

Report:	Validation Report Lateral Flow Tests for Testosterone (Affimedix)
Report No:	36524082023_1
Date:	19.10.2023
Customer:	Dx365 GmbH



IFLb Laboratoriumsmedizin Berlin GmbH



Dx365 GmbH

- vertraulich / confidential -

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1 Identification of parties who carried out the test validation

Signature	Name	Position	Company	Date
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2 Aim of the work

Evaluation the accuracy of point-of-care tests for Testosterone 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	TestNow - Testosterone Quantitative Test by Affimedix, Inc. (San Francisco, CA, USA)	LOT: 23010588 REF: 1173Q 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

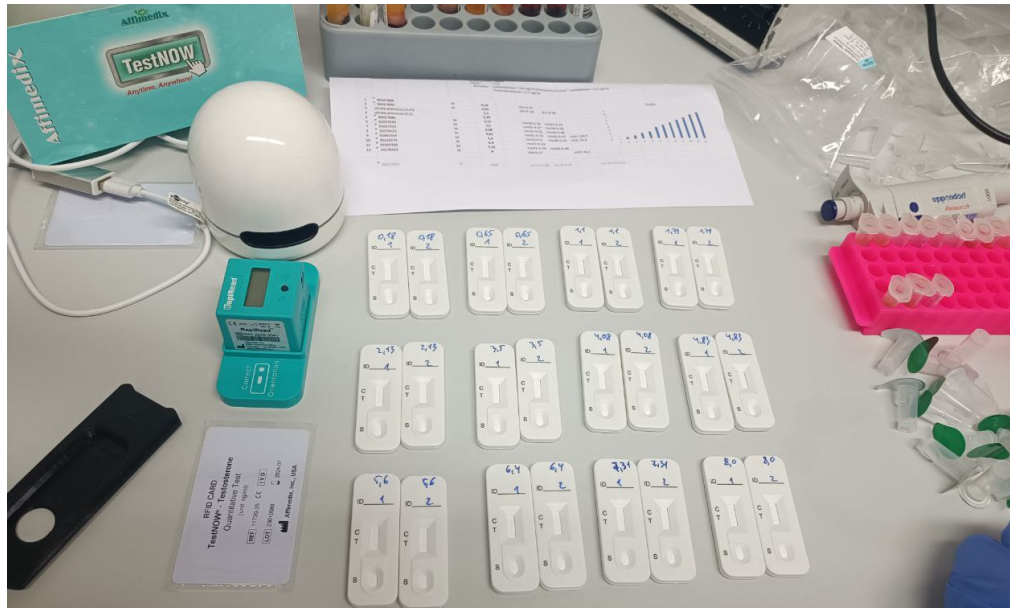


Table 2 Total Testosterone concentrations in clinical samples (measurements by a conventional clinical laboratory analyzer)

Patient number	Total testosterone [ng/ml] ¹
1	0.18
2	0.65
3	1.1
4	1.79
5	2.13
6	3.5
7	4.08
8	4.83
9	5.6
10	6.4
11	7.31
12	8.0

¹ The clinical samples were determined with reference method.

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3.2 Performance characteristics of point-of-care testosterone test

Manufacturer data

Measuring Range CUBE:	1.5 - 25 ng/ml
Lower Detection Limit:	≤ 0.1 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 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

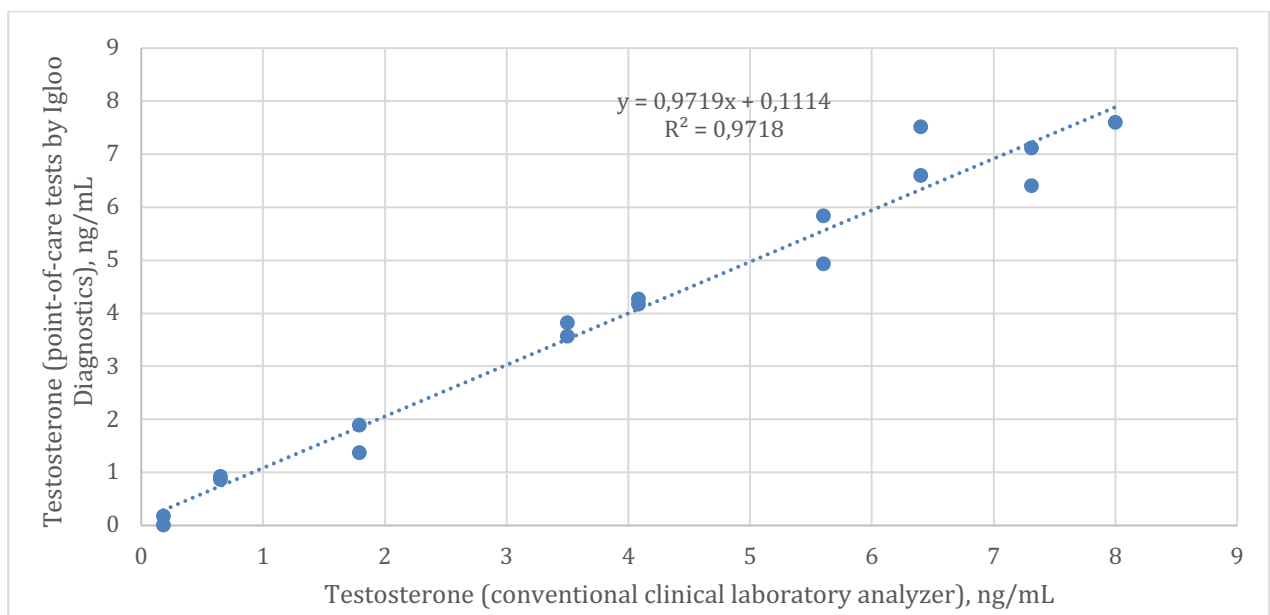
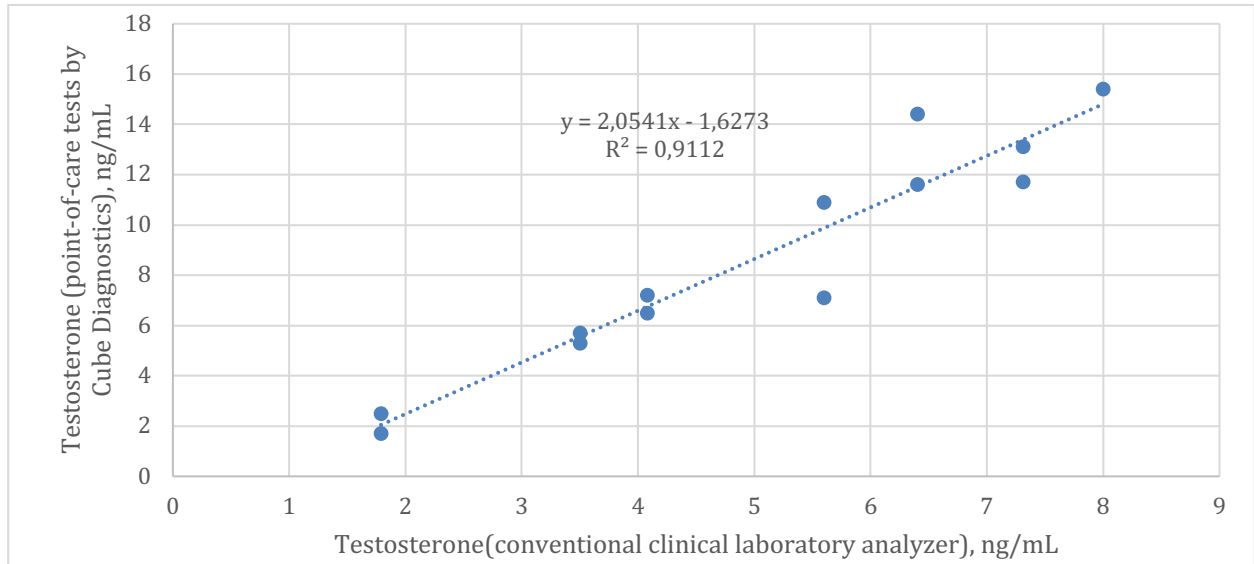
Sample ID	Repeat, №	Expected, ng/mL	Result Cube reader, ng/mL	Result Igloo reader, ng/mL
1	1	0.18	<1.5	0.18
	2	0.18	<1.5	0.008
2	1	0.65	<1.5	0.86
	2	0.65	<1.5	0.92
3***	1	1.1	>25	>max
	2	1.1	>25	>max
4	1	1.79	1.7	1.37
	2	1.79	2.5	1.89
5***	1	2.13	>25	>max
	2	2.13	>25	>max
6*	1	3.5	5.7	3.82
	2	3.5	5.3	3.57
7	1	4.08	6.5	4.17
	2	4.08	7.2	4.27
8*	1	4.83	4.4	2.2
	2	4.83	2.8	1.4
9	1	5.6	10.9	5.83
	2	5.6	7.1	4.93
10	1	6.4	14.4	7.51
	2	6.4	11.6	6.59
11	1	7.31	11.7	6.4
	2	7.31	13.1	7.11
12	1	8.0	15.4	7.6
	2**	8.0	6.4**	3.2**

* - An outlier measurement (we exclude it from the measurements)

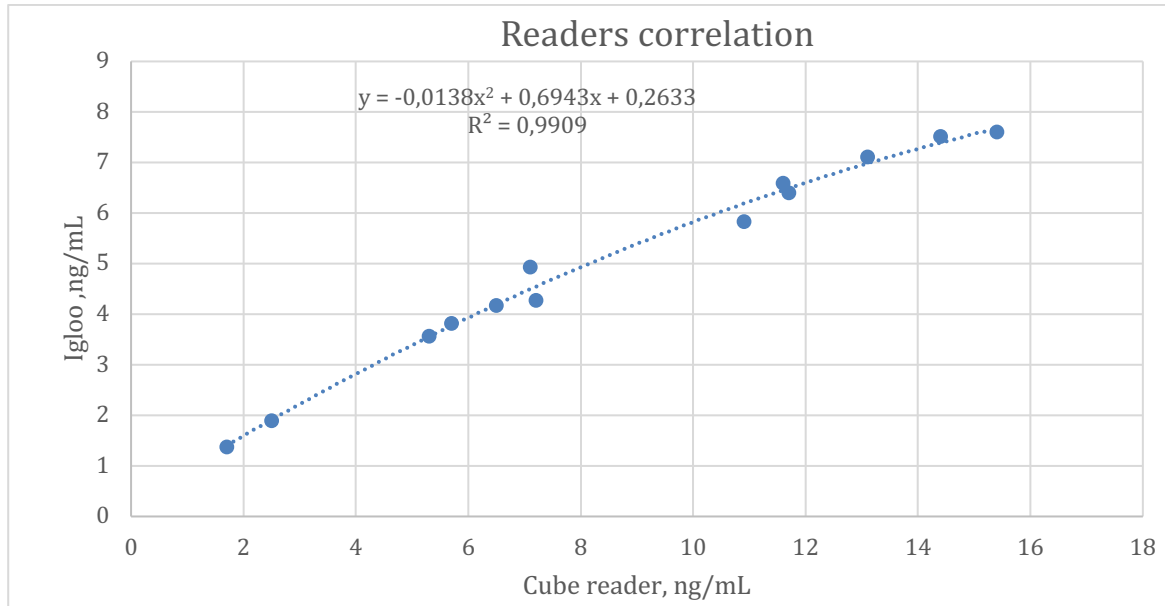
** - Incorrect measurement

*** - Too high sample concentration

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6 Conclusion

We conducted 12 rounds of testing on serum samples. Each round comprised of 2 replicates, with one concentration having triplicates using 5 uL of serum. The analysis revealed a strong correlation between the Cube Reader and the Igloo Reader, exceeding an r^2 value of 0.99. Notably, at two specific points ID (1 and 2), the Cube Reader exhibited a reading of '<1.5', 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 2.00 and bias 1.6 ng/ml, suggesting a need for additional clarification.

Moreover, the correlation between the standard method and the Igloo Reader was found to be robust ($r^2=0.97$), meeting the requirements stipulated for the ELISA method ($r^2=0.84$). Likewise, a correlation was established for the Cube Reader, with data points surpassing concentrations of '<1.5 ng/mL' being omitted from the analysis. The precision of the results also demonstrated compliance with the set criteria, with an $r^2=0.91$.

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 –