

Read entire protocol before use.

ANDROSTENEDIONE-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human 4-Androsten-3, 17-Dione (Androstenedione) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **ANDROSTENEDIONE-RIA-CT** Kit

B. Catalogue number : **BL-32-CT**: 100 tests

C. Manufactured by : Bio-Line S.A.

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III. CLINICAL BACKGROUND

A. Biological activity

4-Androsten-3, 17-dione (Androstenedione) is a C19 steroid. It is produced in the adrenal gland and gonads. Androstenedione is an immediate precursor to both testosterone and estrone, both of which may be subsequently converted to estradiol. Due to the presence of a 17-oxo (rather than hydroxyl) group, Androstenedione has relatively weak androgenic activity, estimated at $\leq 20\%$ of testosterone. Although it is a weak androgen, serum Androstenedione levels may exceed testosterone in both normal and disease states, Androstenedione secretion and production rates exceed those of testosterone in women, and significant extra-adrenal conversion of Androstenedione to testosterone occurs. Furthermore, the affinity of sex hormone-binding globulin for Androstenedione is much less than for testosterone or estradiol (1 – 3).

The physiologic role of Androstenedione is not well defined. Serum Androstenedione levels are high in fetal and neonatal serum, decrease during childhood, and increase during puberty. In normal pubertal and adult men, the major portion of Androstenedione is derived from the testis, either directly or from conversion of testosterone, while in normal adult women, essentially equivalent amounts of Androstenedione are produced by the adrenal gland and ovary (2,3). Increased Androstenedione levels may play a role in the development of secondary sexual hair during adrenarche. Serum Androstenedione levels show significant diurnal variation dependent on the secretion of ACTH. Ovarian Androstenedione production is stimulated by luteinizing hormone, and serum Androstenedione levels vary with the menstrual cycle (3). Adrenal Androstenedione production gradually declines with advanced age in both men and women. In addition, ovarian Androstenedione production decreases after menopause (3).


B. Clinical applications

Measurement of Androstenedione provides a useful marker of androgen biosynthesis : Elevated Androstenedione levels have been demonstrated in virilizing congenital adrenal hyperplasia; additionally Androstenedione levels may have advantages over 17-hydroxy-progesterone levels in monitoring treatment of this condition, e.g. less marked diurnal variation and less suppression after brief glucocorticoid exposure (4). Serum Androstenedione levels are also increased in polycystic ovary syndrome, ovarian stromal hyperthecosis, 3 β -hydroxysteroid dehydrogenase deficiency, and other causes of hirsutism in women (3,6 & 7). By definition, Androstenedione levels are normal in idiopathic hirsutism. Elevated serum Androstenedione levels may also occur in adrenal and ovarian virilizing tumors (3). In a prospective study of over 1000 men, a dose-response relationship of androstenedione and prostate cancer risk was demonstrated (5); additional studies will be needed to confirm these findings.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I labelled Androstenedione competes with the Androstenedione to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. After 1 hour incubation at room temperature on a shaker, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of Working Wash Solution and aspirated. A calibration curve is plotted and the Androstenedione concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	100 Test Kit	Colour Code	Reconstitution		
 Tubes coated with anti Androstenedione	2 x 50	yellow	Ready for use		
<table border="1" data-bbox="119 593 255 638"> <tr> <td>Ag</td> <td>^{125}I</td> </tr> </table> TRACER: ^{125}I iodine labelled Androstenedione (HPLC grade) in buffer with bovine casein and azide (<0.1%)	Ag	^{125}I	1 vial 26 ml 111 kBq	red	Ready for use
Ag	^{125}I				
<table border="1" data-bbox="119 772 255 817"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero calibrator in human serum, thymol and gentamycin	CAL	0	1 vial lyophilized	yellow	Add 0.5 ml distilled water
CAL	0				
<table border="1" data-bbox="119 873 255 918"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators Androstenedione - N = 1 to 5 (see exact values on vial labels) in human serum, thymol and gentamycin	CAL	N	5 vials lyophilized	yellow	Add 0.5 ml distilled water
CAL	N				
WASH SOLN CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).		
<table border="1" data-bbox="82 1220 284 1265"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum, thymol and gentamycin	CONTROL	N	2 vials lyophilized	silver	Add 0.5 ml distilled water
CONTROL	N				

Note : Use the zero calibrator for sample dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 25 μl , 250 μl , 500 μl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tubes shaker (700 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Calibrators** : Reconstitute the calibrators with 0.5 ml distilled water
- Controls** : Reconstitute the controls with 0.5 ml distilled water.
- Working Wash solution** : Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for one week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid successive freezing and thawing.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, storage in aliquots at -20°C is recommended.
- Avoid successive freezing and thawing.
- Serum and plasma provides similar results:

$$Y (\text{Hep. plasma}) = 0.94 \times (\text{serum}) + 0.02 \quad r = 0.98 \quad n = 17$$

$$Y (\text{EDTA plasma}) = 1.01 \times (\text{serum}) - 0.03 \quad r = 0.95 \quad n = 17$$

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls and samples and dispense 25 μl of each into respective tubes.
3. Dispense 250 μl of ^{125}I iodine labelled Androstenedione into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 1 hour at room temperature with continuous shaking.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0 (\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the Androstenedione concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B₀ (%)) values, determine the Androstenedione concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled Androstenedione (B₀/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

ANDROSTENEDIONE-RIA-CT		cpm	B/Bo (%)
Total count		48670	
Calibrator	0.0 ng/ml	14867	100.0
	0.1 ng/ml	13196	88.8
	0.4 ng/ml	9450	63.6
	1.0 ng/ml	5910	39.8
	4.0 ng/ml	2746	18.5
	11.0 ng/ml	987	6.6

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.03 ng/ml.

B. Specificity

The specificity of the polyclonal antibody used in this assay was evaluated by RIA, as the ratio of the amount of Androstenedione which yields 50% inhibition of binding of labelled Androstenedione and the amount of cross-reacting compounds (analogs) giving the same inhibition.

Compound	Cross-Reactivity (%)
Androsterone	0.0566
Cortisol	0.0148
Corticosterone	0.0047
Cortisone	0.0099
DHEA	0.0155
DHEA-SO ₄	0.0009
21-Deoxycortisone	0.0006
Estradiol-17β	ND
Estril	ND
Estrone	0.0270
Etiocholanolone	0.0422
17β-hydroxypregnenolone	ND
17β-Hydroxyprogesterone	0.0840
Isoandrosterone	0.0112
Pregnenolone	ND
Progesterone	0.0168
Spirolactone	0.0006
5α-Dihydrotestosterone	0.0105
Testosterone	0.2406

Note : This table shows the cross-reactivity for the anti Androstenedione.
ND : not detectable

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (ng/ml)	CV (%)	Serum	N	<X> ± SD (ng/ml)	CV (%)
A	10	0.36 ± 0.02	4.5	A	10	0.42 ± 0.04	9.0
B	10	6.04 ± 0.19	3.2	B	10	1.90 ± 0.11	5.9

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
Serum 1	1/1	-	4.64
	1/2	2.32	2.01
	1/4	1.16	0.97
	1/8	0.58	0.55
	1/16	0.29	0.30
	1/32	0.14	0.20
Serum 2	1/1	-	8.94
	1/2	4.47	3.89
	1/4	2.24	2.05
	1/8	1.12	0.98
	1/16	0.56	0.49
	1/32	0.28	0.27
	1/64	0.14	0.18
	1/128	0.07	0.09

Samples were diluted with the zero calibrator.

RECOVERY TEST

Sample	added Androstenedione (ng/ml)	Recovered Androstenedione (ng/ml)	Recovered (%)
Serum	8.0	7.7	96
	4.0	4.0	100
	2.0	1.9	95
	1.0	1.0	100
	0.5	0.4	80

To the best of our knowledge, no international reference material exists for this parameter.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 24 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum (ng/ml)	0'	12'	24'
	0.49	0.55	
	1.96	2.13	
	0.45		0.45
	1.69		2.08

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

The ranges are expressed as 2.5% to 97.5% percentiles.

subjects	N	Mean ng/ml	Range
CHILDREN	25	0.4	0.1 – 0.9
MALES	64	2.0	0.5 – 4.8
FEMALES	250	2.1	0.5 – 4.7
Follicular phase	45	1.9	0.9 – 3.0
Ovulatory peak	14	2.9	1.9 – 4.7
Luteal phase	27	2.0	1.1 – 4.2
Polycystic Ovarian syndrome	25	3.6	2.2 – 6.5
Menopausal	45	1.7	0.3 – 3.7

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE(S) CONTROLS µl
Calibrators (0-5)	-	25	-
Samples, controls	-	-	25
Tracer	250	250	250
Incubation	1 hour at RT with continuous shaking		
Separation Working Wash solution Separation	-	Aspirate 2.0 ml Aspirate carefully	
Counting	Count tubes for 60 seconds		

Bio-Line Catalogue Nr : BL-32-CT	Version: 040702-BL	Revision nr : 031212/1
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