# Automated 25-Hydroxy Vitamin D Immunoassay Comparison With LC-MS/MS Method

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Vitamin D

o Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight.

o Clinicians' 25OHVITD requests from laboratory increase day by day.

o Measurements of 25OHVITD have some difficulties due to the lack of standardization yet.

o 25OHVITD analysis is performed by immunoassay, HPLC and liquid chromatography–tandem mass spectrometry (LC–MS/MS).

o The choice of method for each laboratory remains a balance mainly between turn around time, convenience, cost and the specificity and accuracy of the information obtained.
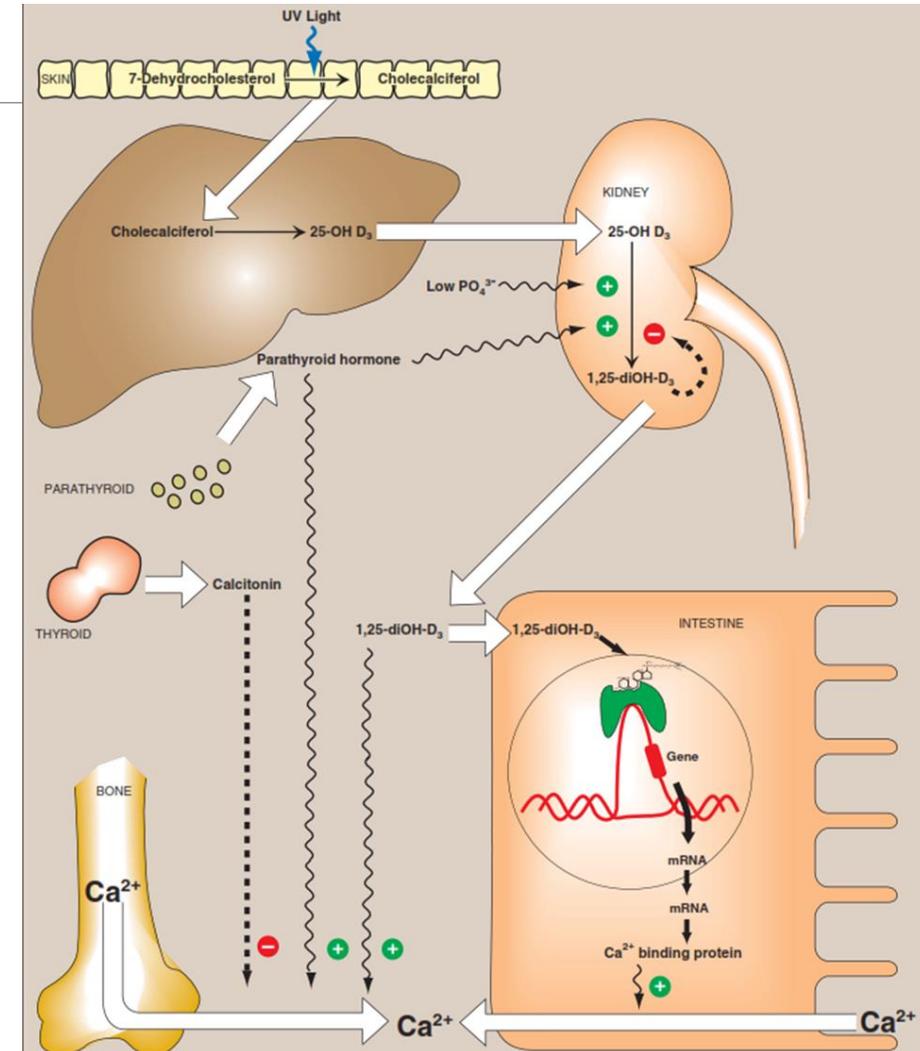
Elizabeta Topic*, Nora Nikolac, Mauro Panteghini, Elvar Theodorsson, Gian Luca Salvagno, Marijana Miler, Ana-Maria Simundic, Ilenia Infusino, Gunnar Nordin and Sten Westgard

How to assess the quality of your analytical method?

Verification of imprecision and bias

A majority of the measurement methods used in laboratory medicine are produced by diagnostic companies, which have already validated them and established that they are fit for the intended purpose [4, 24]. The end-user laboratory, however, is requested to independently verify that the essential performance characteristics, including imprecision and bias of the measurement method and/or measurement system found during manufacturer's validation, can be reproduced locally. Verification is also required when substantial changes occur over time, e.g. change of a measurement system, relocation or when results of IQC or EQA schemes indicate that the performance of the method has worsened with time.

Local consensus on sufficient verification procedures have commonly been agreed and frequently influenced over time, e.g. by accreditation authorities. Published verification procedures have appeared rather recently
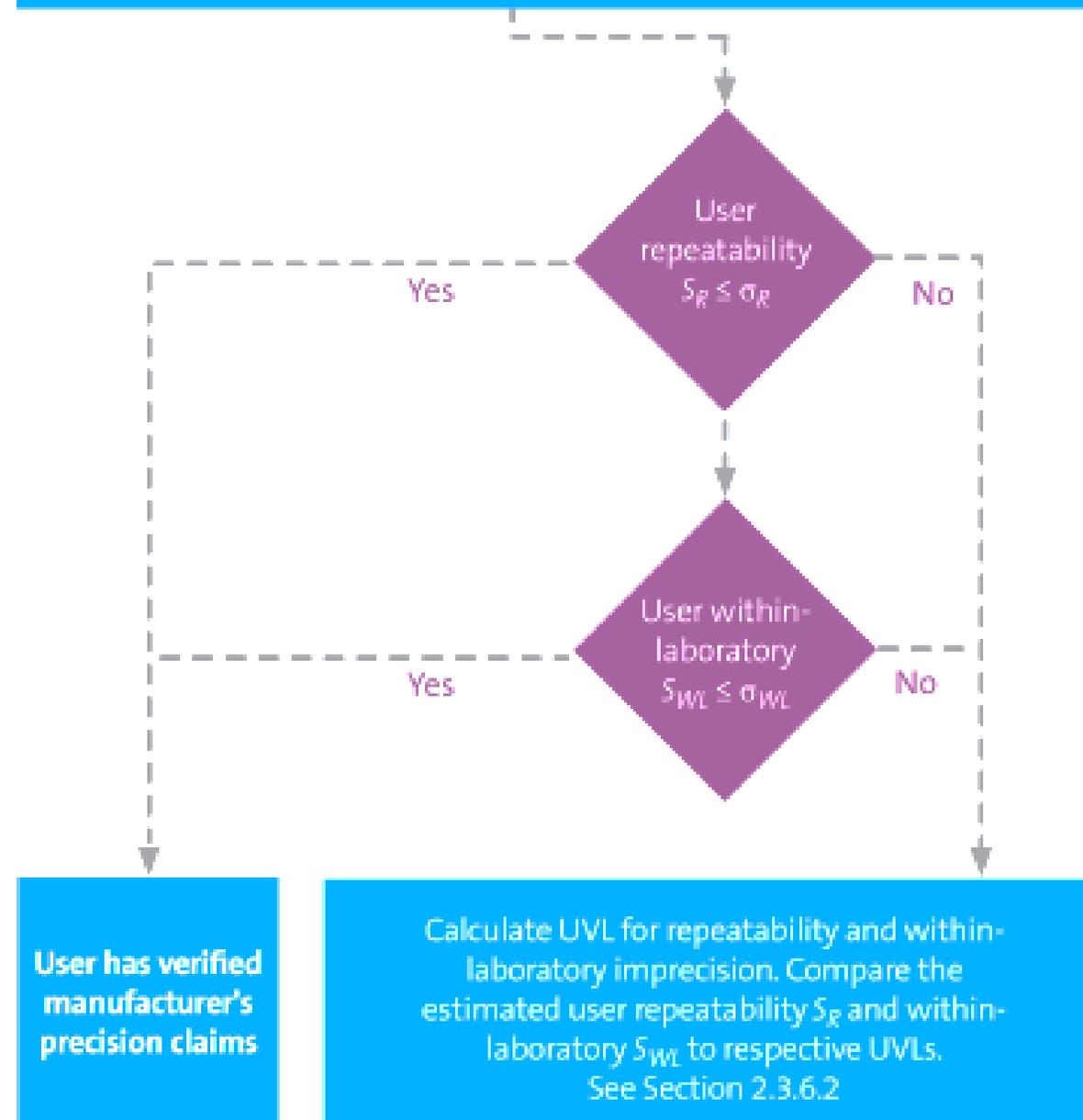
[25–28]. The following is a brief summary of the most widely employed approaches:

– Bias studies. Clinical laboratories commonly measure in the order of 20–200 human samples having as wide a concentration range as possible, using both the comparison ("reference") method and the evaluated method. At least 20 repeated measurements of at least two pooled patient samples may also be used. This latter approach may actually be an advantage when the medical decision limit is close to the detection limit of the measurement method or system.

– Imprecision studies. For estimating imprecision, suitable stable control materials for IQC at two concentration levels are measured in at least two replicates for at least 5 consecutive days each week for 2 weeks.

– Data presentation and analysis. Linear regression, preferably orthogonal linear regression [29, 30], bias plots [31, 32] and analysis of variance [33] techniques are used to quantify bias and within- and between-series imprecision, respectively.

September 2014

EP15-A3

User Verification of Precision and Estimation of Bias; Approved Guideline—Third Edition

Compare estimated user repeatability S_R and within-laboratory S_{WL} to manufacturer's repeatability claim σ_R and within-laboratory claim σ_{WL}, respectively.

User repeatability $S_R \leq \sigma_R$

Yes — No

User within-laboratory $S_{WL} \leq \sigma_{WL}$

Yes — No

User has verified manufacturer's precision claims

Calculate UVL for repeatability and within-laboratory imprecision. Compare the estimated user repeatability S_R and within-laboratory S_{WL} to respective UVLs. See Section 2.3.6.2

Objectives

o In this study, we evaluated the analytical verification of Elecsys Vitamin D total II (VITDT2) assay (Roche Diagnostics GmbH; Mannheim, Germany)

o 25OHVITD method verification was performed by determining precision and trueness according to CLSI EP15-A3 guideline.

Materials and Methods

o Serum 25OHVITD levels were measured on Cobas c602 according to the manufacturer's instruction.

  o Elecsys Vitamin D total II kit (LOT:39192001, REF: 07464215190)

  o Calibrator (LOT:39454101, REF: 07464240190), VITDT 2 Cal1: **3 ng/ml** Cal2: **45 ng/ml**

  o Abnormal control (PCVITDT1 REF: 07464266, LOT: 34262099) (**L1= 13,7 ng/mL**)

  o Normal control (PCVITDT2 REF: 07464266, LOT: 34262199) (**L2 = 28,9 ng/mL**)

o The Elecsys Vitamin D total II assay employs a vitamin D binding protein (VDBP) labeled with a ruthenium complex as capture protein to bind 25-hydroxyvitamin D3 and D2.
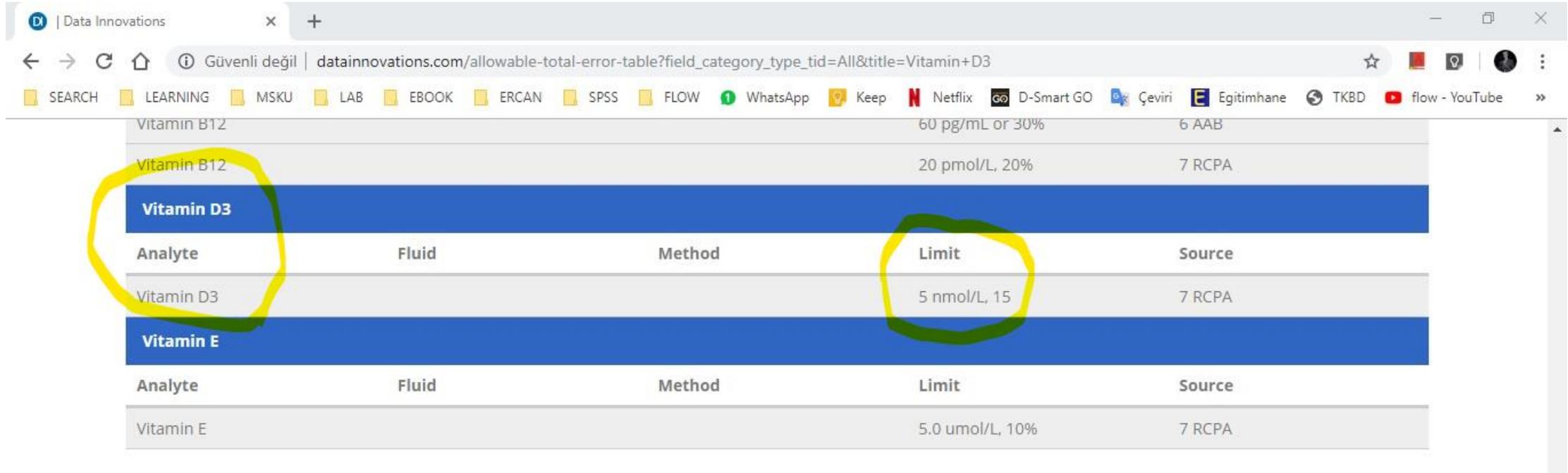
Precision

- We tested precision with 3 repeat analyses in a run over 5 sequential days for 2 levels of IQC materials

- Precision of VITD was considered acceptable if the CV was equal to the Roche rerun method or less.

Trueness

- Trueness was assessed by analyzing 80 patient samples distributed evenly over the entire measuring interval.

- Results from the two methods (Elecsys VITDT2 ECLIA method and LCMSMS method) are compared to determine if significant differences exist.

- Statistical analysis was performed by using MedCalc (Version 15.8, Ostend, Belgium) and EP Evaluator (Data Innovations LLC, USA)

https://datainnovations.com/allowable-total-error-table

PC1

2c03

EP Evaluator
Clinical Laboratory — MSKU

VITDT2
Instrument: COBAS
Sample Name: SERUM

Alternate Precision

Claim Evaluation

User's Concentration: 10,84   Claim Concentration: --

	df	User's % CV	User's	Claim	Verification Value (95%)	Pass/Fail
Within run	--	--	--	0,919	--	--
Between run		6,6	0,72			
Between day		0,0	0,00			
Total	22	6,6	0,72	1,15	1,428	Pass
Medical Req	22	6,6	0,72	1,88	2,33	Pass

The calculated value passes if it does not exceed the verification value.

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

Sample	Mean ng/mL	Mean nmol/L	Repeatability SD ng/mL	Repeatability SD nmol/L	CV %
HS 1	10.5	26.3	0.783	1.96	7.4
HS 2	21.1	52.8	0.968	2.42	4.6
HS 3	24.9	62.3	0.973	2.43	3.9
HS 4	54.9	137	1.72	4.30	3.1
HS 5	94.3	236	2.65	6.63	2.8
PC Vitamin D total II 1	15.9	39.8	0.919	2.30	5.8
PC Vitamin D total II 2	29.4	73.5	1.24	3.10	4.2

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

Sample	Mean ng/mL	Mean nmol/L	Intermediate precision SD ng/mL	Intermediate precision SD nmol/L	CV %
HS 1	10.5	26.3	0.934	2.34	8.9
HS 2	21.1	52.8	1.24	3.10	5.9
HS 3	24.9	62.3	1.23	3.08	4.9
HS 4	54.9	137	2.09	5.23	3.8
HS 5	94.3	236	3.59	8.98	3.8
PC Vitamin D total II 1	15.9	39.8	1.15	2.88	7.2
PC Vitamin D total II 2	29.4	73.5	1.46	3.65	5.0

Precision Plot
(Different plotting symbols represent different runs)
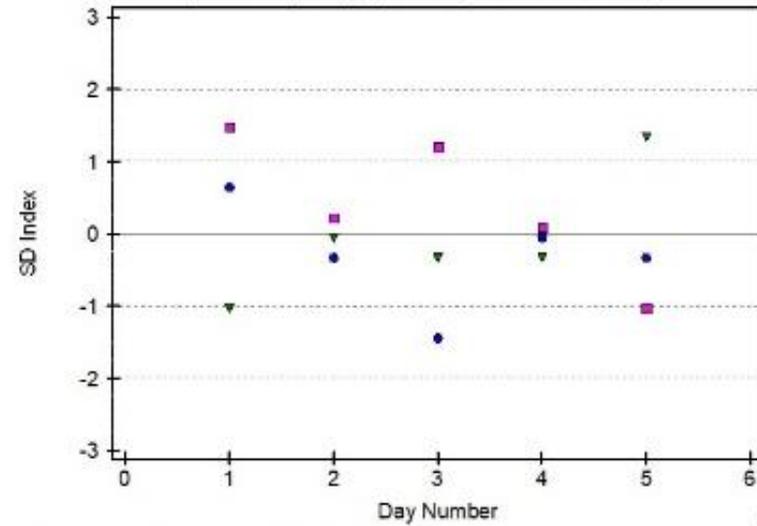
Supporting Data

Analyst	ERCAN SARUHA
Analysis Date	02 Eyl 2019 to 06 Eyl 2019
Days (total/excl)	5 / 0
Runs per Day	3
Reps per Run	1
Critical Value	95%
Units	ng/ml
Verify Mode	Verify Vendor Claim
TEa	12,50 ng/ml
Rand. Err. Budget	15%
Allow Rand. Err.	1,88 ng/ml
Control	PC1
Reagent	11 VITDT2
Calibrators	11 PCT
Comment	

PC2

Alternate Precision

Claim Evaluation

User's Concentration: 24,36   Claim Concentration: --

	df	User's % CV	Standard Deviation User's	Standard Deviation Claim	Verification Value (95%)	Pass/Fail
Within run	--	--	--	1,24	--	--
Between run		2,1	0,52			
Between day		2,9	0,72			
Total	6	3,6	0,88	1,46	2,115	Pass
Medical Req	6	3,6	0,88	1,88	2,72	Pass

The calculated value passes if it does not exceed the verification value.

Precision Plot
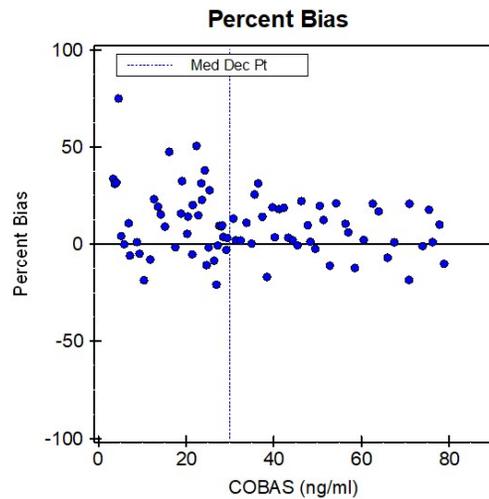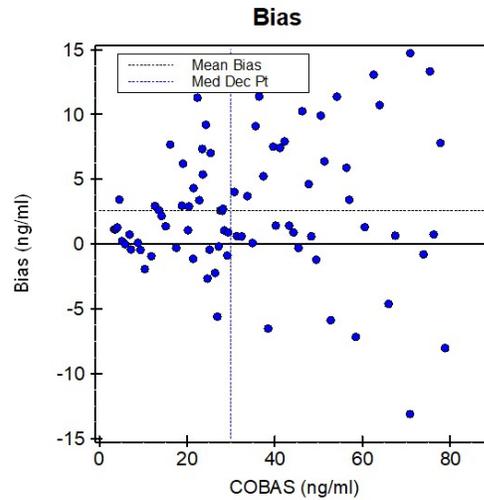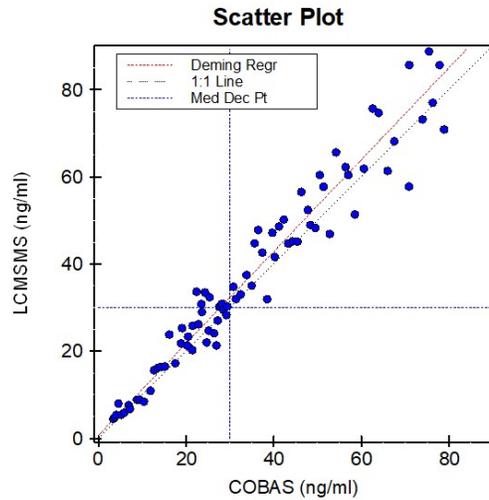(Different plotting symbols represent different runs)
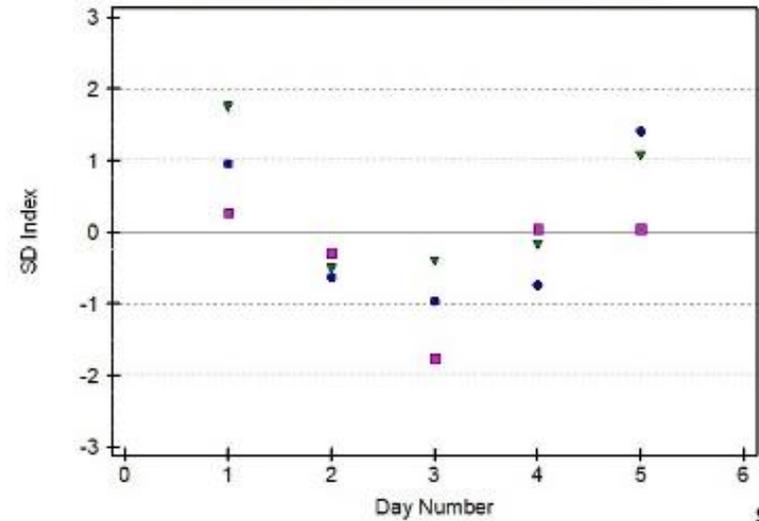
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TEa	12,50 ng/ml
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Control	
Reagent	11 VITDT2
Calibrators	11 PCT
Comment	

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SITKI KOÇMAN ÜNİVERSİTESİ

Alternate (Quantitative) Method Comparison

X Method: COBAS   Y Method: LCMSMS

Scatter Plot

LCMSMS (ng/ml) vs COBAS (ng/ml)
- Deming Regr
- 1:1 Line
- Med Dec Pt

Bias

Bias (ng/ml) vs COBAS (ng/ml)
- Mean Bias
- Med Dec Pt

Percent Bias

Percent Bias vs COBAS (ng/ml)
- Med Dec Pt

Regression Analysis

	Deming	Regular
Slope:	1,054 (0,998 to 1,110)	1,023 (0,968 to 1,079)
Intercept:	0,7233 (-1,5274 to 2,9740)	1,7852 (-0,4482 to 4,0185)
Std Err Est:	5,1938	5,1537

95% Confidence Intervals are shown in parentheses

Medical Decision Point Analysis

Calculated by   Deming Regression (R>=0,9)

| X Method MDP	Y Method Pred. MDP	95% Conf. Limits Low	High
30	32,3	31,2	33,5

Supporting Statistics

Corr Coef (R):	0,9722	Y Mean ± SD:	37,0913 ± 21,8771	Points (Plotted/Total):	80/80
Bias:	2,5835	Std Dev Diffs:	5,1435	Outliers:	Not Tested
X Mean ± SD:	34,5079 ± 20,7884	SubRange Bounds:	None	Scatter Plot Bounds:	None

Experiment Description

	X Method	Y Method
Expt Date:	27 Ağu 2019	27 Ağu 2019
Rep SD:	1	1
Result Ranges:	3,370 to 78,900	4,501 to 88,771
Units:	ng/ml	ng/ml
Reagent		
Calibrators		
Analyst:	ERCAN SARUHA	ERCAN SARUHA
Comment:		

VITD REGRESSION

Chart — LC MS (y-axis) vs ELECSYS VITDT2 (x-axis)

Legend:
- LOW
- NORMAL
- HIGH
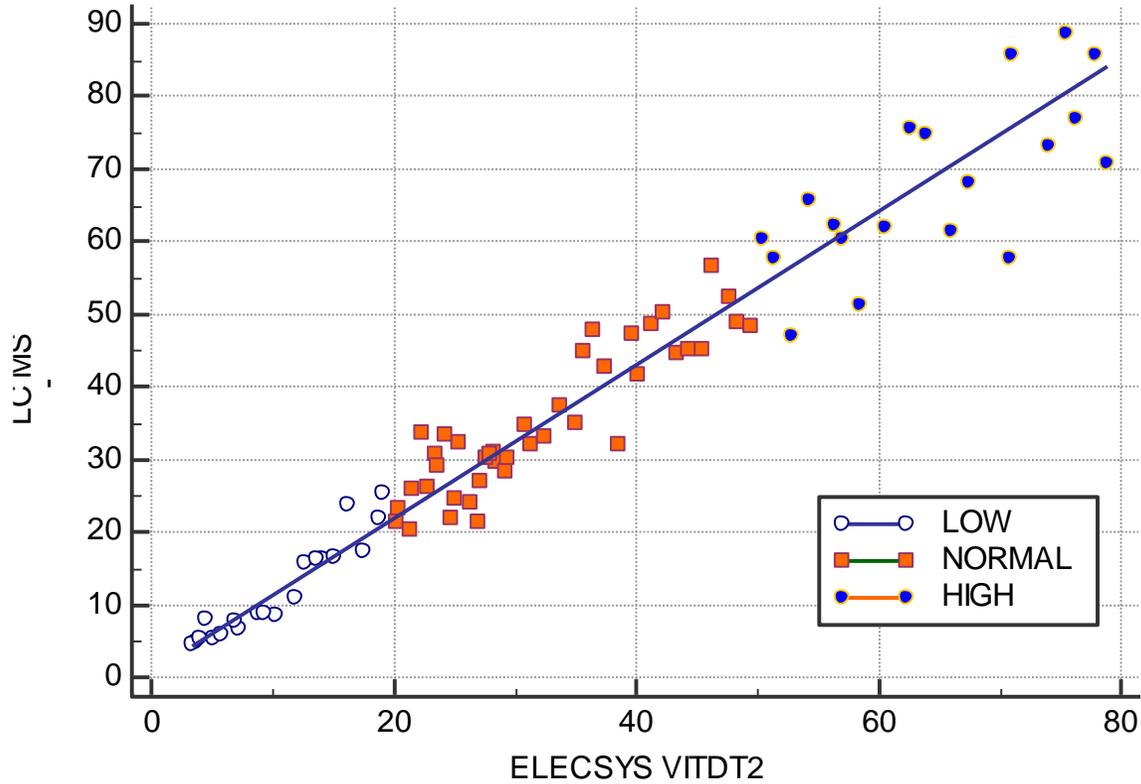
Method comparison

A comparison of the Elecsys Vitamin D total II assay (y) using the CDC Verification Samples with concentrations assigned by the CDC Vitamin D Reference Laboratory by ID-LC-MS/MS (x) gave the following correlations (ng/mL):

Number of samples measured: 111

Deming[27,28]	Passing Bablok[29]
y = 0.954x - 0.707	y = 0.937x - 0.360
r = 0.982	τ = 0.902

The sample concentrations were between 5.6 ng/mL (14 nmol/L) and 93 ng/mL (233 nmol/L).

Regression Equation

y = 0,8339 + 1,0550 x

Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	0,8339	0,5204	0,01939 to 1,6485	1,6024	0,1131
Slope	1,0550	0,02413	1,0069 to 1,1030	43,7258	<0,0001

- The overall correlation was acceptable (**r = 0,9608**).

- The results were linear with slope (a) of **1.055**, intercept (b) of **0.833** ng/mL, a correlation coefficient of **0.9608**

Results

The mean percent difference of Elecsys was **–2.6%** compared to LC–MS/MS.

Conclusions

- Our data show that the Roche Elecsys Vitamin D Total Assay has good correlation with LC-MS/MS.

- Although the LC-MS/MS method is considered reference method, it needs a special instrument and personnel and is thus expensive.

- Therefore, Roche's automated immunoassays for vitamin D total assay is more suitable for evaluating vitamin D status.

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Science: Best Practice
Measurement Verification

Main Document

The document titled "*Measurement verification in the clinical laboratory: A guide to assessing analytical performance during the acceptance testing of methods (quantitative examination procedures) and/or analysers*" is available to download below:

📄 Measurement Verification (*June 2009*)

Editorials

Editorials from Stephen Halloran and David Burnett are available to download in PDF format below. These were originally posted in June 2009.

📄 Stephen Halloran Editorial

📄 David Burnett Editorial

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Thanks for your patience….

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Questions