Diagnostics Biochem Canada

Aldosterone ELISA

REF CAN-ALD-500

ASSAY PROCEDURE

- Bring kit components to room temperature. Mix gently by inversion.
- Prepare working solution.
- Pipette 50 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of the aldosterone-HRP conjugate into each well.
- Incubate on a microplate shaker for 60 minutes at room temperature.
- Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a microplate shaker for 20 minutes at room temperature.
- Pipette 50 µL of stopping solution. Gently shake the microplate by hand to mix.
- Read the plate on a microplate reader at 450 nm.

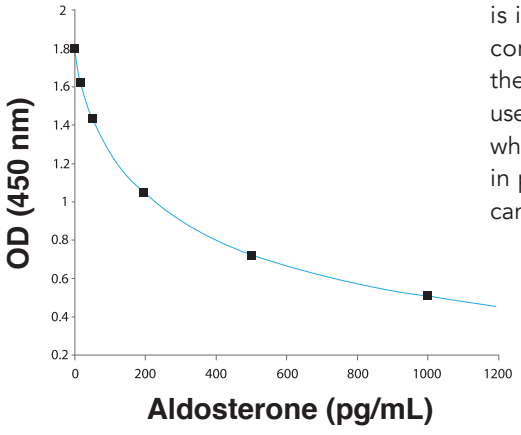
Aldosterone is a potent mineralocorticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the reabsorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension.

Measurement of aldosterone levels in serum in conjunction with plasma renin activity levels can be used to differentiate between primary and secondary aldosteronism.

CONDITION	SERUM ALDOSTERONE	PLASMA RENIN
Primary Aldosteronism	High	Low
Secondary Aldosteronism	High	High

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 wells. 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 with a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of aldosterone in the sample. A set of standards is used to plot a standard curve from which the amount of aldosterone in patient samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 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 **9.1 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with aldosterone cross-reacting at 100%:

Steroid	% Cross-Reactivity
Aldosterone	100
Androsterone	0.01
Cortisol	0.01
Dihydrotestosterone	0.01
11-Deoxycorticosterone	0.075
Testosterone	0.009

The following compounds were tested and cross-reacted at less than 0.001%: Caffeine, Cholesterol, Cortisone and DHEAS.

INTERFERENCE

The following substances were tested and did not show significant interference in the assay: hemoglobin up to 4 g/L, bilirubin conjugated and free up to 125 mg/L and triglycerides up to 30 mg/mL.

INTRA-ASSAY PRECISION

Four serum and four urine samples were assayed 24 times each on the same calibrator curve. The results are tabulated below:

Serum Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	81.2	7.6	9.4
2	284.5	25.9	9.1
3	403.0	22.2	5.5
4	529.5	36.5	6.9

Urine Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	41.2	5.1	12.5
2	335.8	24.9	7.4
3	604.7	36.4	6.0
4	865.8	61.4	7.1

INTER-ASSAY PRECISION

Five serum samples were assayed in 20 different tests in the span of at least ten days. The results are tabulated below:

Sample	Mean (pg/mL)	SD (pg/mL)	CV %
1	80.8	10.4	12.8
2	209.0	22.5	10.7
3	454.5	51.9	11.4
4	677.3	79.1	11.7
5	902.3	68.4	7.6

LINEARITY

Patient serum samples were diluted with Serum and Plasma Diluent. Patient urine samples were diluted with Urine Diluent after an initial dilution of 1:10 in Urine Diluent. The results are tabulated below:

Serum Sample	Obs. Result (pg/mL)	Exp. Result (pg/mL)	Recovery %
1	241.8	-	-
1:2	127.8	120.9	105.7
1:4	65.0	60.5	107.6
1:8	31.6	30.2	104.5
2	840.8	-	-
1:2	456.7	420.4	108.6
1:4	217.1	210.2	103.3
1:8	125.0	105.1	118.9
3	1152	-	-
1:2	624.3	575.9	108.4
1:4	282.3	287.9	98.1
1:8	123.2	144.0	85.6

Urine Sample	Obs. Result (pg/mL)	Exp. Result (pg/mL)	Recovery %
1	320.1	-	-
1:2	178.7	160.1	111.7
1:4	92.8	80.0	116.0
1:8	34.1	40.0	85.1
2	442.3	-	-
1:2	231.9	221.1	104.9
1:4	126.5	110.6	114.9
1:8	48.5	55.3	87.8
3	572.2	-	-
1:2	290.2	286.1	101.4
1:4	149.4	143.0	104.4
1:8	65.2	71.5	91.1

COMPARATIVE STUDIES

The DBC Aldosterone ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 42 serum samples yielded the following linear regression results: y = 0.84x + 3.50, r = 0.96

REFERENCE RANGES

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

SERUM/PLASMA

Group	N	95% Confidence Range (pg/mL)
Normal Salt Intake, Upright	183	ND–199

URINE

Group	N	95% Confidence Range (μg/24 hr)
Normal Salt Intake	42	2.8–13

Ordering Information: REF CAN-ALD-500

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