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  See external label	 2°C-8°C	 Σ=96 tests	 Cat # 1038-17
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Androstenedione ELISA

Cat # 1038-17

Test	Δ4-Androstenedione ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-10 ng/ml
Sample	50ul serum
Specificity	100%
Sensitivity	.04ng/ml
Total Time	~ 80 min
Shelf Life	12-14 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Androstenedione concentration in serum and plasma. **Androstenedione kit is intended for laboratory use only.**

CLINICAL SIGNIFICANCE

Androstenedione (also known as 4-androstenedione) is a 19-carbon steroid hormone produced in the adrenal glands and the gonads as an intermediate step in the biochemical pathway that produces the androgen testosterone and the estrogens estrone and Estradiol. It is the common precursor of male and female sex hormones. Some androstenedione is also secreted into the plasma, and may be converted in peripheral tissues to testosterone and estrogens.

Androstenedione has relatively weak androgenic activity, estimated at ~ 20% of testosterone. However, serum androstenedione levels often exceed testosterone in both normal and disease states. Secretion and production rates also exceed those of testosterone in women in whom significant extra-adrenal conversion of androstenedione to testosterone occurs.

In premenopausal women the adrenal glands and ovaries each produce about half of the total androstenedione (about 3 mg/day). After menopause androstenedione production is about halved, primarily due to the reduction of steroid secreted by the ovary. Nevertheless, androstenedione is the principal steroid produced by the postmenopausal ovary.

Measurement of serum androstenedione provides a useful marker of androgen biosynthesis. Elevated androstenedione levels have been demonstrated in virilizing congenital adrenal hyperplasia. Serum androstenedione levels are also increased in polycystic ovary syndrome, and in case of hirsutism in women. Elevated serum androstenedione levels may also occur in adrenal and ovarian virilizing tumors.

PRINCIPLE

Androstenedione (antigen) in the sample competes with horseradish peroxidase Androstenedione (enzyme-labelled antigen) for binding onto the limited number of anti-Androstenedione coated on the microplates (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.

The enzyme in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.

The colour intensity is inversely proportional to the Androstenedione concentration in the sample.

The enzyme substrate (H_2O_2) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbance are determined.

Androstenedione concentration in the sample is calculated based on a series by a set of standard.

The colour intensity is inversely proportional to the Androstenedione concentration in the sample.

REAGENT, MATERIAL AND INSTRUMENTATION

1. Reagent and material supplied in the kit

1. Androstenedione Standards 6x (1 vial = 1 mL)

STD0	REF	DAS0/1038-17
STD1	REF	DAS1/1038-17
STD2	REF	DAS2/1038-17
STD3	REF	DAS3/1038-17
STD4	REF	DAS4/1038-17
STD5	REF	DAS5/1038-17

2. Control (1 vial = 1ml) **REF DA-Con/1038-17**

3. Conjugate (1 bottle) 21 mL
Androstenedione-HRP conjugate **REF DA-C/1038-17**

4. Coated Microplate :Anti-Androstenedione IgG adsorbed on microplate
(1 microplate breakable) **REF DA-P/1038-17**

5. TMB-substrate (1 bottle) 15 mL H₂O₂.TMB 0.25gr/L
(avoid any skin contact) **REF DA-T/1038-17**

6. Stop solution (1 bottle) 15 mL Sulphuric acid 0.15 mol/L
(avoid any skin contact) **REF DA-S/1038-17**

7. **10 X Conc. Wash Solution (1 vial, 50 ml)**
NaCl 160g/L ; tween-2010 g/L 0.2M Phosphate Buffer, pH 7.4 **REF DA WC/1038-17**

Reagents necessary not supplied
Distilled water.

3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader (450 nm)

Note

Store all reagents between 2- 8C° in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use.

PRECAUTION

- The reagent contain Proclin 300 as preservative
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants.
- Maximum precision is required for dispensation of reagents.
- Do not use different lots of reagents.
- Do not use heavily haemolized samples.
- This method allows the determination of Androstenedione from 0.1 ng/mL to 10.0 ng/mL.
- The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PROCEDURE

1. Preparation of the Standard (S₀,S₁,S₂,S₃,S₄,S₅) and Control
The standard has the following concentration of Androstenedione:

	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅
ng/ml	0	0.1	0.4	1.2	4.0	10.0

Stability: until the expiration date printed on the kit at 2-8 °C.
Once open, the standards are stable six months at 2-8 °C.

2. Preparation of the Sample

The determination of Androstenedione can be performed in plasma as well as in serum .
Store reagent at -20°C if the determination is not performed on the same day of the sample connection.

Dilute the samples higher than 10 ng/mL (1/2) with the “**Sample diluent**”.

3. PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the six points of the standard curve (S₀-S₅), two for each sample, one for Blank.

Reagent	Standard	Sample	Blank
Sample		25 µL	
Standard S0-S5	25 µL		
Control	25 µL		
Conjugate	100 µL	100 µL	
Incubate at +37°C for 1 hour Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted wash solution. Repeat the washing procedure by draining the water completely			
Substrate	100 µL	100 µL	100 µL
Incubate at room temperature 22÷28°C for 15 minutes in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against Blank			

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Androstenedione for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

LIMITATION OF PROCEDURE

1. Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten

minutes to avoid assay drift. If it lasts more than 10 minutes, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

2. Interpretation

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

RESULTS

1. Mean Absorbance

Calculate the mean of the absorbance (E_m) for each point of the standard curve (S0-S5) and of each sample.

2. Standard Curve

Plot the mean value of absorbance (E_m) of the standards (S0-S5) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

3. Calculation of results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

4. Range of Control:

Please refer to the range information from the COA for the given Lot number. The obtained value for the control should fall within the specified range.

REFERENCE VALUE

The serum or plasma Androstenedione reference values are:

WOMAN	Follicular phase	0.75 - 3.1 ng/mL
	Luteinic phase	0.94 - 3.2 ng/mL
MAN		0.60 - 2.7 ng/mL

PERFORMANCE CHARACTERISTICS

1. Precision

1.1.1. Intra Assay Variation

Within run variation was determined by replicate (20x) the measurements of three different control sera in one assay. The within assay variability is $\leq 10.0\%$.

1.1.2. Inter Assay Variation

Between run variation was determined by replicate (10x) the measurements of three different control sera in different lots. The between assay variability is $\leq 9.5\%$.

2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Androstenedione	100 %
5 α -dihydrotestosterone	0,05 %
DHEA	0,05 %
Epitestosterone	0,04 %
DHEA-S	0,027 %
Cortisol	0,008 %
Progesterone	0,007 %
Estrone	0,007 %

3. Accuracy

The recovery of 0.4 - 0.8 - 1.6 - 3.2 ng/mL of Androstenedione added to sample gave an average value (\pm SD) of 100.91% \pm 5.61% with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 - 8 - 16 times gave an average value (\pm SD) of 107.18% \pm 3.03%.

4. Sensitivity

The lowest detectable concentration of Androstenedione that can be distinguished from the zero standard is 0.01 ng/mL at the 95 % confidence limit.

5. Correlation with RIA

The DAI Androstenedione ELISA was compared to another commercially available Androstenedione assay. Serum samples of 16 females and 21 males were analysed according in both test systems.

The linear regression curve was calculated

$$y = 0.928 x + 0.02$$

$$r = 0.946 \text{ (} r^2 = 0.895 \text{)}$$

Androstenedione kit (Y) was compared to the previous Androstenedione Diametra assay (X).

78 serum samples were analysed.

The linear regression curve was calculated:

$$(Y) = 0.79(X) + 0.53$$

$$r^2 = 0.825$$

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY

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Date Adopted	Reference No.
2011-06-30	DA-Androstenedione-2011



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