



ALDOSTERONE RIA CT BL-42-CT

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

Read entire protocol before use.
ALDOSTERONE RIA CT

I. INTENDED USE

RadiolImmunoAssay for the quantitative determination of aldosterone in human serum and urine

II. GENERAL INFORMATION

A. Name: Bio-Line **ALDOSTERONE RIA CT** Kit
B. Catalogue number: BL-42-CT: 100 tests
C. Manufactured by: Bio-Line S.A.
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III. CLINICAL BACKGROUND

Aldosterone is a steroid hormone with molecular weight of 360.4, produced by the zona glomerulosa of the adrenal cortex. It is the most potent regulator of electrolyte excretion in man. Its production is strictly related to certain control mechanisms:

- variations in interstitial, cellular and blood potassium levels. Hypokaliemia inhibits while hyperkaliemia increases aldosterone production.
- variations in interstitial, cellular and blood sodium levels. Hyponatremia increases while hypernatremia inhibits Aldosterone production.
- ACTH secretion. In certain stress situations, ACTH hyperincretion may also affect the adrenal zona glomerulosa. By far, the most significant factor related to aldosterone secretion is:
 - the renin-angiotensin system. Renin is secreted by the juxtaglomerular apparatus of the kidney and leads to the production of angiotensin II, the most potent natural vasopressor produced by the human organism. The changes in body hydration as well as in the amount of circulating fluids brought about by this substance lead to a regulating mechanism, depending upon the degree of aldosterone incretion. The aldosterone assay is extremely important in the diagnosis of primary hyperaldosteronism (Conn's disease), in the so-called essential hypertension, where a verified renal alteration affects the reninangiotensin system and causes aldosterone stimulation, in arterial pressure changes induced by the administration of abnormal amounts of certain types of food (liquorice) or drugs (potassium/sodium), in nephrosis syndromes etc.

Blood levels of aldosterone follow a circadian rhythm with values higher in the morning and lower in the evening. Such levels are also strictly related to states of rest or exercise (body motion stimulates the reninangiotensin system with consequent aldosterone production). Because of these physiological aspects, it is necessary to study blood aldosterone levels by means of stimulation and/or suppression dynamic tests. Testing for urinary aldosterone instead provides an integrated reflection of the daily aldosterone secretion. In any case, both plasma and urinary measurement of aldosterone are useful, depending upon the type of clinical condition we want to investigate.

IV. PRINCIPLES OF THE METHOD

The present method is based on a competitive radioimmunoassay (RIA). During the incubation, the sample/standard aldosterone competes with the aldosterone labeled with Iodine 125 (tracer) for the specific sites of the antiserum coated on the tubes. B/F separation is based on the use of antibody coated tubes, where the anti-aldosterone antiserum is fixed on the tube walls. After aspiration, the radioactivity in the tubes is measured in a gamma counter. The degree of binding will be inversely proportional to the sample/calibrator hormone concentration.

V. REAGENTS PROVIDED

- The reagents are sufficient for 100 tubes.
- Store the kit at 2-8 °C.
- The expiry date of each reagent is shown on the vial label.
- 1 -Coated Tubes: 96 (2 x 48) tubes coated with anti-Aldosterone antibody. Unused tubes should be stored at 2-8 °C protected from moisture with the appropriated cap.
- 2 -Calibrators: 8 vials of Aldosterone in human serum on the vial labels. Preservative : NaN₃ (<0.1%). Lyophilized. Reconstitute the Zero calibrator with 3 ml of distilled H₂O and the calibrators 1-6 with 2 ml of distilled H₂O. After reconstitution, aliquot and freeze unused calibrators at -20 °C.
- 3 -Radioactive Tracer: 1 vial (50 ml) of ¹²⁵I-Aldosterone in phosphate buffer. Radioactivity contents: 85 KBq. Preservative: NaN₃ (<0.1%). Ready for use.
- 4 - Control Serum: 1 vial of Aldosterone in human serum. Preservative: NaN₃ (<0.1%). Lyophilized. Reconstitute with 2 ml of distilled H₂O. After reconstitution, aliquot and freeze unused standards at -20 °C.

VI. SUPPLIES NOT PROVIDED

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

- Plastic test tubes (12 x 75 mm.).
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Automatic pipette adjustable at 1 ml.
- Orbital shaker, adjustable at 150 rpm.
- Aspiration pump or automated tube washing device.
- Scintillation gamma counter.
- Logit-log or semi-log graph paper.
- Distilled H₂O.
- Hydrochloric acid. 0.1 M

In order to obtain correct and reproducible results, the following rules must be observed:

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Use thoroughly clean glassware, free from metal ion contamination or oxidizing substances.
- Use distilled water, stored in perfectly clean containers.
- Carefully avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
- Follow exact incubation times, as described in the "Assay Procedure".

In order to reduce physical, biological and chemical risks, the following precautions must be observed:

- Use protective individual articles (ex.: disposable gloves, lab coats, etc.) while handling radioactive materials and/or potentially infectious material as well as during the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All radiological work should be done in a designated area.
- Radioactive materials should be stored in their original container in a designated area.
- A record book for logging receipt and disposal of all radioactive materials should be kept.
- Any radioactive spills should be taken care of immediately in accordance with established procedures.

VII. SPECIMEN COLLECTION AND PREPARATION

The assay can be performed in serum or urine samples. Since Aldosterone shows a circadian rhythm we suggest to note down the sample collecting time. Highly lipemic or hemolyzed samples must be discarded. Keep samples at 2-8 °C for 1-2 days; for longer periods it is advisable to freeze samples at -20 °C. Repeated freezing and thawing of samples should be avoided.

Urine Samples

Urine collection.

Collect 24-hour urine by adding boric acid (10 g/l) as preservative. Record the volume and store at 2-8 °C for 1-2 days; freeze at -20 °C for longer time periods.

Urine Hydrolysis:

- 1 -Prepare uncoated tubes, one for each urine sample.
- 2 -Pipette 100 µL of urine sample.
- 3 -Add 1 ml of 0.1 M hydrochloric acid into each tube.
- 4 -Incubate for 15-20 hours at 30 °C.
- 5 -Use 50 µL of this solution for the test.

VIII. PROCEDURE

A. Handling notes

- All material of human origin used for the preparation of this kit was tested negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents containing biological material used for the assay must be considered potentially infectious.
- Avoid splashing and aerosol formation; in such cases, carefully wash with a 3% sodium hypochlorite solution. Any cleaning material used for that purpose must be treated as potentially infectious and disposed accordingly.
- The sodium azide used as preservative in some reagents may react with lead and copper plumbing; to prevent build-up of explosive metal azides, the reagents should be discarded by flushing the drain with large amounts of water.
- Acquisition, storage, use and disposal of radioactive material (liquid and solid) are subjected to regulation and ordination of local authorities.

B. Procedure

- Allow reagents and samples to warm up at room temperature.
- Mix samples by inversion before use.
- 1 - Prepare uncoated tubes for Total Activity (T) and Non-specific Binding (NSB) as well as coated tubes for Zero calibrator (Bo), calibrators (1-6), Control Serum and Samples.
- 2 -Pipette 200 µL of each calibrator, Control Serum and Sample into each coated tube.
- 2a -Urine Samples. Pipette 50 µL of Hydrolyzed sample into each tube and add 150 µL of the Zero calibrator.
- 3 -Pipette 200 µL of Zero calibrator into the Non-specific Binding (NSB) tubes.
- 4 -Add 500 µL of Radioactive Tracer into all tubes.
- 5 -Mix test-tube rack manually. Do not use vortex.
- 6 -Incubate: Procedure A: 18-24 hours at room temperature; or

Procedure B: 3 hours at room temperature with an orbital shaker (150 rpm).

- 7 - Carefully aspirate the incubation mixture from all tubes, except those for total activity, with a vacuum pump or decant by drying the edges of the tubes with blot-paper.
- 8 - Count the radioactivity in the tubes for 1 minute by using a gamma counter. We suggest to check the background of the instrument before counting the assay. In order to avoid variations in the sensitivity of the system, the background should be reduced to a minimum or adjusted properly.

ASSAY SCHEME

Tubes Reagent	Total Activity	NSB	Cali- brators	Control	Samples	Urine samples
Calibrator 0	----	200 µl	----	----	----	150 µl
Calibrators	----	----	200 µl	----	----	----
Controls	----	----	----	200 µl	----	----
Samples	----	----	----	----	200 µl	50 µl
Tracer	500 µl	500 µl	500 µl	500 µl	500 µl	500 µl

- Incubate: 18 – 24 h R.T. or 3 h R.T. shaking (150 rpm)
 - Aspirate
 - Count

IX. CALCULATION OF RESULTS

Draw the standard curve on logit-log or semi-log paper, with the standard concentrations on the x-axis and the respective B/Bo % on the y-axis.

Calculate the B/Bo% for each sample and read the concentration by interpolating on the standard curve to obtain the Aldosterone concentration in the tested samples, expressed in pg/ml.

Urine Samples.

The concentration read on the standard curve must be multiplied by factor 44 to obtain the Aldosterone concentration in pg/ml. In order to express Aldosterone concentration in µg/24h:

$$\mu\text{g}/24\text{h} = \frac{\text{Concentration (pg/ml)} \times 44 \times \text{Urine excreted (liters)}}{1000}$$

$$\text{Concentration (pg/ml)} \times 44 \times \text{Urine excreted (liters)} \mu\text{g}/24\text{h} = 1000$$

X. TYPICAL DATA

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

Description	Average cpm	B/BO (%)	Aldosterone (pg/ml)
Total activity (T)	41999	-	-
NSB	154	-	-
CAL 0	19213	100	0
CAL 1	16439	85.4	25
CAL 2	14667	76.1	50
CAL 3	12268	63.6	100
CAL 4	8184	42.1	300
CAL 5	5844	29.9	600
CAL 6	3669	18.4	1500
CONTROL	11263	58.3	133
P1	14905	77.4	45.5
P2	9869	51.0	191
P3	7934	40.8	323

XI. REFERENCE VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

Serum Aldosterone:	
- At rest:	10 - 160 pg/ml
- In motion:	35 - 300 pg/ml
Urinary Aldosterone:	2.8 - 30 µg/24h.

XII. PERFORMANCE AND LIMITATIONS

A. Specificity

The present method has shown the following cross-reactions: 100% with Aldosterone, 17.2% with 3, 5 Tetrahydroaldosterone, 0.12% with 3,5 Tetrahydroaldosterone, less than 0.017% with Prednisolone, less than 0.0033% with Cortisol, Corticosterone, Cortisone, 11-Deoxycortisol, 11-Deoxycorticosterone, 17-Hydroxyprogesterone, Progesterone and less than 0.0017% with Prednisone, Testosterone and Androstenedione.

The percentage of cross-reactivity is calculated by using the Abraham formula: $X/Y \times 100$, X and Y being the weight of the substance to be assayed and the weight of the interfering substance, both able to reduce the binding capacity by 50%.

B. Sensibility

The sensitivity is the lowest concentration of Aldosterone capable of reducing the initial binding capacity by 5%. This amount is 8.8 pg/ ml.

C. Precision

Precision was evaluated based upon intra-and inter-assay variability, in 3 sera at different Aldosterone concentrations.

Intra-assay

Serum	Mean (pg/ml)	±	S.D.	C.V. (%)	N
1	42.3	±	5.8	13.7	20
2	177	±	6.7	3.8	20
3	278	±	14.8	5.3	20

Inter-assay

Serum	Mean (pg/ml)	±	S.D.	C.V. (%)	N
1	38.2	±	7.1	18.6	9
2	169	±	12.6	7.5	9
3	284	±	17.7	6.2	9

D. Accuracy

Accuracy of the method has been checked by the recovery and parallelism tests

Recovery Test

Samples, mixed with equal volumes of each calibrator, were tested.

Added (pg/ml)	Expected (pg/ml)	Measured (pg/ml)	Recovery (%)
S1	-	97.2	-
S1 + CAL 0	48.6	41.6	85.6
S1 + CAL 1	61.1	69.4	113.6
S1 + CAL 2	73.6	88.8	120.7
S1 + CAL 3	98.6	121	122.7
S1 + CAL 4	199	249	125.1
S1 + CAL 5	349	455	130.4
S1 + CAL 6	799	933	116.8
S2	-	142	-
S2 + CAL 0	71.0	68.4	96.3
S2 + CAL 1	83.5	83.4	99.9
S2 + CAL 2	96.0	96.9	100.9
S2 + CAL 3	121	137	113.2
S2 + CAL 4	221	244	110.4
S2 + CAL 5	371	431	116.2
S2 + CAL 6	821	952	116.0

Parallelism Test

Serums with high analyte concentration was tested at different dilutions with the Zero Calibrator.

Dilution	Expected (pg/ml)	Measured (pg/ml)	Recovery (%)
S1 undiluted	-	128	-
1/2	64.1	61.6	96.1
1/4	32.1	30.9	96.3
1/8	16.0	14.9	78.4
S2 undiluted	-	351	-
1/2	176	161	91.5
1/4	87.8	75	85.4
1/8	43.9	36.3	82.7
1/16	22.0	25	113.6

XIII. BIBLIOGRAPHY

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