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Immunoassay

REF CMD0101 / CMD0102 / CMD0103 / CMD0104 / CMD0105

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

Aldosterone CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of Aldosterone in human serum or plasma (EDTA).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP
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CE IVD

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Contact your local dealer for all product-related questions in your local language.

Introduction

Aldosterone is a hormone that plays an important role in maintaining normal sodium and potassium concentrations in blood and in controlling blood volume and blood pressure. Aldosterone is produced by the adrenal glands located at the top of each kidney, in their outer portion (called the adrenal cortex). Aldosterone stimulates the retention of sodium (salt) and the excretion of potassium by the kidneys. Aldosterone is responsible for the reabsorption of about 2% of filtered sodium in the kidneys, which is nearly equal to the entire sodium content in human blood under normal glomerular filtration rates.[1,2,3]

Measurement Principle

This assay is based upon the one-step competitive method. The sample, Mouse anti-rabbit antibody coated microparticles, Antibody Solution and enzyme labeled aldosterone antigen are added. During the incubation, enzyme labeled aldosterone antigen and aldosterone present in the sample compete for binding to the antibodies which had been bind to microparticles. After washing, a complex is generated among the antibodies binding to microparticles, aldosterone in the sample or enzyme-linked antigen by immunological reactions. Chemiluminescent Substrate are then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the concentration of aldosterone in the patient sample.

Materials provided

1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservatives.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2
Antibody Solution	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

Contains Mouse anti-rabbit antibody coated microparticles in PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservatives.

- Enzyme Conjugate

Contains horseradish-peroxidase labeled Aldosterone antigen in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains a selection of preservatives.

- Antibody Solution

Contains Tris-NaCl buffer, Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- Auto Lumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in the washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the Aldosterone calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.
The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirements.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300®may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the calibrators, be certain the vials are securely sealed.
13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be

discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.

4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of samples, or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tubes or cups on the sample rack, 100 μL of sample for each test. But consider the sample container and 150 μLof system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Enzyme Conjugate and Antibody Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of Aldosterone in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tubes or cups and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with an Aldosterone value exceeding 1000 pg/mL may be diluted manually. Low-value Aldosterone sample is used to dilute the samples. After dilution, multiply the result by the dilution factor.

- The concentration of the sample after dilution should not be less than 10 pg/mL.

Measurement Results

The sample test results are determined automatically by the system software. The amount of Aldosterone in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

Controls for the various concentration ranges should be run individually

when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
4. A result within the biological reference interference does not rule out the presence of disease and should be interpreted together with the patient's clinical picture and other diagnostic procedures.
5. Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
6. Any therapeutical decision must also be taken on a case-by-case basis.
7. Aldosterone levels in paediatric age have not been investigated.
8. This test measures concentrations within the range of10-1000 pg/mL. If Aldosterone concentrations above the measuring range to be expected, it is recommended to dilute samples with low concentration Aldosterone sample. The reconmmended dilution is 1:9 of this test, allowing samples to be up approximately to 10000 pg/mL.

Biological Reference Interval

A normal range of 40 pg/mL to 310 pg/mL for upright position and10 pg/mL to 160 pg/mL for supine position(95% confidence interval) were obtained by testing EDTA plasma samples from 412 individuals defined as normal by a clinician.
It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.
1 pmol/L = 0.36 pg/mL

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (pg/mL)	Within-run	Total
			%CV	%CV
1	80	133.87	4.74	6.48
2	80	344.30	2.92	4.71
3	80	738.76	4.47	5.39

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 10.0 pg/mL.
Limit of Detection: 16.32 pg/mL.
Limit of Quantitation: 40.0 pg/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross reaction: The following substances and concentrations were tested and found no cross reaction with the test.

Substance	Concentration (pg/mL)
Allotetrahydrocortisol	$2*10^5$
androstenedione	$2*10^5$
Cortisone	$12.2*10^5$
α-cortol	$2*10^5$
α-cortolone	$5*10^5$
β-cortol	$5*10^5$
β-cortolone	$2*10^5$
Dehydrocorticosterone	$5*10^5$
11-deoxycortisol	$6.75*10^5$
20α-dihydrocortisol	$5*10^5$
20β-dihydrocortisol	$1*10^6$
20α-dihydrocortisone	$1*10^6$
20β-dihydrocortisone	$1*10^6$
11β-hydroxyandrosterone	$1*10^6$
11β-hydroxyetiocholanolone	$5*10^5$
11β-hydroxyprogesterone	$2.5*10^6$
11-keto-androsterone	$1*10^6$
11-keto-etiocholanolone	$1*10^6$
Pregnanetriol	$2*10^5$
Pregnenolone	$7.5*10^5$
Tetrahydrocortisol	$5*10^6$
Tetrahydrocortisone	$2.5*10^6$
Prednisolone	$1.17*10^6$
6-methyl-Prednisolone	$2.5*10^6$
17-hydroxypregenenolone	$1*10^4$
Progesterone	$5*10^4$
Dexamethasone	$1*10^4$
21-hydroxyprogesterone	$1*10^4$

Interference: No interference with 125 mg/dL of haemoglobin, 10 mg/dL of bilirubin, 2000 mg/dL of triglyceride.

4. Trueness by Recovery Test

1 set formed of a low concentration and a high concentration to aldosterone sample (samples X and Y) were mixed in 4:1, 3:2, 2:3 and 1:4 ratios and assayed, measured versus expected aldosterone concentrations were analyzed by linear regression. The correlation coefficients (r)ranged from 0.996 to 1.000.

Dilution	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Recovery %
X	158.905	158.905	-
4:1	319.826	308.624	103.6
3:2	470.394	458.343	102.6
2:3	630.034	608.062	103.6
1:4	778.202	757.781	102.7
Y	907.500	907.500	-

5. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and an Aldosterone Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	60	-31.431	1.1114	0.9694

Literature References

1. Direct Aldosterone Assay and Plasma Aldosterone Activity Assay Compared .Clinical Chemistry,2004 ; 50 (11): 2159-2161.

2. Aldosterone and Aldosterone measurements [Review]. Ann ClinBiochem 2000;37:262-278.

3. Measurement of plasma Aldosterone:a critical review of methodology. JRAAS, 2010;11(2): 89-90.