Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World Since 1973

ANDROSTENEDIONE ELISA

EU: CE IVD USA/CAN: IVD

REF: CAN-AD-208

Version 8.1 (COMB)
Effective: October 17, 2023

INTENDED USE
For the direct quantitative determination of Androstenedione in human serum by an enzyme immunoassay.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of androstenedione in the sample. A set of standards is used to plot a standard curve from which the amount of androstenedione in patient samples and controls can be directly read.

CLINICAL APPLICATIONS
Androstenedione is produced by the adrenals and gonads. As a result, the determination of the level of androstenedione in serum is important in the evaluation of the functional state of these glands. Androstenedione is a precursor of testosterone and estrone. Besides the adrenals, in females, the ovaries have been shown to be an important source of androstenedione. It has been reported that there is a fluctuation day by day of androstenedione during the ovulatory cycle.

The principle production of testosterone in females is from the conversion of other related androgens, especially androstenedione. An abnormal testosterone level in women should be accompanied by the estimation of serum androstenedione. The use of serum testosterone determination in conjunction with the enzyme immunoassay of androstenedione can be used to determine if the source of the excess androgen production is adrenal or ovarian.

PROCEDURAL CAUTIONS AND WARNINGS
1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS
1. All the reagents within the kit are calibrated for the direct determination of androstenedione in human serum. The kit is not calibrated for the determination of androstenedione in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immuno-deficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system. No specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED
1. Rabbit Anti-Androstenedione Antibody-Coated Break-Apart Well Microplate — Ready To Use

Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C.
Stability: 12 months or as indicated on label.

2. Androstenedione-Horseradish Peroxidase (HRP) Conjugate — Ready to Use

Contents: One bottle containing Androstenedione-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 14 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

3. Androstenedione Calibrators — Ready to Use

Contents: Six vials containing androstenedione in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a precise quantity of androstenedione.

* Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.1 ng/mL	0.5 mL
Calibrator C	0.3 ng/mL	0.5 mL
Calibrator D	1 ng/mL	0.5 mL
Calibrator E	3 ng/mL	0.5 mL
Calibrator F	10 ng/mL	0.5 mL

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready To Use

Contents: Two vials containing androstenedione in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of androstenedione. Refer to vial labels for expected value and acceptable range.
Volume: 0.5 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation X10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute wash buffer concentrate 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of wash buffer concentrate in 450 mL of water.

6. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of the Androstenedione-HRP conjugate into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 15–20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 10 ng/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

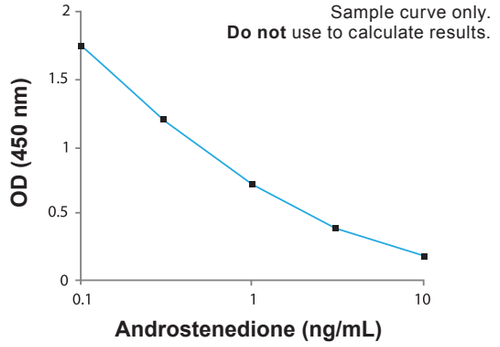
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD	Value (ng/mL)
A	2.443	0
B	1.746	0.1
C	1.195	0.3
D	0.721	1
E	0.385	3
F	0.184	10
Unknown	0.482	2.1

TYPICAL CALIBRATOR CURVE

Sample curve only.
Do not use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows:
LoD = µB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and µB is the mean value of the blank.
The Limit of Detection (LoD) was determined to be **0.04 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with androstenedione cross-reacting at 100%.

Steroid	% Cross Reactivity
Androstenedione	100
DHEA	1.8
Testosterone	0.2
Estrone	< 0.1
Estradiol	< 0.1
Progesterone	< 0.1
17-OH-Progesterone	< 0.1
5α-DHT	< 0.1
Cortisol	< 0.01
DHEA-S	< 0.01

INTRA-ASSAY PRECISION

Four samples were assayed 24 times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.083	0.006	7.1
2	0.832	0.051	6.2
3	3.28	0.193	5.9
4	9.36	0.927	9.9

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.528	0.046	8.7
2	1.534	0.149	9.7
3	5.905	0.457	7.7

RECOVERY

Spiked samples were prepared by adding defined amounts of androstenedione to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1.Unspiked	0.882	-	-
+ 0.75	1.589	1.632	97
+ 1.5	2.521	2.382	106
+ 3.0	4.522	3.882	116
2.Unspiked	1.527	-	-
+ 0.75	2.466	2.277	108
+ 1.5	3.666	3.627	101
+ 3.0	5.756	6.027	96
3.Unspiked	0.585	-	-
+ 0.75	1.268	1.335	95
+ 1.5	1.878	2.085	90
+ 3.0	3.471	3.585	97

LINEARITY

Three patient serum samples were serially diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	2.317	-	-
1:2	1.220	1.158	105
1:4	0.615	0.579	106
1:8	0.329	0.290	113
2	6.594	-	-
1:2	3.212	3.297	97
1:4	1.594	1.648	97
1:8	0.818	0.824	99
3	7.456	-	-
1:2	3.588	3.728	96
1:4	1.835	1.864	98
1:8	0.963	0.932	103

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (ng/mL)	Range (ng/mL)
Males	20	2.0	0.4–3.5
Females	20	1.4	0.3–2.4

REFERENCES

1. Perel E, et al. The Conversion of Androstenedione to Estrone, Estradiol, and Testosterone in Breast Tissue. *J Steroid Biochem.* 1980; 13:89.
2. Bermúdez JA, et al. Stereochemical Approach to Increase the Specificity of Steroid Antibodies. *J Steroid Biochem.* 1975; 6:283.
3. Hampl R, et al. The Use of Iodinated Steroid as Radioligand for Testosterone Radio-immunoassay. *J Steroid Biochem.* 1978; 9:771.
4. Hummer L, et al. An Easy and Reliable Radioimmunoassay of Serum Androstenedione: Age-Related Normal Values in 252 Females Aged 2 to 70 Years. *Scand J Clin Lab Invest.* 1983; 43:301.
5. Hennam JF, et al. A Cost Reductive Method for the Radioimmunoassay of Plasma Androstenedione. A Comparison of Antisera and the Effects of Chromatography. *Acta Endocrinol.* 1974; 76:597.
6. Judd HL, et al. Serum Androstenedione and Testosterone Levels During the Menstrual Cycle. *J Clin Endo Metabo.* 1973; 36:475.
7. Lejeune-Lenair C, et al. A Direct Radio-Immunoassay for Plasma Delta 4-Androstenedione. Application to Children. *Clin Chem Acta.* 1979; 94:327.
8. Parker LN, et al. An Improved Radioimmunoassay for 4-Androstene-3, 17-Dione. *Steroids.* 1977; 29:715.
9. Pizarro MA, et al. Human Testicular Metabolism of Testosterone and Androstenedione in Normal and Infertile Men. *J Steroid Biochem.* 1980; 12:509.
10. Rao PN, et al. Specific Antisera Suitable for Solid-Phase Radioimmunoassay of 11Beta-Hydroxyandrost-4-Ene-3,17-Dione. *Steroids.* 1974; 24:793.
11. Putz Z, et al. A Selective Radioimmunoassay of Androstenedione in Plasma and Saliva. *J Clin Chem Clin Biochem.* 1982; 20:761.
12. Schanbacher BD, et al. Simultaneous Determination of Testosterone, 5Alpha-Androstan-17Beta-Ol-3-One, 5Alpha-Androstane-3Alpha, 17Beta-Diol and 5Alpha-Androstane-3Beta, 17Beta-Diol in Plasma of Adult Male Rabbits by Radioimmunoassay(1). *Endocrinology.* 1975; 97:787.
13. Slaats EH, et al. Interference of Sex-Hormone Binding Globulin in the "Coat-A-Count" Testosterone No-Extraction Radioimmunoassay. *Clin Chem.* 1987; 33:300.
14. Swinkles LM, et al. Salivary and Plasma Free Testosterone and Androstenedione Levels in Women Using Oral Contraceptives Containing Desogestrel or Levonorgestrel. *Ann Clin Biochem.* 1988; 23: 354.
15. Thorneycroft IH, et al. A Radioimmunoassay of Androstenedione. *Steroids.* 1973; 21:111.
16. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. *Gynecol Obstet Invest.* 1995; 40(2):139–40.

DBC-Diagnostics Biochem Canada Inc.
384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: (519) 681-8731
Fax: (519) 681-8734
e-mail: dbc@dbc-labs.com
www.dbc-labs.com
An ISO 13485 Registered Company

MedEnvoy Global B.V.
Prinses Margrietplantsoen 33, Suite 123
2595AM The Hague
The Netherlands

SYMBOLS

CE European Comformity
IVD In vitro diagnostic device
Consult instructions for use
Contains sufficient for <n> tests
Storage Temperature
Legal Manufacturer
Use by
REF Catalogue Number
EC REP Authorized representative
LOT Lot number
X # Dilute 1: # Before use