ANDROSTENEDIONE Elisa

BL-32-E

IN VITRO DIAGNOSTIC USE

CE

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1 INTRODUCTION

The steroid hormone Androstenedione is one of the main androgens, besides Testosterone and Dehydroepiandrosterone. Testosterone, the most important biological active androgen, is derived from peripheral enzymatic conversion of Androstenedione.
In males, androgens are secreted primarily by the Leydig cells of the testes, to some degree also in the adrenal cortex. In females, the androgens are secreted mainly in the adrenal glands and in the ovary. Ca. 10% of the androgens are derived from peripheral conversion, mainly of DHEA. Androstenedione and Testosterone show high diurnal variability. The highest levels are measured in the morning. At the age of puberty serum androstenedione levels rise, after menopause they decline again. High androstenedione levels are measured during pregnancy.
In women, high levels of androstenedione (47-100% above normal) are generally found in hirsutism, mostly in combination with other androgens as testosterone and DHEA-S. Androstenedione overproduction is due to ovarian dysfunction or maybe of adrenal origin. High circulating androstenedione levels are found in women with polycystic ovaries and 21-hydroxylase effect. Significant lower androstenedione levels are found in postmenopausal osteoporosis.

2 PRINCIPLE OF THE TEST

The **Bio-Line Androstenedione ELISA KIT** procedure is an enzyme immunoassay. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on an Androstenedione molecule. An aliquot of patient sample containing endogenous Androstenedione is incubated in the coated well with enzyme conjugate, which is an anti-Androstenedione antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off with aqua dest. The amount of bound peroxidase is proportional to the concentration of Androstenedione in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Androstenedione in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1.		12x8 (break apart) strips, 96 wells Wells coated with anti-Androstenedione antibody
2.	CAL | N	N=1 to 5 5 vials, 1 ml, ready to use See exact values on the vial labels
3.	CAL | 0	1 vial, 1 ml, ready to use 0 ng/ml
4.	Ab | HRP	1 vial, 25 ml, ready to use Anti-Androstenedione antiserum conjugated to horseradish peroxidase
5.	CHROM | TMB	1 vial, 25 ml, ready to use TMB
6.	STOP | SOLN	1 vial, 14 ml, ready to use contains 0.5M H_2SO_4 Avoid contact with the stop solution. It may cause skin irritations and burns.
7.	WASH | SOLN | CONC	1 vial, 30 ml (40X concentrated) see „Preparation of Reagents“

Note: Additional Zero Calibrator for Sample dilution available on request.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Distilled water.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators and Zero Calibrator must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again.

4.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution
Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml. The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line Europe have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

5.1 Specimen collection

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum.

5.2 Specimen storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen dilution

If in an initial assay, a serum specimen is found to contain more than the highest calibrator, the specimens can be diluted 10-fold with Zero Calibrator and reassayed as described in Assay Procedure.

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipet tips for each calibrator, control of sample in order to avoid crosscontamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

6.2 Procedural Notes

- All calibrators, samples, and external controls should be run in duplicate concurrently so that all conditions of testing are the same.
- The concentration of the samples can be read directly from this calibrator curve. Samples with a concentration higher than that of the highest calibrator have to be diluted 1 : 10 with Zero Calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **20 µl** Androstenedione Calibrators, Controls and samples **with new disposable tips** into appropriate wells. Time between distribution of first Calibrator and last sample can be up to 10 minutes without affecting the results.
3. Dispense **200 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **1 hour** at room temperature.
6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **200 µl** of Substrate Solution to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in IU/ml with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration of Androstenedione in ng/ml from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with concentration higher than that of the highest calibrator have to be diluted 1 : 10 with zero calibrator. The dilution factor has to be taken into account.

7 ASSAY CHARACTERISTICS

7.1 Expected values (preliminary)

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

The Performance characteristics are expressed in ng/ml.

To convert to nmol/L: **ng/ml x 3.492 = nmol/L**

POPULATION	n	Absolute Range (2 S.D.) (ng/ml)
FEMALES	27	0.40 - 3.40
MALES	15	0.35 - 3.15

7.2 Specificity

The specificity of the Bio-Line Androstenedione Kit was assessed according to Abraham's method:

Compound	Crossreactivity %
Androstenedione	100
Androsterone	0.00
Cortisol	0.00
Dihydrotestosterone	0.00
Dihydroepiandrosterone	0.01
Epiandrosterone	0.00
Estriol	0.00
16-Epiestriol	0.00
Estradiol	0.00
Estriol-3-glucuronide	0.00
Estriol-16-glucuronide	0.00
Estriol-16-sulfate	0.00
Estrone	0.00
17a-Pregnenolone	0.00
17a-Progesterone	0.00
Progesterone	0.00
Testosterone	0.01

7.3 Sensitivity

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean minus two standard deviations of 20 replicates of the 0 ng/ml androstenedione calibrator is **0.043 ng/ml.**

7.4 Accuracy

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor.

7.5 Precision

Intra and Inter Assay Variation

The within assay variability is shown below:

The **intra-assay** precision was determined from the mean of 6 replicates each.

SAMPLE	n	MEAN (ng/ml)	STANDARD DEVIATION	COEFFICENT OF VARIATION (%)
I	6	0.11	0.01	6.3
II	6	2.46	0.013	5.14
III	6	7.73	0.23	2.99

The **inter-assay** precision was determined from the mean of average duplicates for 3 separate runs.

SAMPLE	n	MEAN (ng/ml)	STANDARD DEVIATION	COEFFICIENT OF VARIATION (%)
I	18	0.13	0.01	8.09
II	18	2.38	0.14	5.95
III	18	7.52	0.49	6.52

7.6 Recovery

Three serum samples containing different levels of endogenous Androstenedione were spiked with known quantities of Androstenedione and assayed.

SAMPLE	ENDOGENEOUS (ng/ml)	ADDED (ng/ml)	EXPECTED (ng/ml)	OBSERVED (ng/ml)	RECOVERY (%)
I	0.078	-			
		5.0	5.078	5.159	101.6
		1.5	1.578	1.576	99.9
		0.5	0.578	0.516	89.3
II	0.98	-			
		5.0	5.98	6.025	100.8
		1.5	2.48	2.463	99.3
		0.5	1.48	1.621	109.5
III	2.37	-			
		5.0	7.37	7.262	98.5
		1.5	3.87	3.84	99.2
		0.5	2.87	2.70	94.1

7.7 Linearity

Three serum samples were diluted with 0 ng/ml Androstenedione calibrator and assayed.

SAMPLE	DILUTION FACTOR	EXPECTED (ng/ml)	OBSERVED (ng/ml)	RECOVERY (%)
I	---	5.1	5.100	
	1: 2	2.55	2.60	102.0
	1: 4	1.28	1.30	102.0
	1: 8	0.64	0.63	98.8
II	---	7.96	7.960	
	1: 2	3.98	3.84	96.6
	1: 4	1.99	2.07	104.0
	1: 8	1.00	0.96	96.2
II	---	9.58	9.58	
	1: 2	4.79	4.81	100.3
	1: 4	2.4	2.37	99.1
	1: 8	1.20	1.20	100.4

8 LIMITATIONS OF USE

8.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters ‘Specimen - Collection’.

8.2 High-Dose-Hook Effect

No hook effect was observed in this test.

9 LEGAL ASPECTS

9.1 Reliability of Results

The test must be performed exactly as per the manufacturer’s instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 9.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.