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Instructions for use

Androstendione Saliva ELISA Free

REF SA E-6700

96

+2 +8 °C

IVD

CE

INTENDED USE

Competitive immunoenzymatic colorimetric method for the quantitative determination of **Androstenedione** concentration in **saliva**.

Clinical Significance

Androstenedione (also known as Δ4-androstenedione) is a 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. 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 produces about half of the total androstendione (about 3 mg/day). After menopause the production of androstenedione decreases by 50%. This is mainly due to the reduction of the steroid secreted by the ovary.Nevertheless, androstenedione is the principal steroid produced by the postmenopausal ovary.
The high serum-saliva correlation for androstenedione suggests that individual differences in serum androstenedione levels may be accurately estimated using saliva as a non-invasive alternative specimen.

PRINCIPLE

Androstenedione (antigen) in the sample competes with horseradish peroxidase androstenedione (enzyme-labelled antigen) for binding onto the limited number of anti- androstenedione (antibody) sites on the microplates (solid phase).
After incubation, the bound/free separation is performed by a simple solid-phase washing.
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 absorbances are determined.
Androstenedione concentration in the sample is calculated based on a series of standard.

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

Reagent, material and instrumentation

Reagent and material supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**SA E-6701**	Standard 0	0 pg/mL	1 ml
STANDARD B	**SA E-6702**	Standard 1	20 pg/mL	1 ml
STANDARD C	**SA E-6703**	Standard 2	100 pg/mL	1 ml
STANDARD D	**SA E-6704**	Standard 3	400 pg/mL	1 ml
STANDARD E	**SA E-6705**	Standard 4	1000 pg/mL	1 ml

INC-BUFF **SA E-6713 Incubation Buffer**

(1 bottle) 30 mL; Phosphate buffer pH 7.5 BSA 1 g/L, stabilizer

CONJUGATE-CONC **SA E-6740 Conjugate**

(1 bottle) 0.4 mL; Androstenedione-HRP conjugate

96 **SA E-6731 Coated Microplate**

(1 microplate breakable); Anti-Androstenedione IgG adsorbed on microplate

SUBSTRATE **MS E-0055 TMB-Substrate**

(1 bottle) 12 mL; H_2O_2-TMB 0.26 g/L (avoid any skin contact)

STOP-SOLN **MS E-0080 Stop Solution**

(1 bottle) 12 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

Reagents necessary not supplied

Distilled water

Auxiliary materials and instrumentation

Automatic dispenser
Microplate reader
Saliva Collection Device

Note

Store all reagents at 2 °C - 8 °C in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use.
The microplate, once opened, it stable until the expiry date of kit. Do not remove the adhesive sheets on the unused strips

PRECAUTION

- The reagents contain Proclin 300 as preservative.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lots of reagents.
- This method allows the determination of Androstenedione from 5 pg/mL to 1000 pg/mL.
- Samples with expected concentrations > 1000 pg/mL should be further diluted (1+1) with S0.
- The clinical significance of Androstenedione determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PROCEDURE

Preparation of the Standard

(S0, S1, S2, S3, S4)

Before use, mix for 5 min. with rotating mixer
The standard has the following concentration of Androstenedione:

	S0	S1	S2	S3	S4
pg/mL	0	20	100	400	1000

When opened it is stable at +4 °C until the expiration date of the kit.
For SI UNITS: pg/mL x 3.487 = pmol/L

Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µL of Conjugate (reagent 3) to 1.0 mL of Incubation Buffer (reagent 2). Mix gently.
Stable for 3 hours at 22 °C - 28 °C.

Preparation of the Sample

The determination of Androstenedione can be performed in saliva.
It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw or SALI SET 100 REF SA D-6100 available from LDN
Do not use sample collector commercially available as "SALIVETTE". Other sample collectors commercially available have not been tested.

Method and Limitations

Collect saliva samples at the times indicated.
If no specific instructions have been given oral fluid (saliva) samples may be collected at any time for saliva collection, the following should be noted:

a. If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth
b. During the day allow 1 hour after any food or drink before collecting saliva samples
c. It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

Saliva Processing Instructions

Let the saliva flow down through the straw into the centrifuge glass tube

1. Centrifuge the sample for 15 minutes at 3000 rpm
2. Store at – 20 °C for at least 1 hour
3. Defrost samples
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2 °C - 8 °C for one week or at – 20 °C for longer time.

Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0-S_4), two for each sample, one for Blank.

Reagent	Standard	Samples	Blank
Standard S0-S4	**50 µL**		
Samples		**50 µL**	
Diluted Conjugate	**150 µL**	**150 µL**	
Incubate at *+37°C* for *1 hour* Remove the contents from each well. Wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely			
TMB substrate	**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**
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

Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipaemic 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 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.

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

Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

Standard Curve

Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (e.g.: Four Parameter Logistic).

Calculation of Results

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

Reference Value

As the values of salivary Androstenedione have a circadian pattern we suggest collecting the samples at the same hour (8 A.M.):
The following values can be used as preliminary guideline until each laboratory established its own normal range.

		pg/mL
WOMEN	Normal	20 – 160
	P.C.O.- Hirsute	120 – 300
MEN		20 - 150

Performance and Characteristics

Precision

Intra Assay Variation

Within run variation was determined by replicate measurements (16x) of two different saliva control in one assay. The within assay variability is ≤ 8.5%.

Inter Assay Variation

Between run variation was determined by replicate measurements (10x) of two different saliva control with different lots of kit. The between assay variability is ≤ 11%.

Accuracy

The recovery of 50 – 200 – 500 pg/mL of Androstenedione added to sample gave an average value (±SD) of 102.60 % ± 13.23 % with reference to the original concentrations.

Sensitivity

The lowest detectable concentration of Androstenedione that can be distinguished from the zero standard is 5 pg/mL at the 95 % confidence limit.

Specificity

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

Androstenedione Saliva	100 %
Testosterone	1.2 %
Epitestosterone	0.2 %
5α-dihydrotestosterone	0.1 %
DHEA	0.1 %
Progesterone	$1x10^{-3}$ %
Estrone	$1x10^{-3}$ %
Cortisol	$1x10^{-3}$ %

Correlation

The Androstenedione saliva ELISA kit was compared to another commercially available Androstenedione saliva assay. 38 saliva samples were analysed according in both test systems. The linear regression curve was calculated:

$y = 0.46x + 5.51$

$r^2 = 0.983$

y = Androstenedione saliva Elisa kit
x = Salivary Androstenedione Salimetrics Elisa kit

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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TROUBLESHOOTING

POSSIBLE ERROR CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

Too high within run (CV%)
- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

Too high between-run (CV%)
- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!