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DEVELOPMENT OF THE VIDAS® TESTOSTERONE II ASSAY

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INTRODUCTION

Testosterone is a steroid hormone which is responsible for the development and maintenance of secondary male sex characteristics ; it circulates in blood bound to transport proteins sex hormone binding globulin and albumin.

In male adult it is secreted by the Leydig cells under the influence of luteinizing hormone and is used in the diagnostic of infertility, erectile dysfunction and reduced libido.

In female it is derived for the most part from peripheral conversion of androstenedione and can be used in the diagnostic of hyperandrogenism.

In children, it can be used in the evaluation of early or delayed puberty.

The aim of the study was to evaluate the analytical and clinical performance of the new VIDAS Testosterone II test.

MATERIAL AND METHODS

The VIDAS® Testosterone II Assay design is a one step competitive immunoassay. Sample is added to a pre-treatment solution to separate the testosterone from carrier proteins. The pretreated sample is then transferred into a well containing alkaline-phosphatase (ALP) conjugated monoclonal specific antibody using a specific device coated with testosterone.

The testosterone of the sample and the one coated on the solid phase compete for binding to the conjugate ; after washing, substrate reagent is added to initiate the reaction. An inverse relationship exists between the amount of Testosterone in the sample and signal detected by the system.

Limit of blank, limit of detection and limit of quantification. The study was performed according to the recommendations of CLSI® document EP17-A2.
The Limit of Blank (LoB) is the 95th 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%. Each of the eight testosterone negative samples was assayed twice per day for 8 days with 3 reagent lots for a total of n=128 values.
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%. The LoD was determined by using 6 low-level testosterone samples (ranging from 0.06 to 0.15 ng/ml). Each sample was assayed 5 times a day in a single run, for 8 days, corresponding to 40 tests per low-level sample. Each sample was assayed with 3 reagent lots for a total of n=240 values.
The Limit of Quantitation (LoQ) or functional detection limit is the lowest concentration of testosterone measured with a level of acceptable inter-assay precision of 20% CV. Using the precision profile generated with LoD samples, the testosterone concentration associated with the desired CV<20% within-laboratory precision was determined.

Linearity was evaluated using serum pools, High Samples and Low Samples selected near the extremes of the calibration range of the VIDAS Testosterone II assay. High and Low samples were sequentially mixed to generate 10 samples of intermediate concentrations. Each sample was tested in triplicate with 3 reagent lots. To determine linearity, the polynomial analysis method was used as described in EP6-A, with a deviation from linearity less than 0.1 ng/mL for samples with dose ≤ 0.8 ng/mL and less than 12% for samples with dose > 0.8 ng/mL.

Precision of the VIDAS Testosterone II assay was determined across the dynamic range using human serum samples according to CLSI protocol EP5-A2. Two replicates of each sample were tested twice per day in separate runs, for 20 days, using 2 reagent lots on 3 different VIDAS systems. Assay precision was determined using samples with testosterone ranging from about 0.20 to 11.00 ng/mL.

Expected values were obtained from a group of apparently healthy subjects 160 males and 156 females with no fertility disorders or sexual dysfunctions) who were not using hormonal contraception or other drug treatments. Analysis was done following CLSI C28-A3 guideline.

Serum/plasma comparison. Whole blood from 48 volunteer study participants was collected into serum plastic tubes, lithium heparin plastic tubes and EDTA plastic tubes.

Method comparison was based on the CLSI EP9-A2. The VIDAS Testosterone II assay was compared to the ID GCMS reference method using 24 frozen samples. Each sample was tested with 3 VIDAS batches and in two replicates in gas spectrometry.

The VIDAS Testosterone II assay was compared to the ROCHE Elecsys Testosterone II assay and to the ABBOTT Architect Testosterone II assay which are FDA approved devices. Frozen patient samples were used. Specimen concentration ranged from about 0.05 ng/mL to 13 ng/mL.

Specificity was evaluated 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, 3.00 ng/mL and 7.00 ng/mL).

RESULTS

Limit of blank, limit of detection and limit of quantification

As per combined results on 3 reagents lots, the **LoB** of the VIDAS Testosterone II assay is **0.02 ng/mL**, the **LoD** is **0.03 ng/mL**, the **LoQ** (CV 20%) is **0.05 ng/mL**.

Linearity

The polynomial analysis method indicated that the assay results demonstrate deviation from linearity less than 0.1 ng/mL for samples with dose ≤ 0.8 ng/mL and less than 12% for samples with dose > 0.8 ng/mL across the claimed range **[0.05 – 13.50] ng/mL**.

Precision

Standard Deviation and CV% were calculated for VIDAS Testosterone II Assay repeatability (precision within-lot, within-run, within-instrument) and reproducibility (precision between-runs, between-days, between-calibration, between-lots, within-instrument). Results are in *Table 1*.

Table 1: Precision analysis

Sample ID	# of replicates	Mean Concentration (ng/mL)	Repeatability (within-run precision)		Reproducibility (between-run iwithin instrument)	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
PP1	240	0.21	0.01	4.8	0.02	10.2
PP2	240	0.94	0.03	3.2	0.06	6.3
PP3	240	2.63	0.09	3.6	0.15	5.8
PP4	240	9.11	0.36	3.9	0.59	6.4
PP5	240	11.35	0.46	4.1	0.69	6.1

Reference values

The non-parametric estimation of the reference value was calculated by sex groups (*Table 2*).

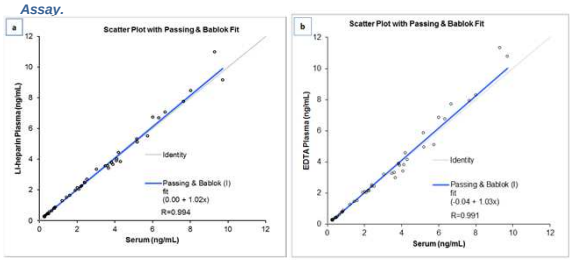
Table 2: Expected values

Subjects	N	Testosterone ng/mL Median	5th percentile	95th percentile
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

Serum/Plasma comparison

Whole blood from 48 volunteer study participant was collected into serum plastic tubes, lithium heparin and EDTA plastic tubes. Results indicate that all sample-types tested are suitable for dosage by VIDAS Testosterone II assay (*Figure 1*).

Figure 1: Serum/plasma comparison. Passing and Bablok Regression plot from plasma (a=Li-Heparin ; b=EDTA) vs. serum samples using the VIDAS Testosterone II Assay.



Method comparison study

A sample correlation study was performed with 24 samples comparing VIDAS Testosterone II to the ID-GC/MS reference method (*Figure 2*).

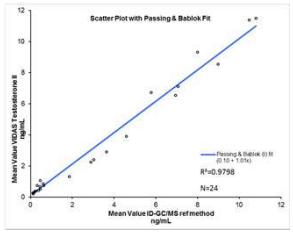
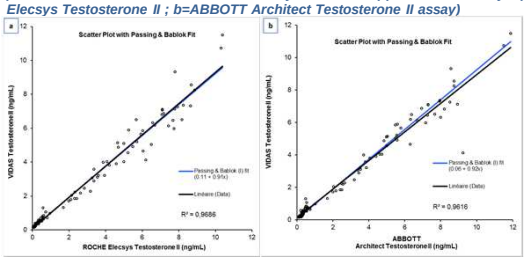


Figure 2: Passing and Bablok Regression plot from the VIDAS Testosterone II assay and ID-GCMS reference method

In another method comparison study, 104 routine specimens were assayed against FDA-approved commercially available Testosterone immunoassays. Passing-Bablock linear regression are presented in *Figure 3*).

Figure 3: Comparison with ID GCMS reference method. Passing and Bablok Regression plot from the VIDAS Testosterone II assay and FDA approved IVD assays (a=ROCHE Elecsys Testosterone II ; b=ABBOTT Architect Testosterone II assay)



Specificity study

Table 3: Cross reactivity results

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%
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%

A cross-reactivity study was performed using 3 levels of pooled human serum samples, each spiked with various testosterone metabolites or similar compounds. The highest observed cross reactivity is summarized in *Table 3*. Cross reactivity has been found significant for 3 of the 26 tested compound : Nandrolone, 11-Ketotestosterone 11β-hydroxytestosterone which are androgens very close to testosterone molecule.

CONCLUSIONS

VIDAS Testosterone II	Ref 414320
Assay duration	40 min
Sample volume	100 µL
Assay range	0.05 – 13.50 ng/mL
Calibration frequency	28 days

The VIDAS® testosterone II Assay exhibits excellent analytical data, very limited cross-reactivity and good correlation with FDA approved commercial Immunoassay. Moreover, this assay is traceable to GC-MS reference method. The assay is a valuable tool in clinical laboratories for the accurate measurement of testosterone in the different physio-pathological conditions.