VIDAS® Testosterone II (TES2)

VIDAS® Testosterone II is an automated quantitative test for use on the instruments of the VIDAS® family for the quantitative determination of total testosterone in human serum or plasma, using the ELFA technique (Enzyme Linked Fluorescent Assay). It is an aid in the diagnosis and monitoring of disease states that cause excess or deficiency of this androgen.

SUMMARY AND EXPLANATION

Testosterone is a steroid hormone from the androgen group. It has a molecular weight of 288 g/mol. In plasma, testosterone circulates bound to transport proteins, predominately SHBG (Sex Hormone-Binding Globulin) and albumin which modulate its bioavailability. Free Testosterone represents 1 to 2% of the total concentration of testosterone. The biological activity of testosterone is reinforced in some target cells when the testosterone is converted to 5α-dihydrotestosterone (DHT) under the influence of the enzyme 5α-reductase. Testosterone levels in human serum (or plasma) are measured by immunoassay or mass spectrometry (1-6).

In males, testosterone is secreted by the Leydig cells of the testes under the influence of luteinizing hormone (LH) and is responsible for the development and maintenance of secondary male sex characteristics.
Its measurement is part of the diagnostic workup for infertility, erectile dysfunction and reduced libido. It is used to diagnose hypogonadism and monitor the effectiveness of substitution therapy (7-8). It can also be used to monitor hormone therapy for prostate cancer.

In females, testosterone is derived for the most part from peripheral conversion of androstenedione (Δ4), and the rest from the ovary.
Testosterone can be measured to identify hyperandrogenism in the presence of cutaneous manifestations (hirsutism and recurrent acne) associated with irregular menstruation or amenorrhea. It therefore aids in differentiating polycystic ovary syndrome, (which is a very common condition affecting 6% to 10% of the female population in Europe) (9), from rare androgen-secreting tumors and non-classical forms of 21-hydroxylase deficiency (10-14).

In children, testosterone measurement contributes to the diagnosis of precocious or delayed puberty (15).

PRINCIPLE

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).
The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.
All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR® several times.
The sample is added to the pre-treatment solution to separate the testosterone from carrier proteins.
The pre-treated sample is transferred into the well containing an alkaline phosphatase-labeled testosterone antibody (conjugate). The antigen in the sample and the testosterone antigen bound to the interior wall of the SPR® compete for the anti-testosterone-specific antibody sites.
Unbound components are eliminated during washing steps.
During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®.
The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of testosterone present in the sample.
At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT - RECONSTITUTION OF REAGENTS (30 TESTS):

30 TES2 Strips	STR	Ready-to-use.
30 TES2 SPR®s 1 x 30	SPR®	Ready-to-use. Interior of SPR®s coated with testosterone.
TES2 Control 1 x 1 mL (lyophilized)	C1	Reconstitute with 1 mL of distilled water. Wait for 5 to 10 minutes and then mix. After reconstitution, the control is stable for 4 weeks at 2-8°C or until the expiration date of the kit when stored at -25 ± 6°C. 5 freeze/thaw cycles are possible. Human* serum + testosterone + preservatives. MLE data indicate the confidence interval in ng/mL ("Control C1 Dose Value Range").
TES2 Calibrator 1 x 2 mL (lyophilized)	S1	Reconstitute with 2 mL of distilled water. Wait for 5 to 10 minutes and then mix. After reconstitution, the calibrator is stable for 4 weeks at 2-8°C or until the expiration date of the kit when stored at -25 ± 6°C. 5 freeze/thaw cycles are possible. Human* serum + testosterone + preservatives. MLE data indicate the concentration in ng/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range).
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, and antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The interior of the SPR® is coated during production with testosterone. Each SPR® is identified by the "TES2" code. Only remove the required number of SPR®s from the pouch and **carefully reseal the pouch after opening**.

The Reagent Strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the TES2 Reagent Strip:

Wells	Reagents
1	Sample well.
2	Conjugate: phosphate buffer + bovine albumin + alkaline phosphatase-labeled anti-testosterone antibody + preservative (300 µL).
3	Pre-treatment solution: phosphate buffer + bovine albumin + dissociation agent + preservative (600 µL).
4 – 5 - 6	Empty wells.
7 – 8 - 9	Wash buffer: Tris + surfactant + preservative (600 µL).
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl-phosphate (0.6 mmol/L) + diethanolamine* (DEA) (0.62 mol/L or 6.6%) pH 9.2 + 1 g/L sodium azide (300 µL).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tips to dispense 1 mL, 2 mL and 100 µL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS® family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use.**
- **For professional use only.**
- **The kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).**
- The kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use SPR®s if the pouch is pierced or if the dot sealing a SPR has come unstuck.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the kit label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS® Testosterone II kit at 2-8°C.
- **Do not freeze reagents, with the exception of calibrators and controls after reconstitution.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR® pouch is correctly sealed and undamaged. If not, do not use the SPR®s.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPR®s and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SPECIMENS

Specimen type and collection:

Human serum or plasma.

Types of tubes validated:

– Plastic tube with clot activator,
– Plastic tube with clot activator and separation gel,
– Plastic tube with lithium heparin,
– Plastic tube with EDTA.

It is recommended to validate collection tubes before use as some contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Specimen preparation

Follow the tube manufacturer's recommendations for use.

The pre-analytical step including the preparation of blood samples is an essential first step when performing medical analyses. In accordance with Good Laboratory Practices, this step is performed under the responsibility of the laboratory manager.

Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

For serum specimens, ensure that complete clot formation has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting times.

Frozen-stored samples: after thawing, these samples must be homogenized before testing. Mix using a vortex-type mixer. Clarify the samples before analysis by centrifugation, if necessary.

Specimen-related interferences

Interferences were studied according to the recommendations of CLSI® document EP7-A2.
None of the following factors have been found to significantly influence this assay:

- hemolysis (after spiking samples with hemoglobin: 0 to 310 µmol/L or 0 to 5 g/L (monomer),
- lipemia (after spiking samples with lipids: 0 to 16.88 mmol/L or 0 to 14.77 g/L equivalent in triglycerides),
- bilirubinemia (after spiking samples with bilirubin: 0 to 510 µmol/L or 0 to 0.3 g/L).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

Specimen stability

Samples (serum and plasma) can be stored in primary tubes at 18-25°C for up to 8 hours or aliquoted at 2-8°C for up to 5 days; if longer storage is required, freeze the sera or plasma at - 25 ± 6°C. These samples can be stored for 3 months at - 25 ± 6°C, with 3 freeze/thaw cycles.

INSTRUCTION FOR USE

For complete instructions, see the User's Manual.

Reading VIDAS® Protocole Test Change (PTC) protocol data and MLE data

When using the assay for the first time:

With the external instrument barcode reader,

1. Scan the PTC barcode(s) at the end of the package insert. or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data on the box label.

Note: If the MLE data have been read before the VIDAS® PTC protocol, read the MLE data again.

When opening a new lot of reagents:

Enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed before initiating the tests, the instrument will not be able to print results.
Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered, and then every 28 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested in **duplicate** (see the User's Manual). The calibration value must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate with S1.

Procedure

1. **Remove the required reagents from the refrigerator. They can be used immediately.**
2. Use one "**TES2**" strip and one "**TES2**" SPR® for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPR®s have been removed.**
3. The test is identified by the "**TES2**" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by C1.
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator and/or the control and the samples using a vortex-type mixer (for serum or plasma separated from the pellet).
6. Before pipetting, ensure that samples, calibrator and control are free of bubbles.
7. **For this test, the calibrator, control, and sample test portion is 100 µL.**
8. Insert the "**TES2**" SPR®s and "**TES2**" strips into the instrument. Check to make sure the color labels with the assay code on the SPR®s and the Reagent Strips match.
9. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
10. Reclose the vials and return them to the required temperature after pipetting.
11. The assay will be completed within approximately **40 minutes**. After the assay is completed, remove the SPR®s and strips from the instrument.
12. Dispose of the used SPR®s and strips into an appropriate recipient.

QUALITY CONTROL

A control is included in each VIDAS® Testosterone II kit.
This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any applicable local regulations.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR® is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR®. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.
The RFV is interpreted by the instrument. The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistics model). Results are expressed in ng/mL or ng/dL.

Conversion factors:

ng/mL x 100 = ng/dL
ng/mL x 3.47 = nmol/L
nmol/L x 0.288 = ng/mL

Calibration of the VIDAS® Testosterone II assay is traceable to ID-GCMS technique (Isotope Dilution - Gas Chromatography Mass Spectrometry) (16).

Samples with concentrations greater than 13.50 ng/mL can be tested after being diluted by 1/2 in the "Serum free" reagent (ref. 66 581) or in physiological saline (NaCl 0.9%). The result provided by the instrument takes into account the dilution factor.

Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components or substances that affect the reaction. For this reason, assay results should be interpreted taking into consideration the patient history, and the results of any other tests performed.

EXPECTED VALUES

The VIDAS® Testosterone II assay reference values were obtained according to the recommendations of the CLSI® document C28-A3, from a group of apparently healthy subjects (160 males and 156 females with no fertility disorders or sexual dysfunctions) not taking hormonal contraception or other drug treatments. The blood samples were collected before 1 p.m.

The following results were obtained:

Subjects	N	Median (ng/mL)	5^{th} and 95^{th} percentiles	
			5^{th}percentile (ng/mL)	95^{th}percentile (ng/mL)
Male	160	5.61	2.27	10.30
Female ≥ 19-50 years	71	0.43	0.23	0.73
Female > 50 years	85	0.32	0.14	0.68

These results are given as a guide; it is recommended that each laboratory establish its own reference values from a rigorously selected population, taking into account age and gender (17).

PERFORMANCE

Studies performed using the VIDAS® Testosterone II assay gave the following results:

Measurement range

The measurement range of the VIDAS® Testosterone II assay is: 0.05 to 13.50 ng/mL. Values below this limit are indicated as < 0.05 ng/mL. Values above this limit are indicated as > 13.50 ng/mL.

Limits of detection and quantitation

Limit of Blank (LoB)	= 0.02 ng/mL
Limit of Detection (LoD)	= 0.03 ng/mL
Limit of Quantitation (LoQ)	= 0.05 ng/mL

The study was performed according to the recommendations of CLSI® document EP17-A2.

The Limit of Blank (LoB) is the 95^{th} percentile value from more than 120 measurements of analyte-free samples. The LoB is the concentration below which analyte-free samples are found with a probability of 95%. LoB = 0.02 ng/mL

The Limit of Detection (LoD) is the lowest concentration of testosterone that can be distinguished from the analyte-free sample with a probability of 95%. LoD = 0.03 ng/mL

The Limit of Quantitation (LoQ) or functional detection limit is the lowest concentration of testosterone measured with a level of acceptable precision of 20% CV. LoQ = 0.05 ng/mL

Linearity

The VIDAS® Testosterone II assay is linear over its measuring range (0.05 to 13.50 ng/mL), evaluated according to the recommendations of CLSI® document EP6-A.

Precision

A study was performed according to the recommendations of CLSI® document EP5-A2. A panel of 5 human samples covering the measurement range was tested as follows: each sample was tested in duplicate in 2 separate runs per day, over 20 days, using 2 reagent lots (10 days per reagent lot) on 3 instruments located at the same site (N=240 values per sample). Two calibrations were used for each reagent lot (5 test days per calibration for each lot). The repeatability (within-run precision) and reproducibility (within-instrument, between-lot) were calculated for each sample using this protocol and are reported in the following table:

Sample	Concentration Mean (ng/mL)	Repeatability		Reproducibility (within-instrument, between-lot)	
		Standard deviation (ng/mL)	CV (%)	Standard deviation (ng/mL)	CV (%)
Sample 1	0.21	0.01	4.8	0.02	10.2
Sample 2	0.94	0.03	3.2	0.06	6.3
Sample 3	2.63	0.09	3.6	0.15	5.8
Sample 4	9.11	0.36	3.9	0.59	6.4
Sample 5	11.35	0.46	4.1	0.69	6.1

The VIDAS® Testosterone II assay has been developed with the focus on providing a reproducibility ≤ 12% (within-laboratory total CV) for samples with a concentration between 0.3 ng/mL and 3 ng/mL and ≤ 10% (within-laboratory total CV) for samples with a concentration greater than 3 ng/mL.

Specificity

The specificity of the VIDAS® Testosterone II assay was established by testing cross-reactive compounds according to the recommendations of CLSI® document EP7-A2. Cross-reactivity was evaluated by spiking the cross-reactive compounds with serum samples containing testosterone (approximately 0.70 ng/mL to 7.00 ng/mL).
The results of this study are reported in the following table:

Tested compound	Concentration	Cross-reactivity %
5α-androstane-3 β, 17β diol	1 mg/L	≤ 0.88%
5α-androstene 3β, 17β diol (Androstenediol)	1 mg/L	≤ 0.14%
Androstenedione (Δ4)	0.1 mg/L	≤ 2.15%
Cortisol	1 mg/L	≤ 0.03%
Cortisone	2 mg/L	≤ 0.02%
Danazol	1 mg/L	≤ 0.23%
Dehydroepiandrosterone (DHEA)	1 mg/L	≤ 0.05%
Dehydroisoandrosterone sulfate (DHAS)	50 mg/L	0%
11-Deoxycortisol	1 mg/L	≤ 0.03%
Dexamethasone	0.6 mg/L	≤ 0.02%
5α-dihydrotestosterone (5α-DHT)	0.5 mg/L	≤ 0.86%
Epitestosterone	0.029 mg/L	≤ 1.54%
17β-Estradiol (E2)	1 mg/L	≤ 0.18%
Estriol (E3)	1 mg/L	≤ 0.13%
Estrone (E1)	1 mg/L	≤ 0.04%
Ethisterone (Proluton C, Pranone)	0.01 mg/L	≤ 4.84%
Ethynylestradiol	0.1 mg/L	≤ 0.21%
11β-hydroxytestosterone	0.0015 mg/L	≤ 34.44%
17α-hydroxyprogesterone	0.5 mg/L	≤ 0.08%
11-Ketotestosterone	0.0015 mg/L	≤ 28.80%
Levonorgestrel (Norlevo)	1 mg/L	≤ 0.54%

Tested compound	Concentration	Cross-reactivity %
Nandrolone (19-Nortestosterone)	0.001 mg/L	≤ 279.00%
Prednisolone	3 mg/L	≤ 0.01%
Prednisone	0.3 mg/L	≤ 0.11%
Progesterone	1 mg/L	≤ 0.02%
Testosterone propionate	0.1 mg/L	≤ 5.82%

Interference

The VIDAS® Testosterone II assay was evaluated for interference consistent with CLSI® document EP7 A2.

None of the following factors have been found to significantly influence this assay:

Rheumatoid factors	800 IU/mL
HAMA	1.18 µg/mL

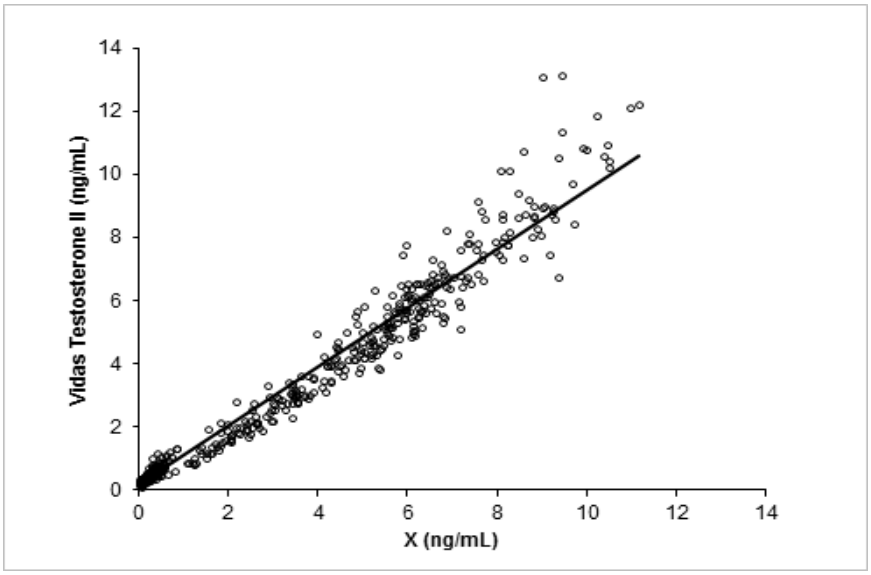
Method comparison

1) A comparison of the VIDAS® Testosterone II (Y) assay with another commercial immunological kit (X) gave the following results:

Number of samples tested: 516

Equation for Passing-Bablok regression: Y = 0.93 X + 0.15

Coefficient of correlation: 0.96



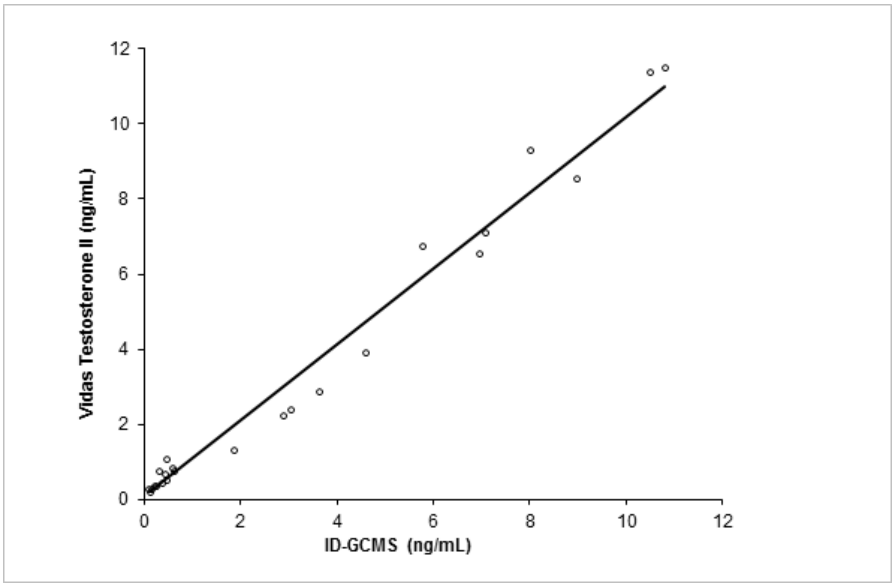
The concentrations of samples tested with the VIDAS® Testosterone II assay ranged between 0.08 ng/mL and 13.10 ng/mL.

2) Another comparison of the VIDAS® Testosterone II assay (Y) with the ID GC-MS method (X) gave the following results:

Number of samples tested: 24

Equation for Passing-Bablok regression: Y = 1.01 X + 0.10

Coefficient of correlation: 0.98



The concentrations of samples tested with the VIDAS® Testosterone II assay ranged between 0.12 ng/mL and 10.80 ng/mL.

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	9307142C	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT - RECONSTITUTION OF REAGENTS (30 TESTS): WARNINGS AND PRECAUTIONS
2015/06	9307142D	Technical	CONTENT OF THE KIT (60 TESTS) INSTRUCTION FOR USE

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