Report:	**Validation Report Lateral Flow Tests for 25-OH-VD (Affimedix)**
Report No:	**36524082023_1**
Date:	**27.10.2023**
Customer:	**Dx365 UG**

IFLb Das Labor.

IFLb Laboratoriumsmedizin Berlin GmbH

Dx365 UG

- vertraulich / confidental -

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1 Identification of parties who carried out the test validation

Signature	Name	Position	Company	Date
	Alexander Taran	CEO	Dx365 UG	27.10.2023
	Dr. Jürgen Reiner	General manager	IFLb GmbH	27.10.2023
	Dr. Oleg Tsuprykov	Research associate	IFLb GmbH	27.10.2023

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2 Aim of the work

Evaluation the accuracy of point-of-care tests for 25(OH)-vitamin D by Affimedix Diagnostics by comparing its measurement results with the ones by a conventional clinical laboratory analyzer.

3 Materials and reagents

Table 1. Materials and reagents

	Description	**Lot/SN**
Cube Reader	Colorimetric	**SN:** 94022460041 **Manuf**.: 2022-11
Igloo Reader	Colorimetric	**UDI**: 0104251766300002 21E0AC2043000019M
Tests	Rapid - Vitamin D Quantitative Test by Affimedix, Inc. (San Francisco, CA, USA)	**LOT:** 23010355 **REF:** 1155Q **Exp**: 07.2024
Biomaterial (clinical samples)	Clinical samples of venous serum, n = 12, stability of serum at 4 °C: 5 days. POCT tests were performed in not later than 24h after the measurements by a conventional clinical laboratory analyzer (samples were stored by at 4 °C in between).	
Conventional clinical laboratory analyzer	Abbott Alinity (Abbott Laboratories, Illinois, United States)	

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3.1 Clinical samples

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Table 2. 25(OH)-vitamin D concentrations in clinical samples (measurements by a conventional clinical laboratory analyzer

Patient number	25(OH) vitamin D [ng/ml][1]	Sample volume, uL
1	5.52	10
2	7.64	10
3	14.84	10
4	20.6	10
5	26.44	10
6	35.72	10
7	43.56	10
8	49.24	10
9	59.72	10

[1] The clinical samples were determined with reference method.

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10	67.96	10
11	91.72	10
12	152.8	10
13	9.76	5
14	22.52	5
15	31.36	5
16	42.48	5
17	51.4	5

3.2 Performance characteristics of point-of-care 25-OH-VD test

Manufacturer data

Measuring Range:	10 - 100 ng/ml
Lower Detection Limit:	≤ 3 ng/ml
Precision:	≤ 15 %
Specificity:	-
Sensitivity:	-
ELISA Correlation:	92% (r^2=0.84)
LC-MS/MS:	98% (r^2=0.96)

4 Procedure

4.1 Test procedure

- Transfer (10 or 5 µl) of the serum to the sample buffer tube.
- Fully insert Blood Collection into buffer tube and push firmly to close tightly. Mix gently.
- Transfer 3 drops of mixing fluid to the test cassette.
- Incubate for 15 minutes and then take measurements.

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5 Results

Sample ID	Repeat, №	Sample size, uL	Expected, ng/mL	Result Cube reader, ng/mL	Result Igloo reader, ng/mL
6	1	10	71.44	29.5	55.27
	2	10	71.44	39.5	68
	3	10	71.44	36.6	62
1	1	10	11.04	8.8	14.38
	2	10	11.04	7.6	17.74
2	1	10	15.28	7.7	16.10
	2	10	15.28	7.1	15.67
3	1	10	29.68	20.8	38.31
	2	10	29.68	29	52.5
4	1	10	41.2	24.1	44.01
	2	10	41.2	28.2	52.21
5	1	10	52.88	19.9	39.35
	2	10	52.88	36.5	63.9
7*	1	10	87.12	100**	76.4
	2	10	87.12	26.8	54.7
8*	1	10	98.48	73.8	117
	2	10	98.48	100**	132
9	1	10	119.44	80.7	115
	2	10	119.44	85.5	111
10	1	10	135.92	86.4	122
	2	10	135.92	96.1	132
11	1	10	183.44	100	175
	2	10	183.44	100	186
12***	1	10	305.6	100	157
	2	10	305.6	100	130
13	1	5	9.76	3.5	11.12
	2	5	9.76	4.3	12.29
14	1	5	22.52	18.2	32
	2	5	22.52	25	43
15	1	5	31.36	21.2	39
	2	5	31.36	22.9	39
16	1	5	42.48	35.3	57
	2	5	42.48	26.9	45
17	1	5	51.4	32.2	54
	2	5	51.4	28.5	49

*- An outlier measurement (we exclude it from the measurements)
** - Incorrect measurement
*** - Too high sample concentration

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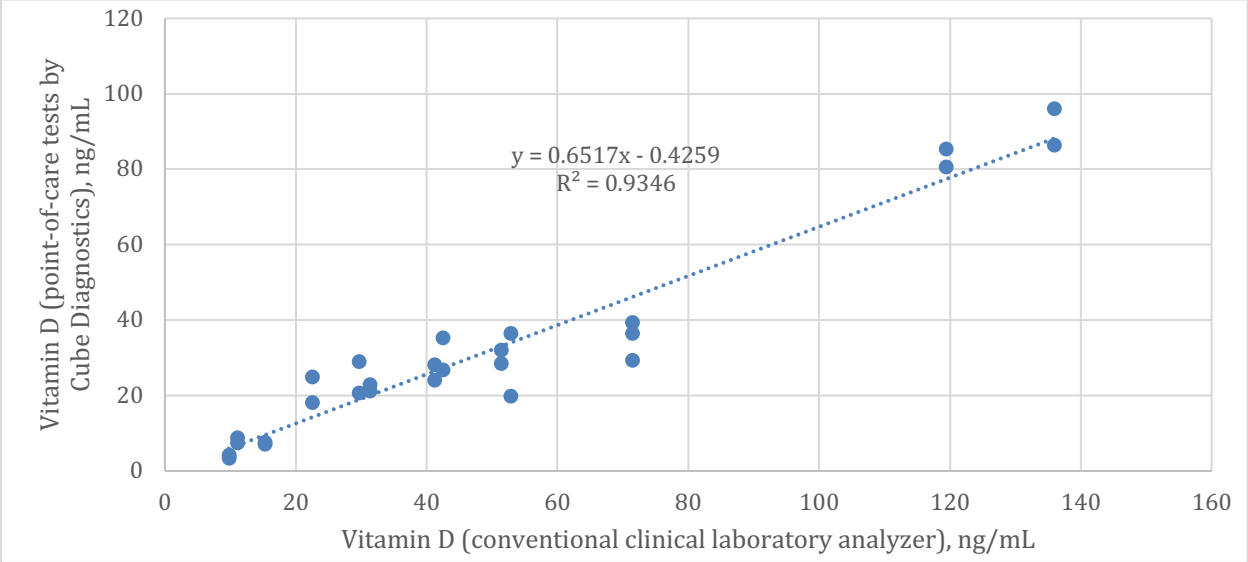

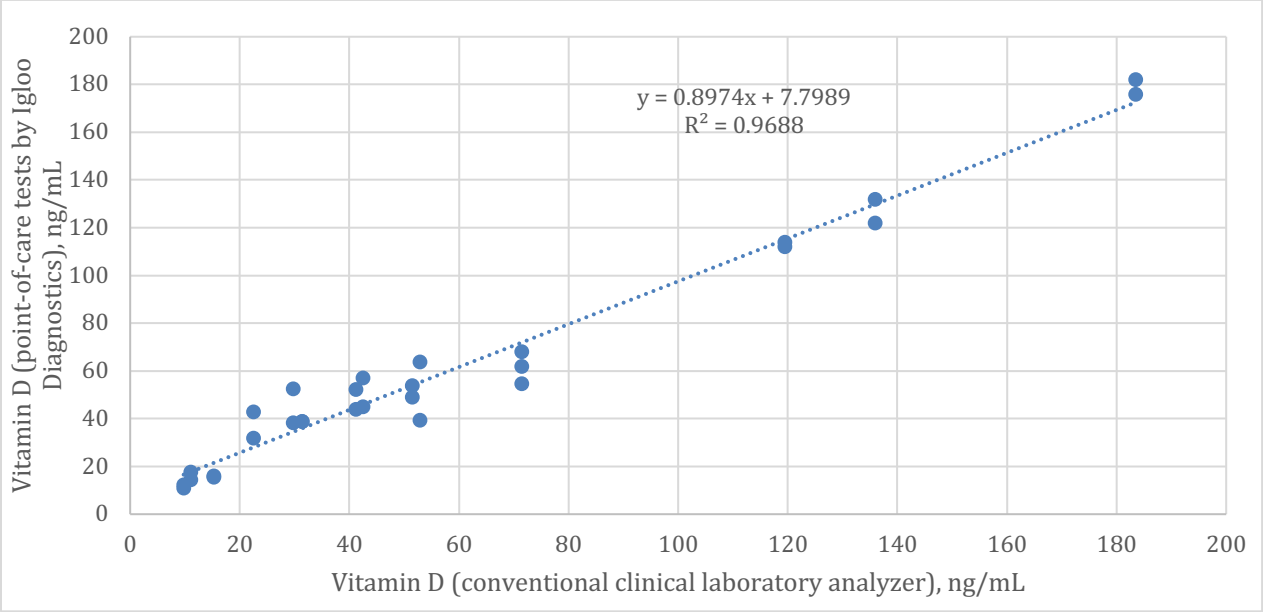

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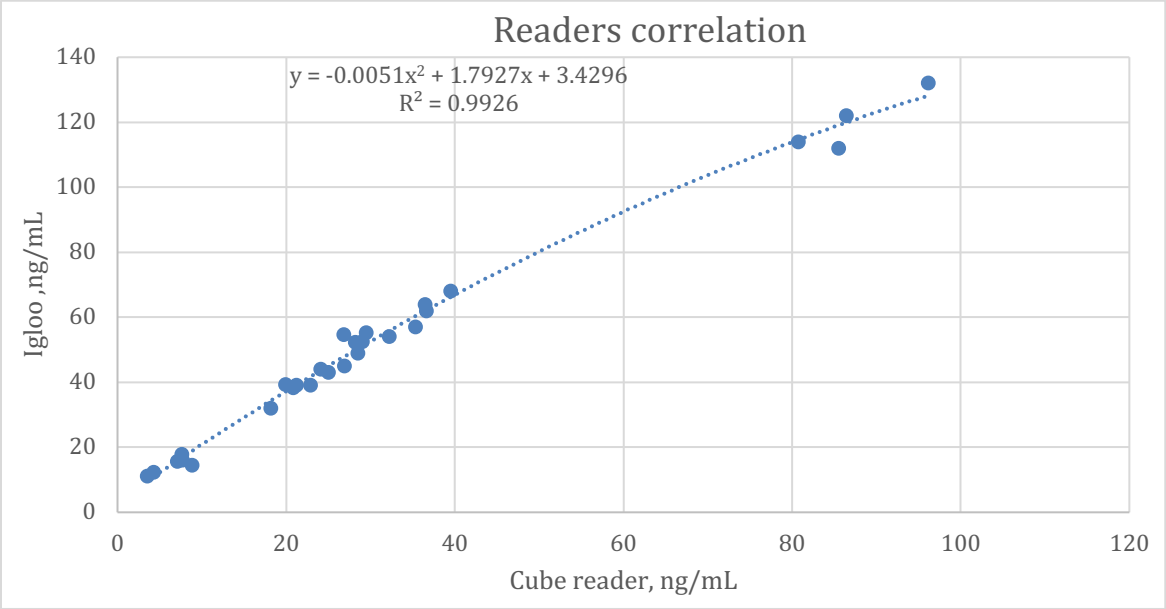

6 Conclusion

We conducted 11 rounds of testing on serum samples. Each round comprised of 2 replicates, with one concentration having triplicates using 10 uL of blood. Additionally, we performed 5 rounds with 2 replicates each, utilizing 5 uL of serum. The analysis revealed a strong correlation between the Cube Reader and the Igloo Reader, exceeding an r2 value of 0.99. Notably, at two specific points ID (7 and 8), the Cube Reader exhibited a reading of '>100', while the Igloo Reader displayed a normal concentration level.

Further investigation indicated that the Cube Reader significantly underestimated the output concentration at a coefficient of 0.66, suggesting a need for additional clarification.

Moreover, the correlation between the standard method and the Igloo Reader was found to be robust (r2=0.97), meeting the requirements stipulated for the ELISA method (r2=0.84). Likewise, a correlation was established for the Cube Reader, with data points surpassing concentrations of '>100ng/mL' being omitted from the analysis. The precision of the results also demonstrated compliance with the set criteria, with an r2=0.93.

Consequently, the quality of the conducted research is deemed to be satisfactory, adhering to the precision and accuracy standards specified by the manufacturers.

- End of Report –