Performance Evaluation Report

Free Testosterone ELISA

IVD

CE

REF **EIA-2924**

Σ **96 wells**

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This document consists of Analytical Performance Report, Scientific Validity Report and Clinical Performance Report.

Content:

1 INTENDED PURPOSE

Free Testosterone ELISA is a manual *in vitro* diagnostic device intended for the quantitative determination of free testosterone in human serum or plasma.
Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis and monitoring of disorders involving the male sex hormones (androgens).

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

2 CLINICAL SIGNIFICANCE

Testosterone is found in circulation predominantly linked to carrier proteins, the most common of which being sex-hormone binding globulin (SHBG). Testosterone plays a key role in the development of primary and secondary sexual characteristics in males and is involved in the production of female sexual hormones.
Only 1 – 2% of testosterone in circulation is not bound to any protein and is biologically active – this is referred to as 'free testosterone' (FT). Bioavailable testosterone refers to the sum of FT and the testosterone bound to serum albumin, since it is bound with low affinity and readily able to dissociate to become available for its biological function.
In males elevated levels of testosterone are associated with several conditions such as, early (precocious) puberty, congenital adrenal hyperplasia (CAH), androgen insensitivity syndrome (AIS), steroid use and testicular or adrenal tumours. Whereas the major causes of supressed levels include Klinefelter's syndrome, testicular damage, pituitary disorders etc. In females of all ages, elevated testosterone levels can be associated with a variety of virilising conditions including adrenal tumours and polycystic ovarian syndrome (PCOS).
These clinical conditions are associated with either a lack or excess of testosterone in circulation (hypoandrogenism or hyperandrogenism). Diagnosis of these disorders involve the quantification of total testosterone (TT) in association with other clinical evidence and laboratory data. However, clinical manifestations of androgen disorders are often associated with normal levels of TT. In such cases, additional information may be gained by the assessment of the biologically active, FT level. Several androgen disorders can be caused by alteration of SHBG production which affects the levels of FT available in serum.
Measurement of FT can be considered useful in the diagnosis of several conditions including androgen deficiency in men and androgen excess in women[1]. Assessment of free testosterone levels may prove beneficial[2] and may avoid an incorrect diagnosis of hypogonadism in cases when low concentrations of total testosterone are determined and alterations of SHBG are suspected.
There is an observed and well documented circadian variation of testosterone levels in men with circulating concentrations being higher in the morning and declining throughout the day[3]. Testosterone levels also decline in ageing males (andropause) and is associated with loss of muscle and bone mass, leading to osteoporosis, loss of libido, erectile dysfunction. Depression and impaired cognitive function[4].

2.1 Literature

1. Shea JL, Wong PY, Chen Y. Free testosterone: clinical utility and important analytical aspects of measurement. Adv Clin Chem. 2014;63:59-84.
2. Diver MJ. Analytical and physiological factors affecting the interpretation of serum testosterone concentration in men. Ann Clin Biochem. 2006 Jan;43(Pt 1):3-12.
3. Brambilla DJ, Matsumoto AM, Araujo AB and McKinlay JB. The Effect of Diurnal Variation on Clinical Measurement of Serum Testosterone and Other Sex Hormone Levels in Men. J Clin Endocrinol Metab. 2009 Mar; 94(3): 907–913.
4. Rajfer J. Decreased Testosterone in the Aging Male. Rev Urol. 2003;5(suppl 1):S1–S2.
5. Basic QC Practices On-line Course; http://www.Westgard.com.
6. Boscato, LM. and Stuart, MC., 'Heterophilic antibodies: a problem for all immunoassays'. *Clin Chem*, 34, 1988, pp 27–33

3 PRINCIPLE OF THE TEST

The Free Testosterone ELISA is a competitive enzyme immunometric assay (ELISA) where free testosterone (antigen) in the sample competes with the antigenic testosterone conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti-testosterone coated on the microplate (solid phase).
After the incubation, the bound/free separation is performed by a simple solid phase washing. Then, the enzyme HRP in the bound fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is inversely proportional to the free testosterone concentration of in the sample.
Free testosterone concentration in the sample is calculated through a calibration curve.

4 MATERIALS PROVIDED

1. ***Standards*** **(Standard 0 – 5)** (6 vials, 1 mL each)
2. ***Controls*** (Control A and Control B) (2 vials, 1 mL)
   Control concentration is indicated on the Certificate of Analysis
3. ***Enzyme Conjugate*** (1 vial, 15 mL)
   Testosterone conjugated with Horseradish peroxidase (HRP)
4. ***Microtiterwells*** (1 breakable microplate)
   Anti-Testosterone antibody adsorbed on microplate
5. ***Substrate Solution*** (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
6. ***Stop Solution*** (1 vial, 15 mL)
   Sulphuric acid 0.15 M *(avoid any skin contact)*
7. ***Wash Solution***, 10X Conc. (2 vials, 25 mL)
   0.2 M Phosphate buffer, pH 7.4

4.1 Reagent Preparation

4.2 Preparation of Standards and Controls

Before use, mix for 5 minutes with a rotating mixer.
The Standards are ready for use and have the following concentration of Testosterone:

	S0	S1	S2	S3	S4	S5
pg/mL	0	0.2	1.0	4.0	20.0	100.0

4.3 Preparation of the Wash Solution

Dilute the content of the two vials "*Wash Solution"* with distilled water to a final volume of 500 mL prior to use (for both vials, 25 mL + 25 mL + 450 mL).
For smaller volumes respect the 1:10 dilution ratio.

It is possible to observe the presence of crystals within the concentrated wash solution; in this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely by rinsing of the bottle, then mix until crystals are completely dissolved.

4.4 Preparation of Samples

The determination of free testosterone can be performed in human serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.
The Controls are ready to use.

5 SAMPLE COLLECTION AND STORAGE

The assay should be performed using

serum (standard sampling tubes or tubes containing serum separating gel) or
plasma (lithium heparin, sodium heparin or potassium EDTA) samples.

Sample Storage	Duration
2 °C - 8 °C	24 hours
Freeze/thaw cycles	1 cycle

6 ASSAY SPECIFICATIONS

6.1 Verification (Evaluation of Assay Characteristics)

6.1.1 Test Procedure

Please also refer to the IFU for further remarks.

- **Allow all reagents to reach room temperature (22 °C - 28 °C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2 °C - 8 °C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2 °C - 8 °C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (S0 - S5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample / Control	Blank
Standard S0-S5	20 µL		
Sample / Control		20 µL	
Enzyme Conjugate	100 µL	100 µL	
Incubate at 37 °C for 1 hour. Remove the content from each well; wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate Solution	100 µL	100 µL	100 µL
Incubate at room temperature 22 °C – 28 °C for *15 minutes* in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

6.1.2 Specificity

Cross-reactivity was measured in a similar manner to interference under CLSI EP7-A3
One batch (5396A) was used throughout the study. The assay was performed according to the IFU.
To determine the level of cross-reactivity, two base serum sample pools (low and high) were used. The concentrations of low and high base sample pools were about 0.80 and 15.00 pg/mL.
Samples were assayed spiked with the test substance and un-spiked (spiked with the solvent of the test substance only) in 18 replicates.

Cross-reactivity is calculated as per guidelines for interference, with the known concentration of the cross-reactivity species spiked into the sample, as per the equation below:

$$\%\ Cross-reactivity = 100 * \frac{measured\ value - true\ value}{concentration\ of\ cross-reactant}$$

Results:

Cross-Reagent	Target Concentration	Result low	Result High
11-keto-testosterone	100 ng/mL	**0.1%**	**0.0%**
11-β-hydroxy-testosterone	100 ng/mL*	**> ULoQ**	**> ULoQ**
	10 ng/mL	**0.2%**	**0.2%**
17α-OH-Progesterone	500 ng/mL	**0.0%**	**0.0%**
Aldosterone	3000 ng/mL	**0.0%**	**0.0%**
Androstenedione	100 ng/mL	**0.0%**	**0.0%**
Cortisol	1000 ng/mL	**0.0%**	**0.0%**
Cortisone	1000 ng/mL	**0.0%**	**0.0%**
Danazol	1000 ng/mL	**0.0%**	**0.0%**
Dexamethasone	2000 ng/mL	**0.0%**	**0.0%**
DHEA	1000 ng/mL	**0.0%**	**0.0%**
DHEA-S	10000 ng/mL	**0.0%**	**0.0%**
5α-dihydrotestosterone (DHT)	500 ng/mL	**0.0%**	**0.0%**
Estradiol	1000 ng/mL	**0.0%**	**0.0%**
Estriol	100 ng/mL	**0.0%**	**0.0%**
Estrone	1000 ng/mL	**0.0%**	**0.0%**
Ethisterone	1000 ng/mL*	**> ULoQ**	**> ULoQ**
	100 ng/mL	**0.0%**	**0.0%**
Norgestrel	1000 ng/mL*	**> ULoQ**	**> ULoQ**
	100 ng/mL	**0.0%**	**0.0%**
Prednisone	1000 ng/mL	**0.0%**	**0.0%**
Pregnenolone	5000 ng/mL	**0.0%**	**0.0%**
Progesterone	1000 ng/mL	**0.0%**	**0.0%**
Testosterone propionate	1000 ng/mL	**0.0%**	**0.0%**

11-β-hydroxy-testosterone, Ethisterone and Norgestrel exceeded the high assay range limit (100 pg/mL) and for this reason, the concentrations were reduced to have results within the measurable range.

Conclusion:

No cross-reactivity of the assay to structurally related substances is detected.

6.1.3 Limit of Blank (LoB) and Limit of Detection (LoD)

60 replicates of blank samples and low positive samples were measured (5 days, 6 samples, 2 replicates per sample). Two batches (5377RD2, 5519RD) were used throughout the study. The assay was performed according to the IFU. The acceptance criteria are as follows:

- LoB ≤ 0.1 pg/mL
- LoQ ≤ 0.25 pg/mL

Results:

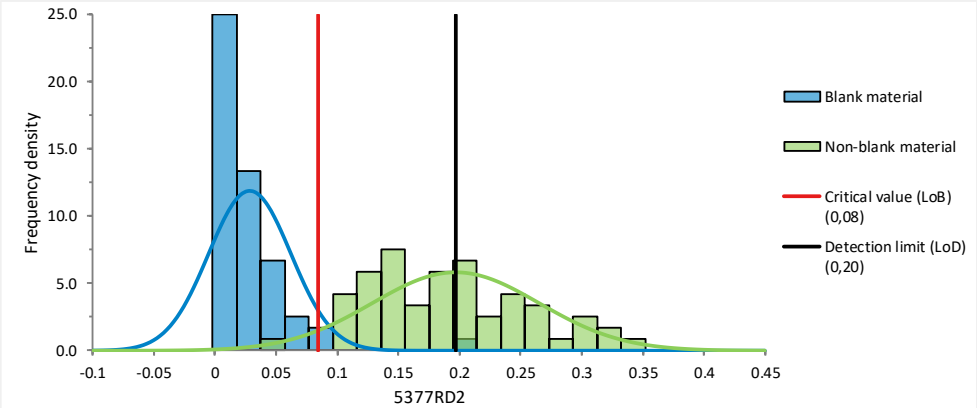
LoB – LoD lot 5377RD2

Detection Decision

	N	Mean	SD	Alpha	Critical value (LoB)
Blank material	60	0.028	0.034	5%	0.08

Detection Limit

	N	Pooled SD	Beta	Detection limit (LoD)
Non-blank material	60	0.068	5%	0.20



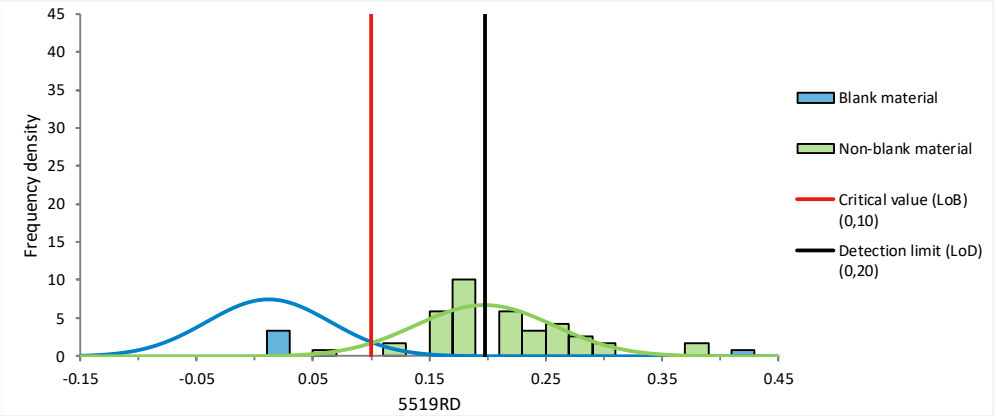
LoB – LoD lot 5519RD

Detection Decision

	N	Mean	SD	Alpha	Critical value (LoB)
Blank material	60	0.012	0.053	5%	0.10

Detection Limit

	N	Pooled SD	Beta	Detection limit (LoD)
Non-blank material	60	0.059	5%	0.20



Lot	LoB (pg/mL)	LoD (pg/mL)
5377RD2	0.08	0.20
5519RD	0.10	0.20

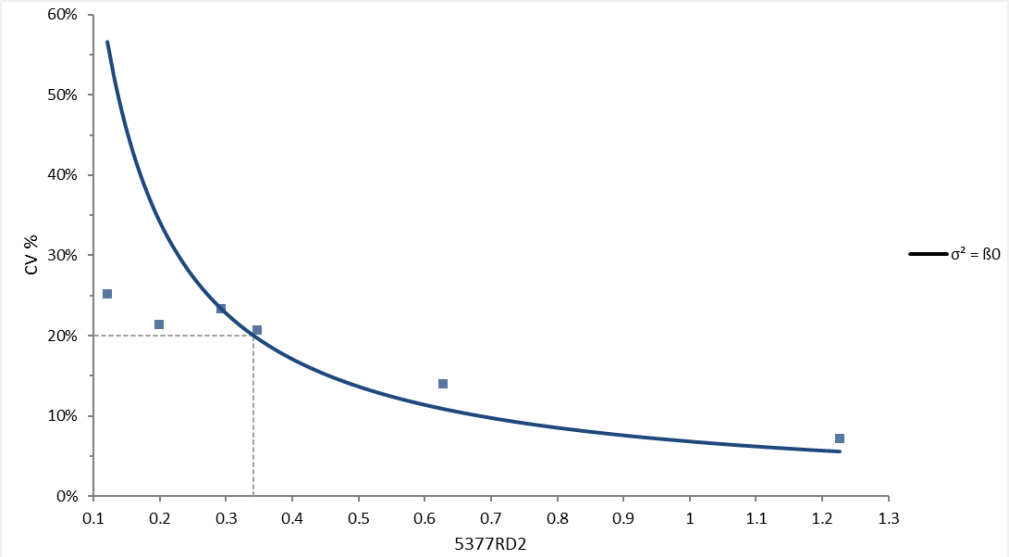
Conclusion:

LoB and LoD both meet the required specifications for both lots.

6.1.4 Limit of Quantification (LoQ)

LOQ is defined as the lowest concentration of analyte that can be detected with precision equal to the within-laboratory precision CV at 20 %. The sensitivity was determined on the basis of CLSI EP17-A2.
Results from low positive samples from LoD, LoB analysis were used to calculate LoQ.
The acceptance criteria is: LoQ ≤ 0.5 pg/mL.

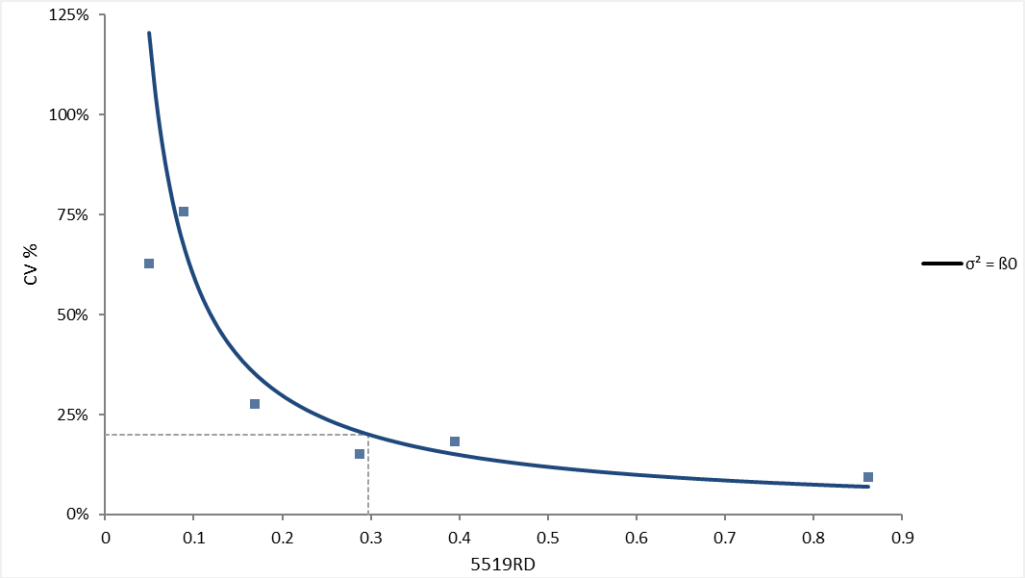
LoQ lot 5377RD2



Inverse Prediction

CV	U
20.0%	0.34

LoQ lot 5519RD



Inverse Prediction

CV	U
20.0%	0.30

Lot	LoQ (pg/mL)
5377RD2	0.34
5519RD	0.30

Conclusion:

LoQ meets the required specification for both lots.

The assay measuring range (AMR) is 0.34 – 70.0 pg/mL (1.18 – 242.9 pmol/L).

6.1.5 Traceability (Trueness)

Meteorological traceability has been defined as per CLSI EP32R.

After a search on the Joint Committee of Traceability and Laboratory Medicine (JCTLM) database, no higher-order reference materials or reference measurement methods/procedures have been recognized for Free Testosterone parameter.

Since the Free Testosterone is an established non-standardized assay, a predicate device was selected to guarantee the trueness and therefore the metrology and traceability of the assay.

According to an analysis of other assays on the market, the Accubind Free Testosterone ELISA by Monobind 5325-300A was selected, taking into consideration both the analytical performances (FDA 510k Number: K181017; decision date 20 July 2018) and the presence on different countries (EU and extra-EU). The following traceability chain describes the materials and the procedures used to establish the traceability of the calibrators to the Free Testosterone AccuBind ELISA 5325-300A.

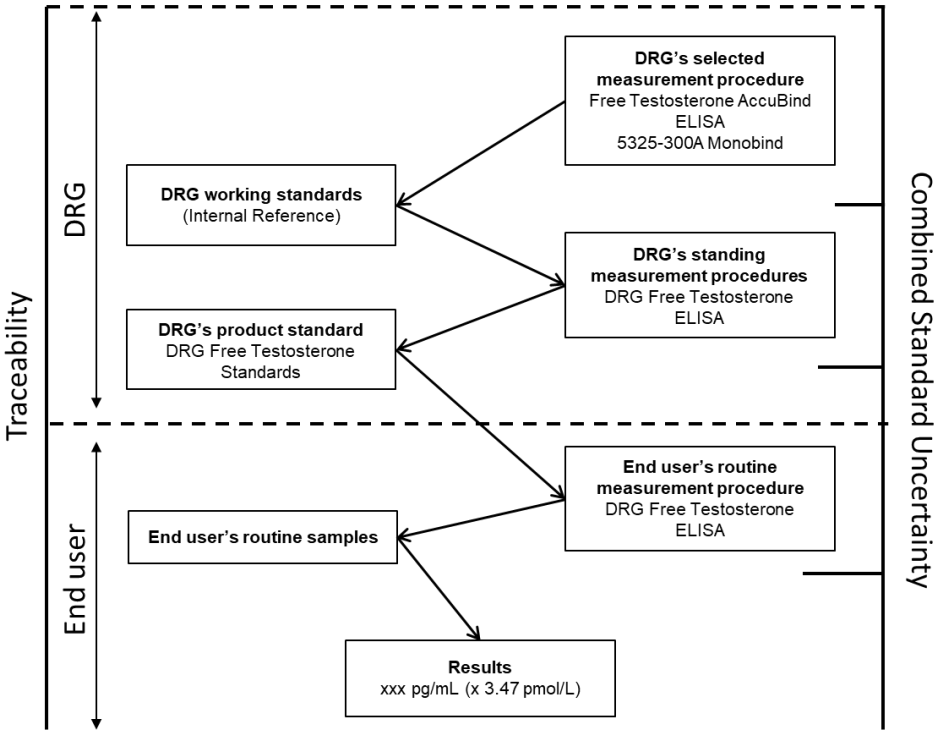


Figure 1: Traceability chain

6.1.6 Precision

The precision study was designed according to CLSI EP05-A3 guideline.
The precision was determined using the following variables:

- Within-run assay (repeatability)
- Between-run precision (reproducibility)

Precision studies were performed using two different reagent lots. Tests were performed by three operators. Six samples covering the assay range were assayed once a day, five replicates per samples were used to provide a minimum of 75 total replicates per sample. Two types of washing (automatic and manual) were tested.
The acceptance criteria are as follows:

- Within-run (repeatability) ≤ 15%
- Between-run(reproducibility 1 Lot) ≤ 20%

Results:

Precision lot 5377RD2

Sample	n	Mean (pg/mL)	Repeatability CV%	Repeatability SD (pg/mL)	within-lot Reproducibility CV%	within-lot Reproducibility SD (pg/mL)
sample 1	75	0.82	10.9%	0.09	11.5%	0.09
sample 2	75	1.86	7.0%	0.13	7.6%	0.14
sample 3	75	5.72	3.8%	0.22	6.3%	0.36
sample 4	75	11.00	3.5%	0.39	7.2%	0.79
sample 5	75	39.83	6.7%	2.68	11.7%	4.65
sample 6	75	78.97	7.0%	5.49	12.7%	10.05

Precision lot 5519RD

Sample	n	Mean (pg/mL)	Repeatability CV%	Repeatability SD (pg/mL)	within-lot Reproducibility CV%	within-lot Reproducibility SD (pg/mL)
sample 1	75	0.68	11.3%	0.08	14.2%	0.10
sample 2	75	1.62	5.9%	0.10	13.6%	0.22
sample 3	75	5.25	3.7%	0.19	9.5%	0.50
sample 4	75	10.43	4.6%	0.48	10.2%	1.07
sample 5	75	34.99	3.3%	1.17	9.2%	3.20
sample 6	75	68.65	6.7%	4.62	12.3%	8.47

Between-lot/within Laboratory (reproducibility 2 Lots) is presented in chapter 6.2.2 Between-lot precision.

Conclusion:

All precision data are below the respective acceptance limits.

6.1.7 High Dose Hook Effect

The hook effect is typically detected in one-step sandwich immunoassays, while is absent in competitive immunoassays. Thus, the hook effect test was not performed for Free Testosterone ELISA assay.

6.1.8 Matrix Interference

Interferences was measured under CLSI EP7-A3.

Increasing amounts of bilirubin, hemoglobin, and triglycerides (1:10 dilution of 10-fold stock solutions) were added to 4 samples. After determination of the analyte concentrations in the bilirubin-, hemoglobin-, and triglyceride-spiked samples, the results were compared with the acceptance ranges.
To determine the level of interference, two base serum sample pools (low and high) were used. The concentrations of low and high base sample pools were about 00.80 and 15.00 pg/mL.
Samples were assayed spiked with the test substance and un-spiked (spiked with the solvent of the test substance only) in 18 replicates. The interference is calculated according to EP7-A3:

$$\%\ Interference = \frac{(Mean\ spiked\ concentration - Mean\ Nonspiked\ Control\ Concentration)}{Mean\ Nonspiked\ Control\ Concentration} \times 100$$

Results:

Interferent	Target Concentration	Result Low	Result High
Bilirubin unconjugated	15 mg/dL	0.1%	2.0%
Bilirubin conjugated	15 mg/dL	-5.5%	-3.3%
Hemoglobin	200 mg/dL	-5.4%	-10.0%
Triglycerides	500 mg/dL	-0.3%	0.0%
Total protein	7 g/dL	-1.9%	-14.1%

Conclusion:

In general, hemolytic, icteric or lipemic samples should be avoided, but can be tolerated up to 15 mg/dL unconjugated bilirubin, 15 mg/dL conjugated bilirubin, 200 mg/dL hemoglobin, 500 mg/dL triglycerides, and 7 g/dL total protein. The bias for all interfering substances was below 15 %.

6.1.9 Sample Collection

A Matrix Comparison study has been performed according to CLSI GP34-A.
One batch (5396A) was used throughout the study. The assay was performed according to the IFU.
Serum, SST, EDTA plasma, Li-heparin plasma and Na-heparin plasma were used for this study. Individual matched sample sets were assayed in the same assay. Samples were stored frozen.

Results:

	SST	K2 EDTA	Li Heparin	Na Heparin	Acc. crit.	Status
n	21	21	21	21	≥ 20	**passed**
Correlation - r	0.99	0.99	1.00	0.99	≥ 0.90	**passed**
Intercept	-0.02	0.27	-0.01	-0.01	< LoQ	**passed**
Slope	0.97	1.03	0.99	0.93	0.90 – 1.10	**passed**

Conclusion:

Serum, SST, EDTA plasma, Li-Heparin plasma, and Na-Heparin can be used for this assay.

6.1.10 Sample Stability – freeze-thaw cycles

The study was designed according to CLSI EP25-A.
One batch (5396A) was used throughout the study. The assay was performed according to the IFU.
The sample stability studies were conducted on serum samples and on EDTA plasma samples.
Human serum and EDTA plasma samples were tested to establish the number of freeze thaw cycles samples without any impact on measured concentration.
A total of 3 samples for each type were used (approx. concentrations 0.80 - 10.00 - 30.00 pg/mL).
The test was performed in duplicate and one freeze/thaw cycle was tested.

Results:

	day 0	T1 (-20 °C) – 1 freeze-thaw cycle	
Serum Samples	pg/mL	pg/mL	Recovery (%)
Serum 1	0.91	0.90	98.9
Serum 2	8.50	10.02	117.9
Serum 3	24.91	26.07	104.7
Plasma Samples	pg/mL	pg/mL	Recovery (%)
Plasma 1	0.71	0.77	108.5
Plasma 2	8.38	8.07	96.3
Plasma 3	28.08	30.86	109.9

Conclusion:

Samples can be subjected to 1 freeze-thaw cycle without affecting the measured concentrations.

6.1.11 Sample Stability – short-term

The study was designed according to CLSI EP25-A.
One batch (5396A) was used throughout the study. The assay was performed according to the IFU.
The sample stability studies were conducted on serum samples and on EDTA plasma samples.
Human serum samples and EDTA plasma samples were tested to establish the length of time a sample can be stored at 2 °C – 8 °C without any impact on measured concentration.
A total of 3 samples for each type were used (approx. concentrations 0.80 - 10.00 - 30.00 pg/mL).
The test was performed in duplicate. Samples were stored for 24 h ± 1 hour.

Results:

	day 0	1 day storage at 2 °C – 8 °C	
Serum Samples	pg/mL	pg/mL	Recovery (%)
Serum 1	0.91	0.92	101.1%
Serum 2	8.50	9.84	115.8%
Serum 3	24.91	26.48	106.3%
Plasma Samples	pg/mL	pg/mL	Recovery (%)
Plasma 1	0.71	0.82	115.5%
Plasma 2	8.38	7.99	95.3%
Plasma 3	28.08	29.41	104.7%

Conclusion:

Samples can be stored at 2 °C – 8 °C for 24 hours without any impact on the measured concentration.

6.1.12 Preliminary shelf life

Introduction:

The kits were stored at 25 °C ± 3°C and 37 °C ± 3°C and compared with the reference kits stored at 2 °C – 8 °C, by testing them for several timepoints, defined in the table below.

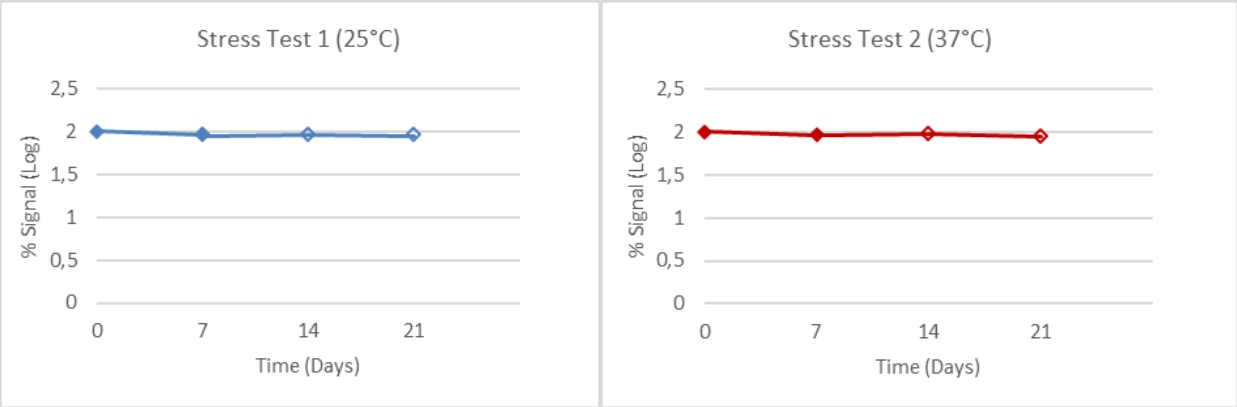
T0 is the day when the reagents are placed at the elevated temperatures.

The study can be stopped before T4 only if two consecutive fails are observed.

Day	Time point
0	T0
7	T1
14	T2
21	T3
28	T4

Two batches (5377RD2; 5519RD) were used throughout the study. The assay was performed according to the IFU.

Results:



estimated storage temperature	1/T	Log Time (2)	calculated shelf life (days)	calculated shelf life (years)
2 °C – 8 °C (4 °C) 277.15 K	0.003608154 1/K	2,746803288	558	1.5

Conclusion:

Using Arrhenius calculation, a shelf life of 1.5 years is expected.

6.1.13 Transport Stability

Introduction:

All components of DRG ELISA kit were stored at 2 °C - 8 °C as recommended in IFU. One kit is then successively exposed to the following temperatures:

- 56 h at 37 °C ± 2 °C,
- 24 h at 2 °C - 8 °C,
- 72 h at 37 °C ± 2 °C,
- > 24 h at 2 °C - 8 °C.

After transport simulation, the results of the kit are compared to the results of a kit, which was stored at 2 °C – 8 °C as recommended.
One batch (5519RD) was used throughout the study. The assay was performed according to the IFU.

Results:

Controls / Samples	storage at 2 °C - 8 °C		test after transport simulation			Acceptance Range 100 +/- 20% pg/mL	
	OD_{450}	Conc. pg/mL	OD_{450}	Conc. pg/mL	Status	Min	Max
Control A	1.754	0.95	1.469	1.29	**passed**	0.78	1.57
Control B	0.379	14.95	0.300	17.41	**passed**	9.30	19.55
Sample 1	2.128	0.47	2.009	0.48	**passed**	0.38	0.76
Sample 2	1.185	2.53	1.009	2.86	**passed**	2.28	3.45
Sample 3	0.469	11.25	0.396	11.89	**passed**	9.81	14.34
Sample 4	0.173	39.66	0.148	43.88	**passed**	31.07	50.17

Conclusion:

The kit is stable after transport simulation.

6.1.14 Stability of opened kits

The stability study was designed according to DIN EN ISO 23640.
A complete DRG ELISA Kit and 3 samples were measured at day 0 (all vials must be opened and used). Thereafter, all vials are closed again and reevaluated after 6 months storage at 2 °C - 8 °C. Samples are stored at - 20 °C.
One batch (5377RD2) was used throughout the study. The assay was performed according to the IFU.

Results:

Condition	T0			T1			
Date	2020-10-27			2021-04-30			
Standards	pg/mL	OD	Acc. Range	pg/mL	OD	Acc. Range	**Status**
S0	0.0	2.923	> 1.170	0.0	2.514	> 1.170	**passed**
S1	0.2	2.524	> 1.010	0.2	2.080	> 1.010	**passed**
S2	1.0	1.867	> 0.745	1.0	1.600	> 0.745	**passed**
S3	4.0	0.824	> 0.330	4.0	0.630	> 0.330	**passed**
S4	20.0	0.264	> 0.105	20.0	0.244	> 0.105	**passed**
S5	100.0	0.073	> 0.030	100.0	0.082	> 0.030	**passed**
Sample	pg/mL	Acceptance range		pg/mL	Acceptance range		**Status**
Ctrl NEG	1.48	0.94 – 2.36		1.04	0.94 – 2.36		**passed**
Ctrl POS	15.73	9.30 – 18.47		13.88	9.30 – 18.47		**passed**
Sample 1	2.33	1.48 – 2.75		1.83	1.48 – 2.75		**passed**
Sample 2	10.40	7.84 – 14.56		8.60	7.84 – 14.56		**passed**
Sample 3	24.88	17.34 – 32.21		21.55	17.34 – 32.21		**passed**

Conclusion:

Opened kit stability could be demonstrated for all samples for 6 months.

6.2 Validation

6.2.1 Method Comparison

Since free testosterone is an established non-standardized assay, the trueness of measurement of DRG Free Testosterone ELISA (EIA-2924) was demonstrated by performing a method comparison test with the same predicate device reported in the metrology and traceability chain: the Free Testosterone Accubind ELISA 5325-300A by Monobind.
One batch (5396A) was used throughout the study. The assay was performed according to the IFU.
95 serum samples were tested in duplicate, and the mean of the replicates was used for the analysis.

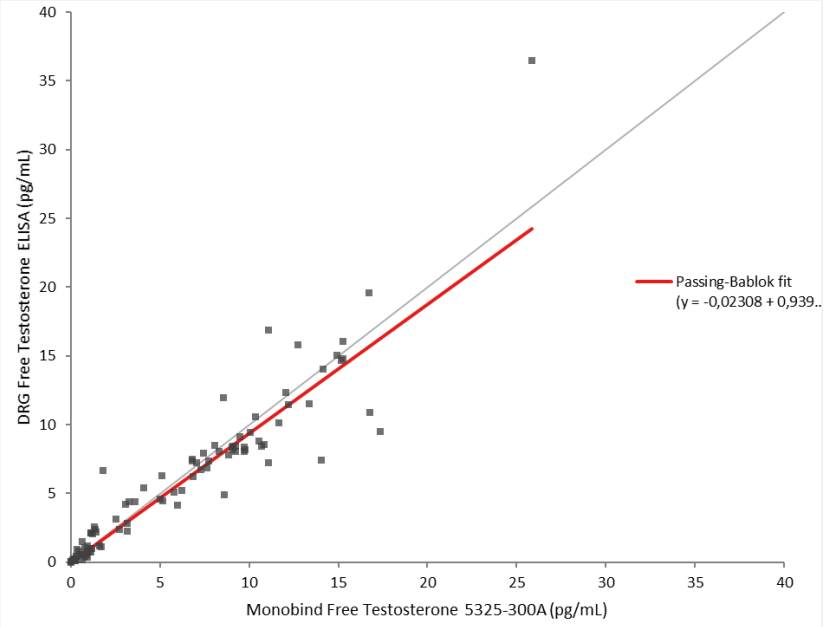
Results:

A comparison of DRG Free Testosterone ELISA EIA-2924 (y) and reference method (x) using clinical samples gave the following correlation:

n = 95
r = 0.93
y = 0.94x – 0.02

DRG performed a Passing-Bablok regression analysis, resulting in the following results.

	N	Slope	95% CI	Intercept	95% CI	Correlation Coefficient (r)
results	95	0.94	0.88 to 0.99	-0.02	-0.14 to 0.06	0.93
acceptance ranges	-	0.90 – 1.10	--	> LoQ	-	≥ 0.90
status	-	**passed**	-	**passed**	-	**passed**



Conclusion:

The trueness of measurement of the DRG Free Testosterone ELISA (EIA-2924) was demonstrated through the method comparison to Monobind Free Testosterone AccuBind ELISA 5325-300A.

6.2.2 Between-lot Precision

The values from two lots from chapter “precision” were combined to evaluate the between-lot precision.

Results:

Sample	n	Mean (pg/mL)	Within Lot CV%	Within Lot SD (pg/mL)	Total CV%	Total SD (pg/mL)
PS1	150	0.75	11.1%	0.08	18.0%	0.14
PS2	150	1.74	6.6%	0.11	14.1%	0.24
PS3	150	5.48	3.8%	0.21	9.4%	0.52
PS4	150	10.72	4.1%	0.44	8.7%	0.94
PS5	150	37.41	5.5%	2.07	12.9%	4.83
PS6	150	73.81	6.9%	5.08	14.7%	10.83

Conclusion:

Between-lot precision of all samples was below the acceptance limit of 20 %.

6.2.3 Reference Values – Healthy Population (VII 3a)

Introduction:

Reference values study was performed to establish clinical reference interval for DRG Free Testosterone ELISA. The reference interval evaluation was performed according to CLSI guideline C28-A3C and analysed by using the Analyse-it software.
It is strongly recommended, that each laboratory should determine its own reference values.

Materials and Methods:

One batch (5519RD) was used throughout the study. The assays were performed according to the IFU.

Results:

	Males 21 - 49 years	Males > 50 years	Pre-menopausal Females (21 years and older)	Post-menopausal Females
n	120	120	120	120
Mean (pg/mL)	14.50	12.63	0.67	0.87
SD (pg/mL)	5.44	4.30	0.41	0.58
Median (pg/mL)	14.13	12.75	0.55	0.75
Expected values, central 95% range of results (pg/mL)	5.01 - 27.78	4.11 - 21.85	<0.34 - 1.70	<0.34 - 2.34

Conclusion:

The reference values were established.
The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.