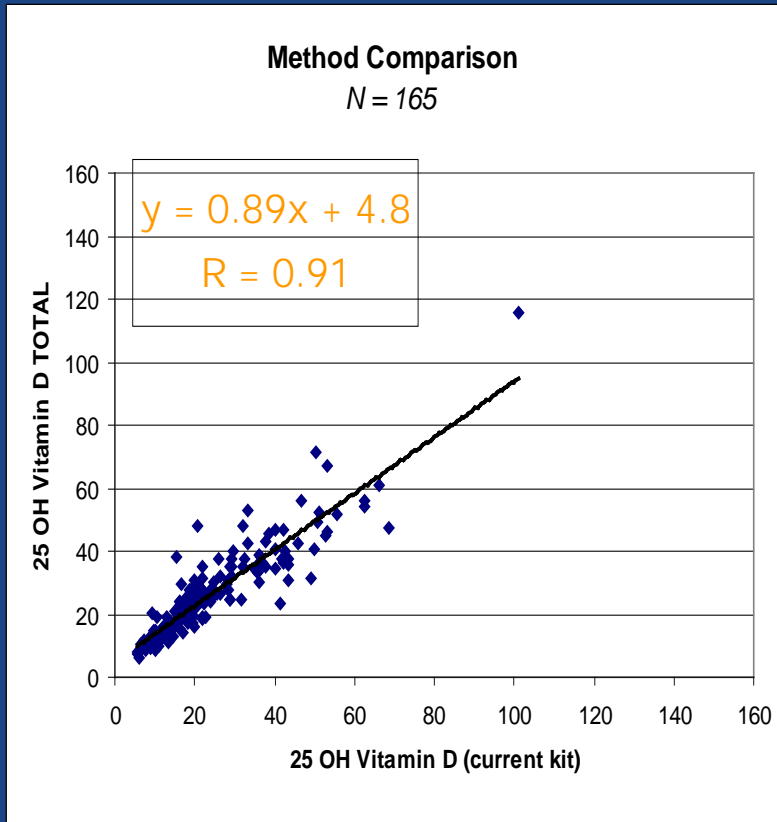
Precision-duplicates



Precision was evaluated following CLSI EP5-A2. Samples containing different concentrations of analyte were assayed in duplicate, two assays per day, over 20 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay variability).

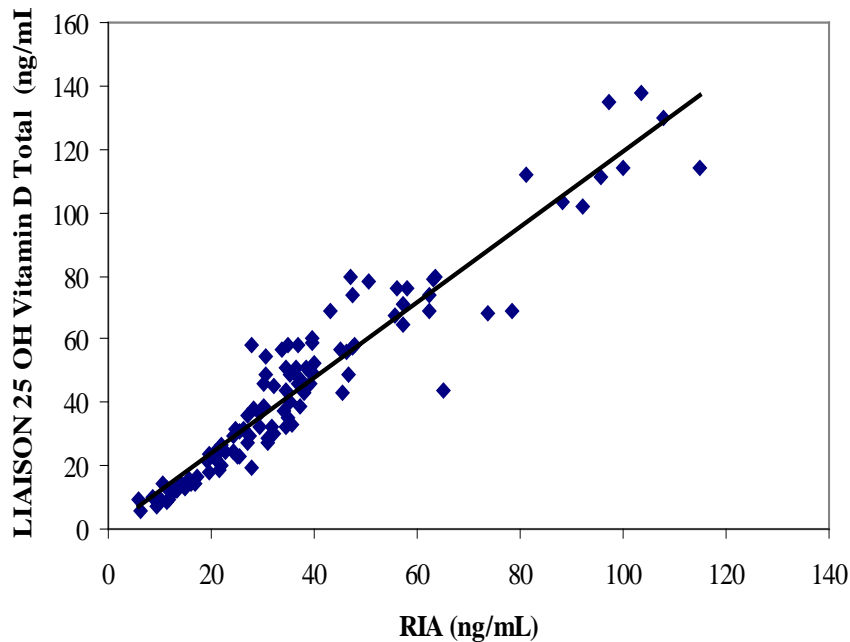
LIAISON® 25 OH Vitamin D TOTAL

Method Comparison to the 'old' LSN (164 samples)



The Bland-Altman plot indicates that the LIAISON® 25 OH Vitamin D TOTAL assay has a slight tendency to give lower results below ~25 ng/mL or 63 nmol/L, but no bias in higher values.

Correlation to DiaSorin RIA

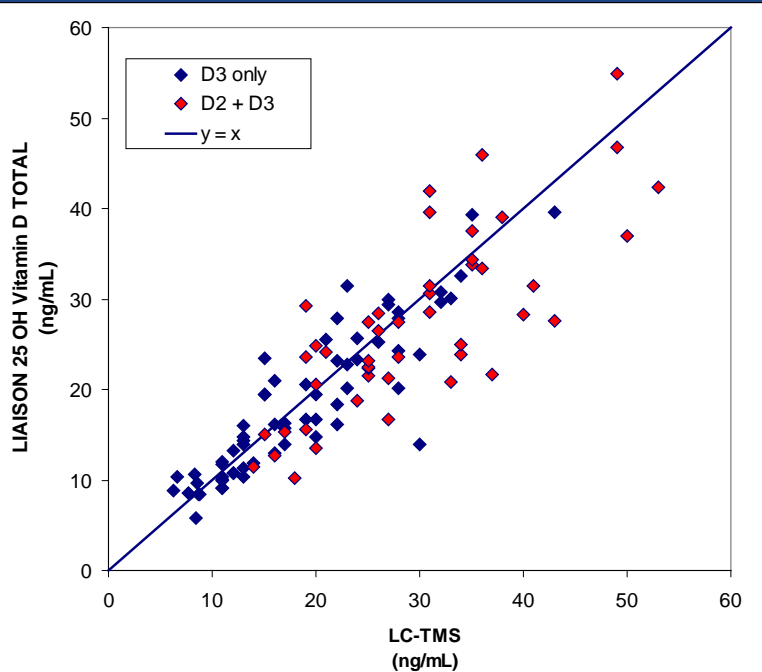


A total of 109 samples was tested by LIAISON[®] 25 OH Vitamin D TOTAL and by a radioimmunoassay method (RIA).

The resulting regression equation was:

$$\text{LIAISON}^{\text{®}} = 1.19 (\text{RIA}) + 0.31$$
$$R = 0.95$$

Correlation to LC-MS D₂ and D₃ specificity



Samples containing only 25-OH D₃ (n=64):
LIAISON[®] = 0.88(LC-TMS) + 1.9;
R = 0.90

Samples containing both 25-OH D₃ and 25-OH D₂
(n=46):
LIAISON[®] = 0.80(LC-TMS) + 3.6;
R = 0.78

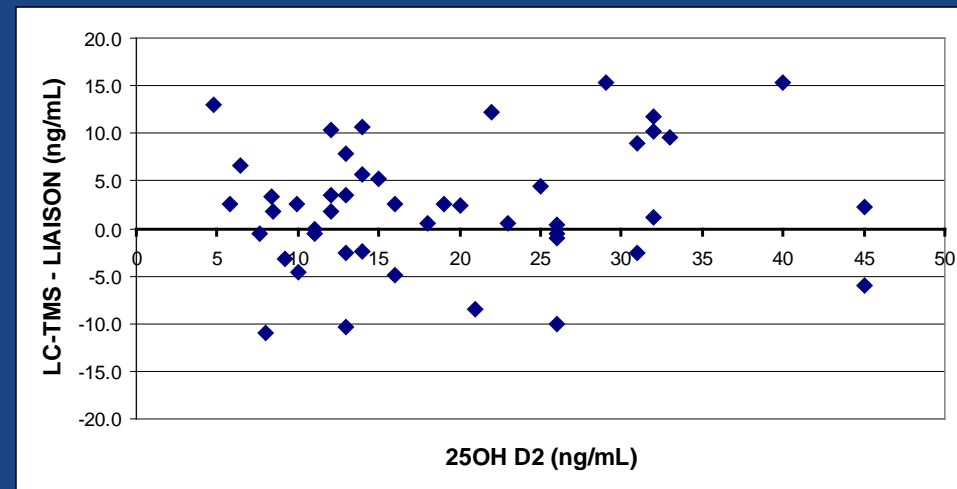
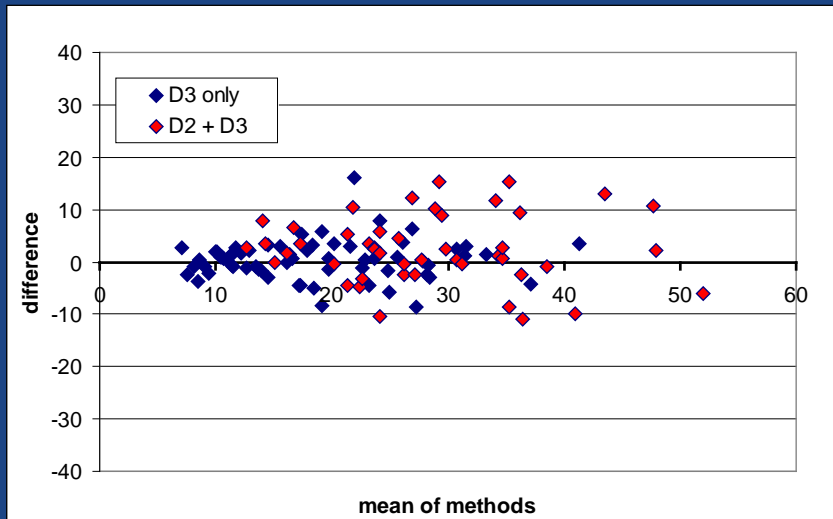
All samples (n=110):
LIAISON[®] = 0.83(LC-TMS) + 2.7;
R = 0.87

LIAISON® 25 OH Vitamin D TOTAL

Correlation to LC-MS D2 and D3 specificity



The Bland-Altman plot shows no systematic bias in the results:



nor is there a systematically increasing bias
in the results with increasing 25OH D₂:

LIAISON® 25 OH Vitamin D TOTAL

125 serum samples from African Americans by three different assay methods:
liquid chromatography-tandem mass spectrometry and
LIAISON® 25 OH Vitamin D TOTAL

Distribution of DBP (Gc) Allele Frequencies

From Kamboh & Ferrell, Hum. Genet. 1986, 72(4):281-93 [1]

Population	Gc-1F	Gc-1S	Gc-2
Europe	0.06-0.20	0.51-0.61	0.24-0.41
Africa – North & East	0.26-0.50	0.35-0.67	0.01-0.21
Africa – South & West	0.68-0.88	0.08-0.12	0.02-0.16
USA – Whites	0.11-0.28	0.49-0.57	0.21-0.31
USA – Blacks	0.67-0.79	0.12-0.18	0.08-0.13



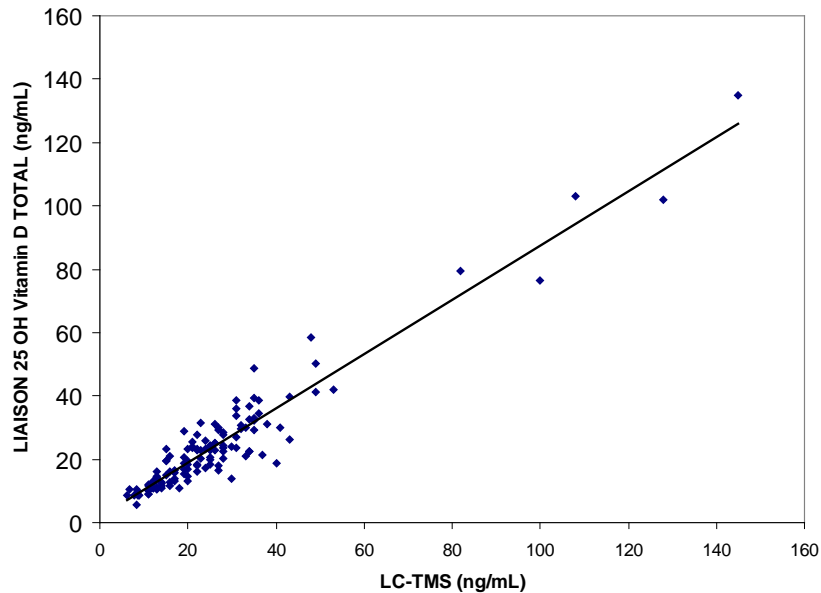
The form of DBP that is most common in African Americans has been reported to exhibit a higher affinity for vitamin D metabolites than the forms most common in Europeans (Braun A, et al. Electrophoresys 1990, Arnaud et al. Hum Genet 1993)
Other reports conclude that there is no difference in affinity between the various isoforms (Boutin B et al. J Ster Biochem 1989, Kawakami M et al Biochem J 1979)

LIAISON[®] 25 OH Vitamin D TOTAL

Correlation to LC-MS in African American and Caucasian

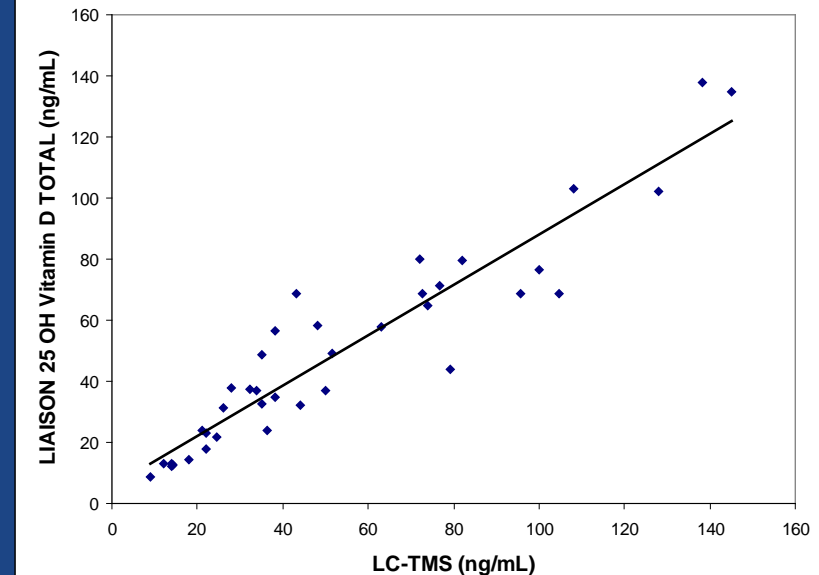


Samples from African Americans



$$\text{LIAISON}^{\text{®}} = 0.86(\text{LC-TMS}) + 1.7;$$
$$R = 0.96$$

Samples from Mixed Population



$$\text{LIAISON}^{\text{®}} = 0.82(\text{LC-TMS}) + 5.6;$$
$$R = 0.94$$

LIAISON[®] 25 OH Vitamin D TOTAL

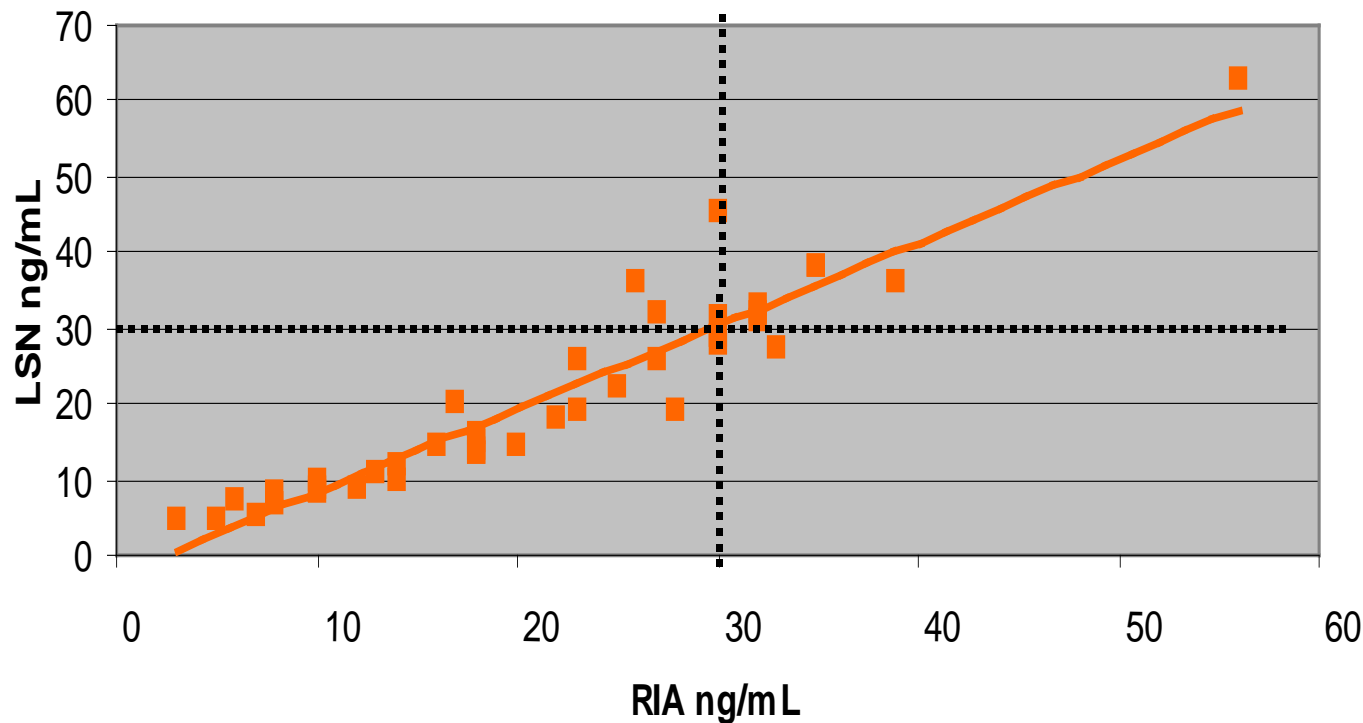
External evaluation on DEQAS control sera run in two customer sites in Europe

DEQAS	RIA	old LSN	TOTAL D site 1	TOTAL D site 2
nmol/L				
286	44,4	34	43,3	
287	46,1	34,1	44,1	
288	20,3	15,7	18,2	
289	66,1	51,5	65,9	
290	63,2	55,7	64	
301	90,3	95,2	104	92
302	77,2	78,2	77,9	80,3
303	60,5	53	63,9	64
304	62,7	60,1	55,5	49,8
305	27,2	21,5	26,2	
306	21,3	19,4		17,1
307	39,6	34,1		45,5
308	91	90,9		103
309	57,4	52,5		64,3
310	75,3	70,1		84,5

LIAISON[®] 25 OH Vitamin D TOTAL

External evaluation run in France

Blind correlation to results obtained with the DiaSorin RIA kit



$$y = 1,0986x - 2,6432$$

$$R = 0,8953$$

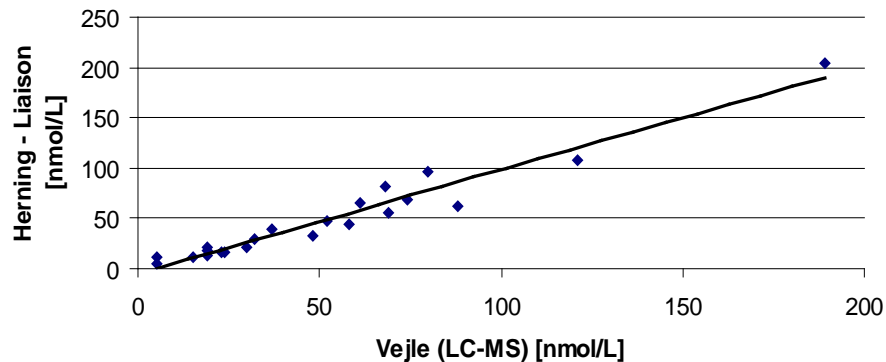
n = 41

External evaluation run in Danemark Regional Hospital, Herning correlation to tandem mass spect on routinary samples

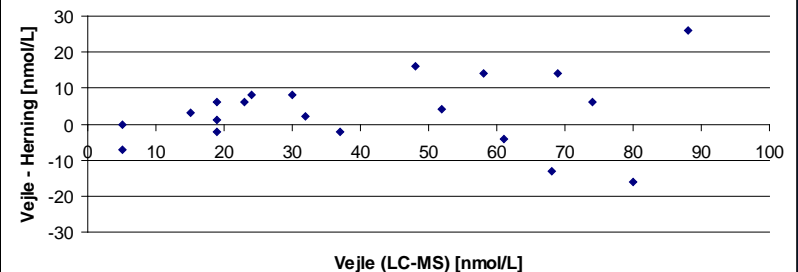
XY-plot metodesammenligning

$$y = 1.0303x - 4.6092$$

$$R^2 = 0.9474$$



Differensplot metodesammenligning; område <100 nmol/L



Bland Altman evaluation run on samples
< 100nmol/L

LIAISON® 25 OH Vitamin D TOTAL

External evaluation run in Danemark Regional Hospital, Herring Limit of quantification

1	11.5
2	14.6
3	13.0
4	12.6
5	14.1
6	12.3
7	12.3
8	11.5
9	11.5
10	14.1
11	12.9
12	11.4
13	10.5
14	10.6

Mean:	12.35	nmol/L
SD:	1.287	nmol/L
CV:	10.4	%

14 determinations every run

REMIND

Functional sensitivity is defined as the levels at which CV% is lower than 20%

External evaluation run in UK Kings College Hospital precision test, intra-assay evaluation

	Level 1 (ng/mL)	Level 2 (ng/mL)	Level 3 (ng/mL)
1	6.47	34.9	41.4
2	5.43	32.7	46.8
3	5.78	33.7	47
4	5.23	37.3	45.8
5	7.32	36	44.3
6	6.0	37.7	47.6
7	7.03	34.9	49.3
8	4.86	33.9	49.4
9	5.39	36.6	41.2
10	5.9	37	46.8
Mean	5.95	35.47	45.96
S.D	0.79	1.70	2.88
% C.V	13.31	4.80	6.26

LIAISON® 25 OH Vitamin D TOTAL

External evaluation run in UK Kings College Hospital precision test, Quality Control evaluation

	Low QC	High QC	ng/mL
1	15.30	44.00	
2	16.70	44.70	
3	15.40	50.50	
4	17.10	45.20	
5	15.20	43.80	
6	16.90	44.80	
7	18.00	45.30	
8	17.10	50.00	
9	17.70	47.00	
10	17.80	47.60	
Mean	16.72	46.29	
S.D	1.06	2.41	
%C.V	6.35	5.20	

Assay methods on LIAISON®



LIAISON® 25 OH Vitamin D TOTAL

direct, competitive chemiluminescence immunoassay in serum or EDTA plasma.

- 1st incubation, 10'
25 OH Vitamin D dissociated from its binding protein binds to the specific antibody on the solid phase
- 2nd incubation, 10'
Tracer 25 OH vitamin D linked to an isoluminol derivative added.
- Washing
- Starter reagents
- Flash chemiluminescent reaction

LIAISON® 1,25 dihydroxyvitamin D

Indirect competitive chemiluminescence immunoassay in serum or EDTA plasma

- Off-line extraction and purification of vitamin D metabolites through C18OH "Extra Clean" cartridges
- 1st Incubation, 30'
Extracted sample binds to the polyclonal antibody on the solid phase
- 2nd incubation, 10'
Tracer 1,25-(OH)₂ vitamin D linked to an isoluminol derivative added.
- Washing
- Starter reagents
- Flash chemiluminescent reaction

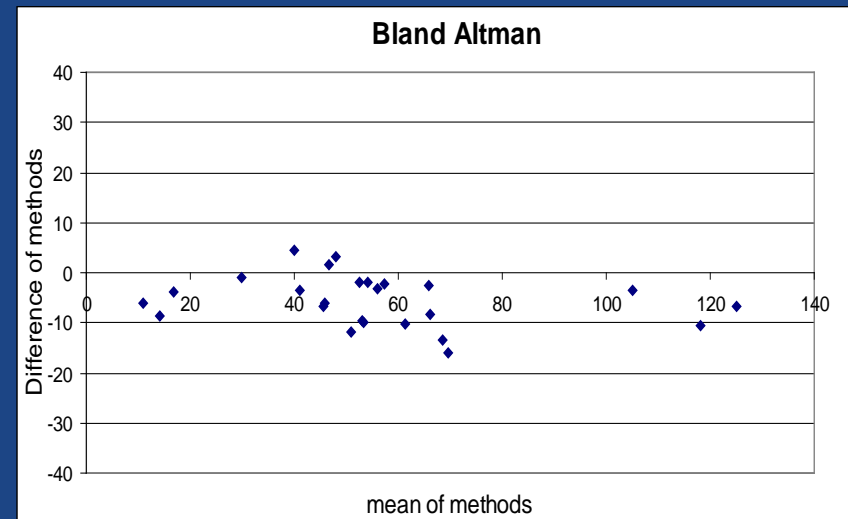
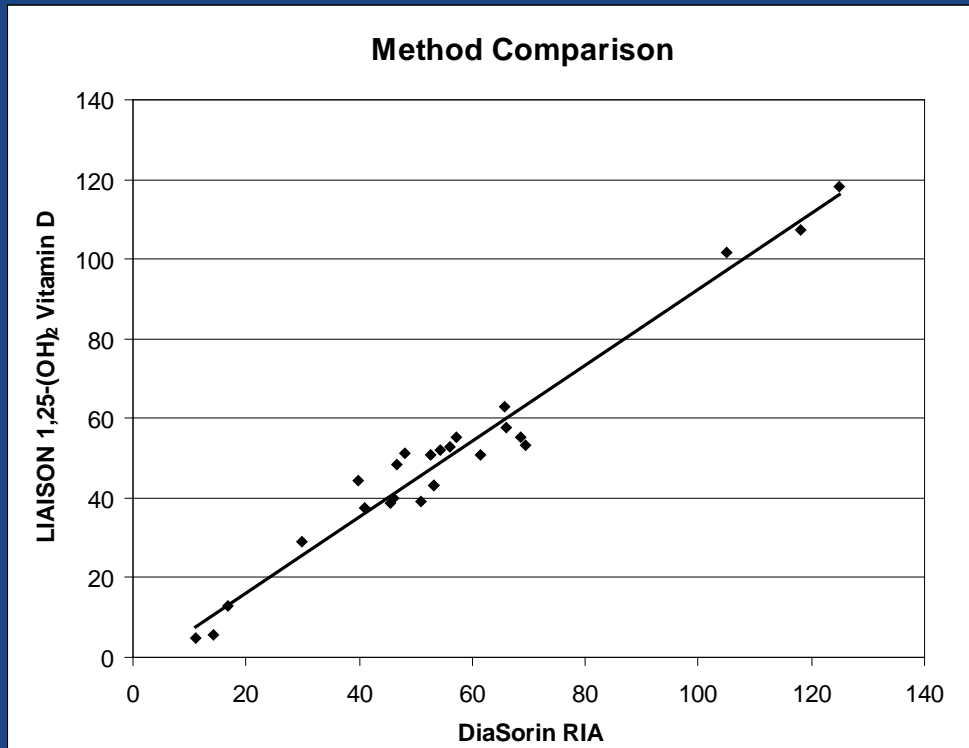
Available on LIAISON
Autumn 2007

Method Comparison LIAISON® 1,25-(OH)₂ Vitamin D

25 clinical samples tested versus the 1,25-Dihydroxyvitamin D ¹²⁵I RIA

Linear regression equation: $LIAISON = 0.95 * (RIA) - 3.0$

$R = 0.98$



CONCLUSIONS

- ✓ **good performance characteristics, with excellent precision and sensitivity**
- ✓ **assay automated on the LIAISON[®] System assuring quick availability of the results for the clinicians**
- ✓ **100% cross-reactivity versus Vitamin D₂ and Vitamin D₃ allows the optimal characterization of the nutritional status and of the 25 OH Vitamin D supplementation**
- ✓ **correlation of the LIAISON TOTAL D assay to the DiaSorin RIA demonstrates a very tight association of the two methods**
- ✓ **correlation of the LIAISON TOTAL D assay to the LC/LC-MS reference method in Caucasian and African American shows an optimized extraction technique from the binding protein**

CONCLUSIONS

- ☀ **convenient methods for the routine measurement of Vitamin D metabolism with automated non-isotopic immunoassays**
- ☀ **with only one blood sample both 25 OH Vitamin D and 1,25(OH)₂ can be performed**
- ☀ **fast analytical protocols allow the physicians to get results in 1 hour time from the drawing in order to better assess the patient treatment**
- ☀ **close correlation to the gold standard methods assures full clinical consistency of the results obtained**
- ☀ **100% recovery to D₂ and D₃ metabolites assures reliable follow-up of therapy for insufficiency and deficiency**

DiaSorin



The Diagnostic Specialist