



1. NAME AND INTENDED USE

ALDO-RIACT is a kit for the radioimmunoassay of aldosterone in serum, plasma and urine.

2. INTRODUCTION

Aldosterone is a steroid hormone with a molecular weight of 360.4. It is secreted by the glomerulosa zone of the adrenal gland and controls regulation of hydromineral metabolism.

In the distal part of the nephron, aldosterone encourages reabsorption of Na⁺ and Cl⁻ ions and secretion of K⁺ and H⁺ ions in blood vessel lumen. Thus there is an increase in extra-cellular osmolarity and water retention.

Regulation of aldosterone secretion is linked to the body's water balance and brings three factors into play. These are the renin-angiotensin system, the plasmic Na⁺/K⁺ ratio and, as a secondary factor, the ACTH.

Physiologically, there seems to be circadian rhythm for levels of aldosterone in the blood, which may be linked to changes in body posture. It has also been found that aldosterone levels are higher for children than for adults, and increase during pregnancy.

In pathology, lowered levels are found in cases of adrenal insufficiency and in some congenital adrenal hyperplasia cases with enzyme deficiencies. However, aldosterone assay is mainly indicated for complete etiological examinations related to arterial hypertension (AHT), when a high level of aldosterone is a sign of hyperaldosteronism, either primary (adrenal adenoma) or secondary (malignant AHT, renal artery stenosis...).

3. PRINCIPLE

The principle of the assay is based on competition between a fixed amount of 125 iodine aldosterone and aldosterone contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites bound to the solid phase (coated tubes).

After incubation and removal of the unbound tracer, the amount of 125 iodine aldosterone bound to the antibody is inversely proportional to the amount of unlabelled aldosterone present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
COATED TUBES : ready for use Anti-aldosterone antibody coated on the bottom of the tube.	2 packs of 50 tubes	2-8°C until the expiry date.
ALDOSTERONE 125 I : ready for use 125I labelled aldosterone, buffer, bovine albumin, rabbit serum, sodium azide, red dye, non specific immunoglobulins. ≤ 111 kBq (≤ 3 µCi) per vial	1 50 ml vial	2-8°C until the expiry date.
STANDARD 0 : lyophilized Human aldosterone-free serum, preservative. Reconstitute the vial's content with 4 ml of distilled water.	1 qs 4 ml vial	2-8°C until the expiry date. After reconstitution : 1 week at 2-8°C or 1 month at -20°C.
STANDARDS : lyophilized** Human serum, preservative, aldosterone 25, 60, 180, 500, 1500 pg/ml* (69, 166, 498, 1385, 4150 pmol/l). Reconstitute the vial's contents with 1 ml of distilled water.	5 qs 1 ml vials	2-8°C until the expiry date. After reconstitution : 1 month at -20°C .
CONTROL : lyophilized** Human serum, preservative, aldosterone 110 pg/ml* (304 pmol/l). Reconstitute the vial's contents with 1 ml of distilled water.	1 qs 1 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at -20°C.

(*) The values shown above are only target values : the true value of each standard or control is shown on its label.

(**) Standards and control should be frozen and thawed only once.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.



5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Do not manipulate much than 100 tubes at the same time.

Avoid any microbic contamination of the reagents or of the water.

Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

6.1. Serum or plasma

The assay is performed directly on serum or plasma; haemolyzed or hyperlipemic samples should not be used. If the test is to be carried out within 24 hours, the samples must be refrigerated at 2-8 °C. Otherwise, they should be divided into aliquots and deep frozen (-20 °C) until needed.

Dilutions

Should elevated aldosterone levels be suspected, the Standard 0 found in the kit is used for dilution.

It is recommended to carry out the dilutions using disposable plastic tubes.

6.2. Urine

Collect 24-hour urine, measure and record the volume. Mix well before drawing off an aliquot to assay. Add 1g boric acid / 100 ml of urine and store at 2-8 °C, or at - 20 °C for extended storage.

Urine hydrolysis

Aldosterone can be assayed in urine samples after acid hydrolysis of aldosterone 18-glucuronide. In the conditions recommended for the assay, hydrolysis is complete.

- Mix 500 µl urine and 1 ml 0.1 N HCl. Use glass tubes.

- Cap tubes and incubate for 15-20 hours at 30 ± 2 °C.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 10 µl, 200 µl, 500 µl, 1 ml and 4 ml (± 1%). Their calibration should be checked regularly.

Distilled water.

Disposable plastic tubes.

Vortex-type mixer.

Circular horizontal shaker (400 rpm).

Gamma scintillation counter calibrated for 125 iodine measurement.

Equipment suitable for this assay is available from CIS bio international ; information on request.

7.2. Protocol

All reagents must be brought to room temperature (18-25 °C) at least 30 minutes before their use. Standards and control must be reconstituted 15 minutes before use. Dispensing of the reagents into the tubes is carried out at room temperature (18-25 °C).

The assay requires the following groups of tubes :

Standard "0" group, for the determination of maximum binding (Bo).

Standard groups, to establish the standard curve.

Control group for the control.

Sx groups, to test serum or plasma samples.

Ux groups, to test urine samples.

It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.

Strictly observe the order in which reagents are to be added :

Standards, control or serum and plasma samples



Dispense 200 µl of standards, control or samples to be assayed, into the corresponding tubes.

Urine samples

Dispense 200 µl of standard 0 + 10 µl of hydrolysed urine samples to be assayed into the corresponding tubes.

Add 0.5 ml of ¹²⁵I Aldosterone to each tube.

Mix each tube gently with a Vortex-type mixer.

Incubate for 3 h ± 5 mn at room temperature (18-25 °C) under agitation (400 rpm).

Aspirate the contents of the tubes as completely as possible. Any trace of red dye should disappear.

Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter calibrated for 125 iodine measurement.

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.

Calculate the B/Bo ratio for each standard and unknown sample as follows :

$$B/Bo (\%) = \frac{\text{Std cpm or Sx cpm or Ux cpm}}{\text{Std 0 cpm}} \times 100$$

Draw up the standard curve by plotting the standard's B/Bo against their concentrations.

Serum or plasma

Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

Urine

Multiply the read value on the calibration curve by 63 to obtain the urinary aldosterone concentration in pg/ml. The factor 63 results from the dilution factor of hydrolyzed urine (factor 3) multiplied by the (standard+sample)-to-sample ratio (factor 21).

Daily aldosterone excretion expressed as µg of aldosterone/24 hours is calculated by thus :

$$\text{pg/ml} \times \text{ml urine/24 h} \times 10^{-6} = \mu\text{g/24 hours}$$

Conversion of µg aldosterone in nmol is : 1 µg = 2.77 nmol.

Typical standard curve (example only) : these data must not be substituted for results obtained in the laboratory.

Tube groups	Mean (cpm)	B/Bo (%)	Concentration (pg/ml)
Standard 0	24802	100	0
Standard 1	20387	82.2	30
Standard 2	18179	73.3	71
Standard 3	13889	56.0	195
Standard 4	9399	37.9	530
Standard 5	5878	23.7	1400
Control	16245	65.5	116
Sx	9722	39.2	489
Ux	18924	76.3	(55 x 63) = 3465

10. PROCEDURAL LIMITATIONS

Samples containing fibrin, gross haemolysis, gross lipemia or turbidity may give misleading results.

Falsely elevated levels may be obtained for patients undergoing substitute corticotherapy.

Do not attempt to extrapolate sample values beyond the last standard. Dilute the samples and retest.

11. EXPECTED VALUES

The ranges given below are only indicative for a normal population with a normal sodium intake ; each laboratory should establish its own reference ranges.

NORMAL SUBJECTS	PLASMA (pg/ml)		
	5 th percentile	Median	95 th percentile
Supine position* (n=25)	42	99	201.5
Standing position (n=41)	97	201	626

* In supine position for at least one hour.

Conversion of pg/ml aldosterone in pmol/l is : 1 pg/ml = 2.77 pmol/l.



Because of possible serie interferences, this method, without preliminary sample extraction, can give some different values compared to an extraction method.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 2 samples with different concentrations. They were tested either 29 times in the same series of assays, or in duplicate in 15 different series.

Sample	Mean pg/ml	Within-run CV %	Between-run CV %
1	30.1	7.7	8.4
2	656	8.3	5.0

12.2. Specificity

The antibody used in this assay guarantees a measurement which is completely specific for aldosterone. It shows very low cross-reactions with some other compounds.

Aldosterone	100 %	Dexamethasone	N.D. %
Androstene-dione	0.00217 %	Estradiol	0.00037 %
Androsterone	0.00065 %	Estriol	N.D. %
Dehydroepi-Androsterone	0.00016 %	Estrone	0.00019 %
Canrenone	N.D. %	Prazosin-HCl	N.D. %
Cortisolone (11-deoxycortisol)	0.0052 %	Prednisolone	0.00035 %
Corticosterone	0.04 %	Prednisone	0.00022 %
11-deoxy-Corticosterone	0.065 %	Pregnanetriol	N.D. %
18-hydroxydeoxy-Corticosterone	0.012 %	Pregnenolone	0.00043 %
Cortisol	0.0022 %	Progesterone	0.027 %
Cortisone	0.0018 %	17 α -hydroxy-Progesterone	0.0025 %
9 α -fludro-Cortisone	N.D. %	Spirolactone	0.00037 %
		Testosterone	0.0026 %

N.D. = Not detectable

12.3. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 7 pg/ml.

ASSAY FLOW CHART

Tubes	Standard 0 μ l	Standards Control Serum or Plasma Samples μ l	Hydrolysed urine samples μ l	Aldosterone ¹²⁵ I μ l	Mix	Count
Standard 0	200			500	Incubate 3 H \pm 5 mn at 18-25°C under agitation	
Standards		200		500		
Control		200		500		
Serum Sx		200		500	Aspirate	



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Urine Ux	200		10	500		
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