

Evaluation of the updated 25-Hydroxy Vitamin D assay on the Cascadion SM Clinical Analyzer

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INTRODUCTION

Evidence of the skeletal and nonskeletal effects of vitamin D, coupled with recognition that vitamin D deficiency is common, has raised interest in what is called a vitamin but is in fact a hormone. Vitamin D is produced by the action of ultraviolet B radiation on skin or obtained from dietary sources, including supplements. Persons commonly at risk for vitamin D deficiency include those with inadequate sun exposure, limited oral intake, or impaired intestinal absorption. Vitamin D sufficiency is best determined by measurement of 25-Hydroxy Vitamin D in serum, which allows precise evaluation of Vitamin D sufficiency as well as monitoring of vitamin D supplementation. In measuring 25-Hydroxy Vitamin D it is desirable that the C₃ epimers should be separated, as significant epimer concentrations have been observed not only in newborns but also in adults and have a biological activity different from vitamin D itself.

This presentation details analytical data of the updated Thermo Scientific™ Cascadion™ 25-Hydroxy Vitamin D assay designed for the fully automated Cascadion SM Clinical Analyzer. This includes the lower limit of quantitation (LLOQ), the Analytical Measurement Range (AMR), calibration, response linearity and stability, accuracy, precision, and specificity including epimer separation. Data is from studies performed by Viollier AG (Switzerland) comparing an immunoassay-based Vitamin D assay with the LC-MS/MS technology used by the Cascadion SM Clinical Analyzer and the Cascadion SM 25-Hydroxy Vitamin D Assay.

MATERIALS

- Proprietary reagents for the Cascadion Analyzer: solvents (methanol and water containing 5mM ammonium formate and 0.05% formic acid; and mixture of acetonitrile, isopropyl alcohol and acetone) and probe wash solutions (aqueous and solvent mixtures containing acetonitrile, isopropyl alcohol, acetone and 0.1% formic acid)
- Cascadion SM 25-Hydroxy Vitamin D Calibrators (6 levels across a concentration range of 3.4-132 ng/mL)
- Cascadion SM 25-Hydroxy Vitamin D Controls (3 levels across the calibration range)
- Quick Connect Cartridge (quick and simple to install) containing a TurboFlow™ extraction column for sample clean-up and an analytical column for component separation
- Cascadion SM 25-Hydroxy Vitamin D Internal Standard solution containing stable isotope labeled 25-Hydroxy Vitamin D₂ (VitD₂) and 25-Hydroxy Vitamin D₃ (VitD₃)
- NIST reference materials 968f L1-L2 and 972a L1-L4
- CDC Reference serum patient samples traceable to NIST reference material SRM 972a

METHODS

Analytical Method: Cascadion SM Clinical Analyzer preparatory LC-MS/MS method

- Sample preparation: protein precipitation followed by on-line extraction using TurboFlow technology
- Analytical separation obtained for Vitamin D₂ and Vitamin D₃ and their C₃ epimers
- Analyte detection and identification with two SRM transitions per analyte for ion ratio calculation

Data Calculations and Acceptance Criteria

- Linear calibration curve with accuracy criteria of ±15%
- Accuracy of calibration curve confirmed with system QC samples: accuracy ±15% for Quality Control material
- Analytes with concentrations above 20 ng/mL confirmed with ion ratio calculations

Assay Performance Validation

Validation procedures followed CLSI guidelines:

- Method comparison: Analysis of 115 CDC-certified reference samples. Two replicates of each sample run on each of 3 days.
- Spike Recovery: three patient samples with concentrations across quantification range were spiked and analyzed in six replicates (data not shown).
- Limit of Quantitation (LOQ): Four individual patient samples diluted with blank matrix and analyzed in duplicates daily for five days on two Cascadion SM Clinical Analyzers.
- Precision (Within Run and Total): Six patient samples analyzed in duplicates, twice daily for 21 days (n=84) (Table 2).
- Accuracy: NIST reference materials 968f and 972a analyzed in four replicates (Table 4).
- Linearity: bracketed target quantitation range of 3.4 – 132 ng/mL tested by mixing high and low concentration patient samples (data not shown).
- Carryover: experiment analyzing low level sample (3.6 ng/mL) after high concentration sample (750 ng/mL) in alternating sequence for a total 20 of measurements (data not shown).
- Interference study: evaluation of 84 compounds at concentrations listed in CLSI guideline, FDA safety communication or tested by predicate devices were spiked into patient samples and tested for interference with the Cascadion SM 25-Hydroxy Vitamin D assay (data not shown).
- Evaluation of plasma/serum collection tubes and equivalency between serum and plasma matrices: analysis of patient samples collected in 7 types of serum and 9 types of plasma collection tubes (data not shown).
- Stability of calibration curve: quantification of QC samples against calibration curve stored for 1-30 days.

Data Innovations EP Evaluator® software and Analyse-It for Microsoft Excel 5.11 were used to process the data following CLSI protocols.



Figure 1. Cascadion SM Clinical Analyzer

RESULTS

RESULTS FROM VALIDATION & VERIFICATION

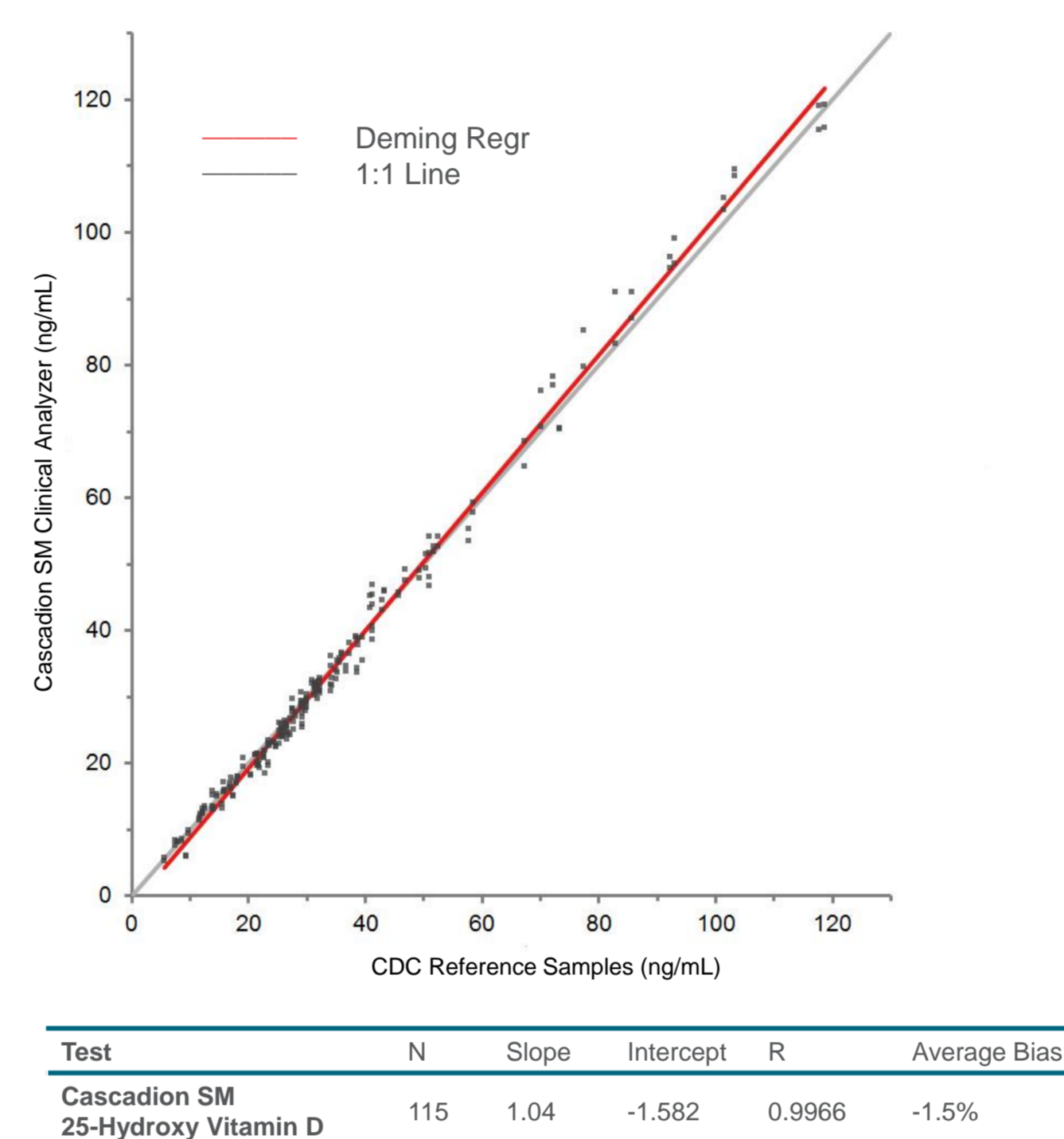
Basic method performance

Table 1. Basic method performance data from validation & verification

	Vitamin D ₂	Vitamin D ₃	Total Vitamin D
Lower limit of analytical measuring range	3.4 ng/ml	3.4 ng/ml	3.4 ng/ml
Analytical measuring range (AMR)	3.4 – 132 ng/ml	3.4 – 132 ng/ml	3.4 – 264 ng/ml
Stability of calibration	30 days	30 days	30 days
Day-to-day precision (CV)	3.2 – 6.1 %	2.9 – 7.6 %	2.3 – 7.6 %
Accuracy of CDC samples			1.04 x CDC – 1.582 ng/ml R=0.9966

Accuracy

Figure 2: Accuracy of CDC Reference samples traceable to NIST reference material SRM 972a



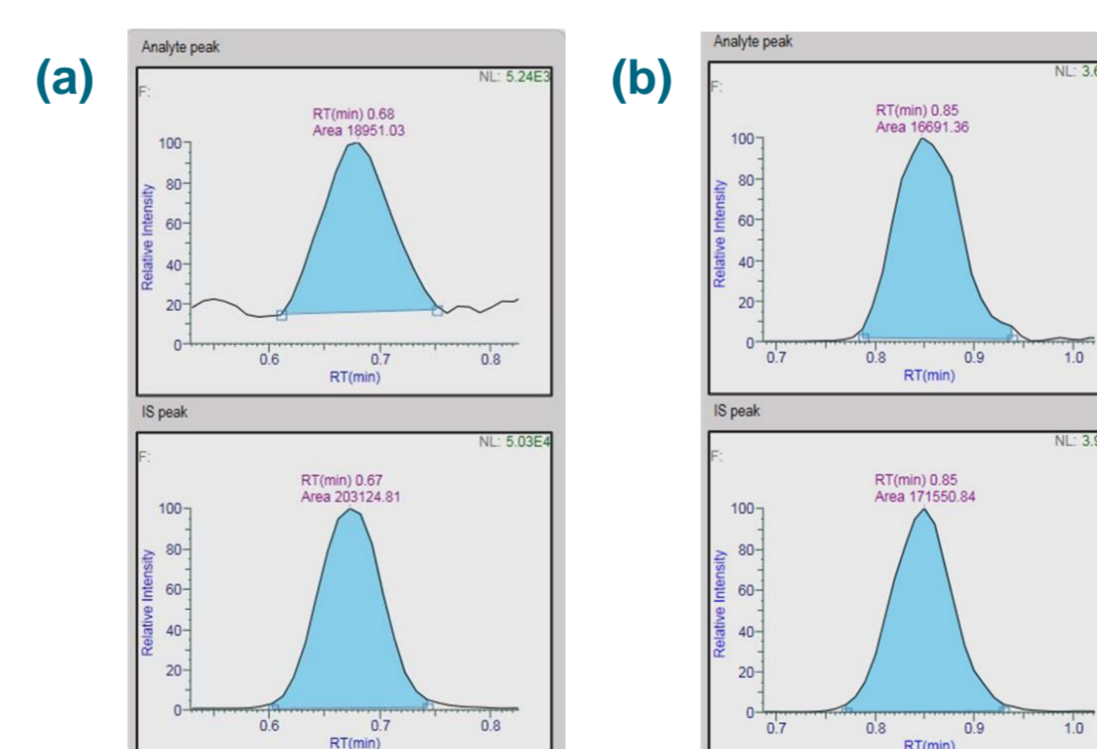
Precision

Table 2: Precision data presented by sample and analyte

Sample	N	25-OH Vitamin D ₂			25-OH Vitamin D ₃			25-OH Vitamin D (D ₂ + D ₃)		
		Mean Value (ng/ml)	SD	Total %CV	Mean Value (ng/ml)	SD	Total %CV	Mean Value (ng/ml)	SD	Total %CV
1	84	No measurable D ₂ concentration			5.95	0.454	7.6	5.95	0.454	7.6
6	84	No measurable D ₂ concentration			14.1	0.678	4.8	14.1	0.678	4.8
4	84	5.24	0.320	6.1	24.1	0.701	2.9	29.3	0.766	2.6
2	84	26.2	0.897	3.4	29.9	0.950	3.2	56.1	1.313	2.3
3	84	8.57	0.391	4.6	102.9	3.428	3.3	111.5	3.474	3.1
5	84	104.5	3.313	3.2	10.1	0.561	5.6	114.6	3.455	3.0

Signal quality at lower limit of analytical measuring range (LLOQ)

Figure 3: Representative chromatograms of the analyte and internal standard at the lower limit of analytical measuring range: (a) 25-OH VitD₃ (3.4 ng/mL) (b) 25-OH VitD₂ (3.4 ng/mL)



RESULTS FROM VIOLLIER AG

Stability and accuracy of calibration in routine clinical laboratory

Figure 4: Viollier AG Vit D₃ calibration Six consecutive calibration runs on both channel 1 and channel 2

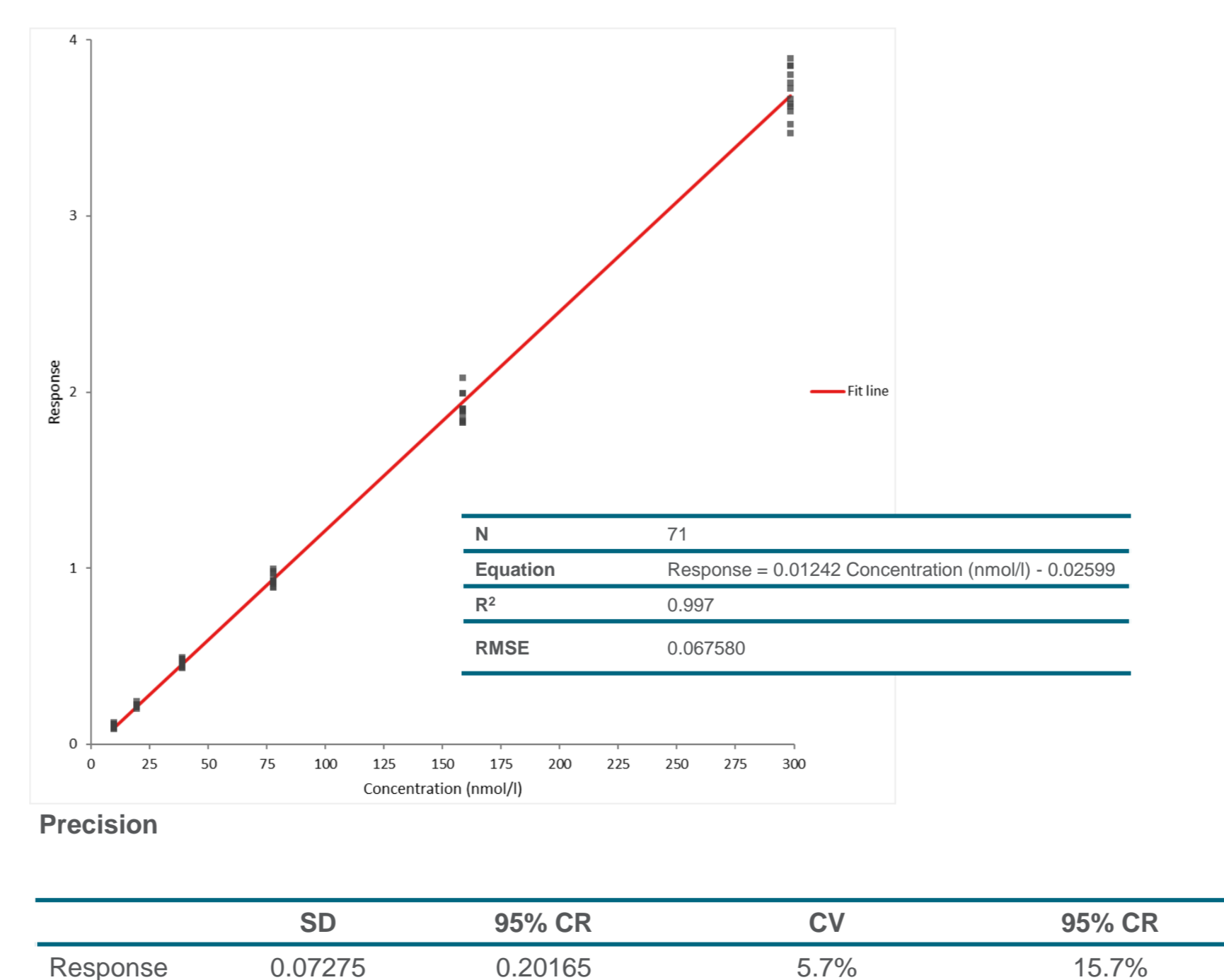


Table 3: Accuracy and precision of Quality Control over 30 days at Viollier AG

Vit D ₃ Control	Mean Value nmol/l	Repeatability SD	Repeatability CV%	Within Laboratory SD	Within Laboratory CV%
VitD Ctrl 1	25.6	1.2	4.6	1.2	4.7
VitD Ctrl 2	74.1	2.5	3.4	2.6	3.5
VitD Ctrl 3	225.3	6.7	3.0	6.7	3.0

Vit D ₂ Control	Mean Value nmol/l	Repeatability SD	Repeatability CV%	Within Laboratory SD	Within Laboratory CV%
VitD Ctrl 1	23.3	1.1	4.6	1.1	4.7
VitD Ctrl 2	69.3	2.3	3.3	2.3	3.3
VitD Ctrl 3	210.1	7.3	3.5	7.3	3.5

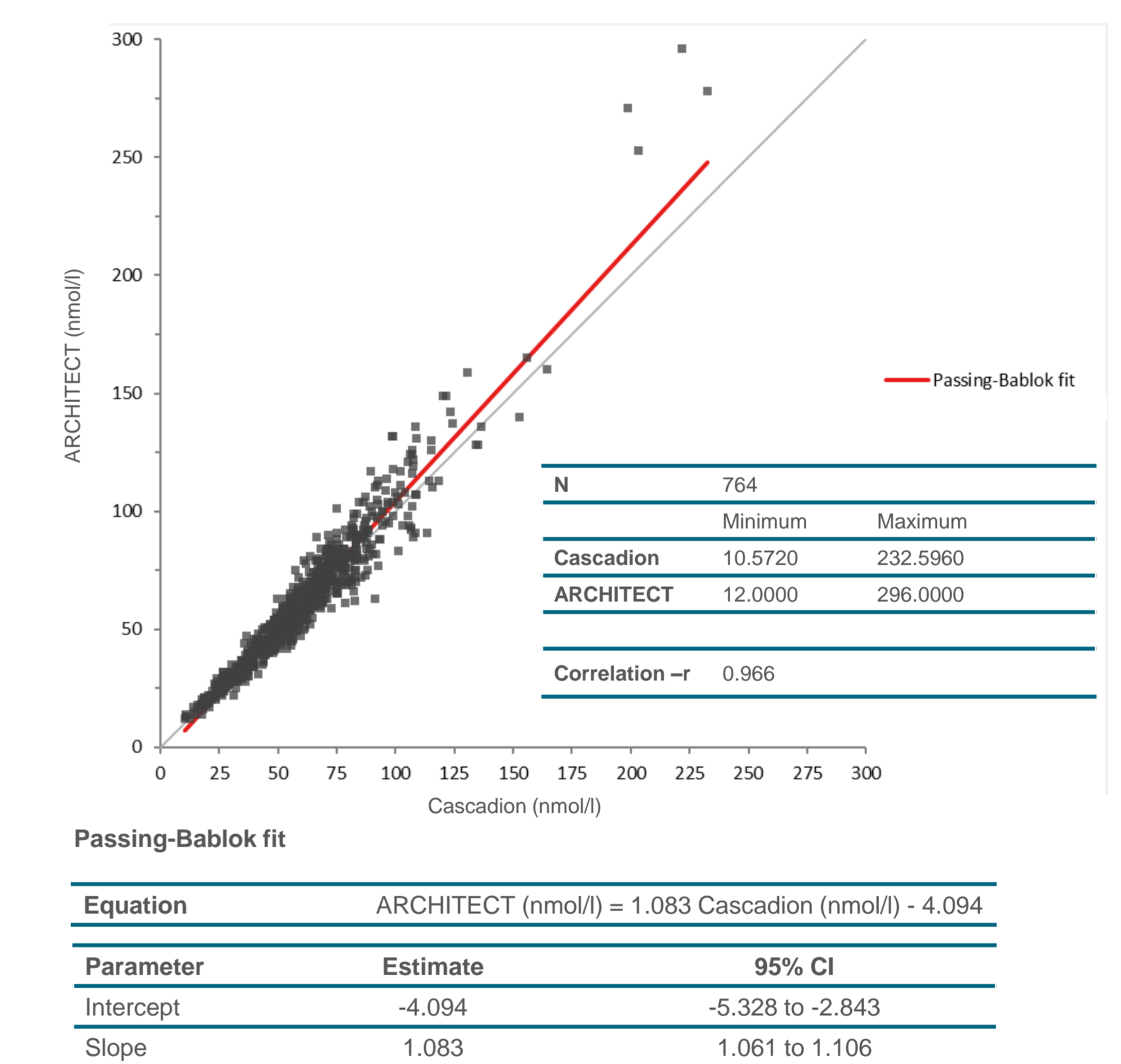
Table 4: Comparison of NIST 968f and NIST 972a Cascadion Vitamin D vs. Target Value in Viollier AG

Id	N	Interlab Value (nmol/l)	Mean	Mean SE	Recovery%
NIST 968f L1	4	34.9	32.9	0.61	94.2
NIST 968f L2	4	42.2	43.7	0.42	103.5

Id	N	Assigned Value (nmol/l)	Mean	Mean SE	Recovery%
NIST 972a L1	4	71.8	70.1	1.04	97.6
NIST 972a L2	4	47.1	45.2	2.42	95.9
NIST 972a L3 D ₂	4	32.3	31.0	0.66	96.0
NIST 972a L3 D ₃	4	49.5	50.1	1.42	101.1
NIST 972a L4	4	73.4	71.3	1.42	97.1

Figure 5: Cascadion 25-Hydroxy Vitamin D method comparison to predicate method at Viollier AG.

ARCHITECT™ Vitamin D Total vs. Cascadion 25-Hydroxy Vitamin D₃



CONCLUSIONS

The Cascadion SM Clinical Analyzer provides superior accuracy and precision with its 25-Hydroxy Vitamin D assay. Using gold-standard LC-MS/MS technology without requiring manual sample preparation it delivers accurate and reproducible sample concentrations for VitD₂ and VitD₃ without interference from epimers. Evaluation in the clinical laboratory by Viollier AG demonstrated high levels of accuracy and precision on reference samples or a predicate immunoassay.

REGULATORY STATUS

The Cascadion SM Clinical Analyzer and Cascadion 25-Hydroxy Vitamin D assay are CE IVD marked but not 510(k)-cleared and not yet available for sale in the U.S. Availability of product in each country depends on local regulatory authorization status.

TRADEMARKS

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