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Instructions for use

Aldosterone ELISA

REF **MS E-5200**

2°C – 8°C Σ 96 IVD CE

Aldosterone ELISA

1. INTENDED USE

The **Aldosterone ELISA** is a manual enzyme immunoassay for the quantitative measurement of Aldosterone in human serum or plasma (K_2 EDTA, K_3 EDTA, Li-heparin or citrate plasma 3.2%) and urine.
For *in vitro* diagnostic use only. For laboratory professional use.
The device is **intended to be used** as an aid to diagnosis of primary and secondary aldosteronism.
The device is **not intended** for the diagnosis of adenomas.

2. SCIENTIFIC VALIDITY REPORT

The steroid hormone aldosterone is a potent mineral corticoid that is produced by the zona glomerulosa of the adrenal cortex in the adrenal gland. The synthesis and release are controlled by the renin-angiotensin-aldosterone system (RAAS)[1], as well as by plasma potassium concentration [2,12], the pituitary peptide ACTH, and by the blood pressure via pressure sensitive baroreceptors in the vessel walls of nearly all large arteries of the body [3,12]. Aldosterone binds to mineralocorticoid receptors (MR) and triggers the transcription of hormone-responsive genes. In consequence, aldosterone increases the blood pressure by reabsorption of sodium and water from the distal tubules of the kidney into the blood, secretion of potassium into the urine, and elevation of circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension. Aldosterone activity is reduced in Addison's disease and increased in Conn's syndrome.
Primary hyperaldosteronism, which may be caused by aldosterone-secreting adrenal adenoma/carcinomas or adrenal cortex hyperplasia, is characterized by hypertension accompanied by increased aldosterone levels, hypernatremia, and hypokalemia. Secondary hyperaldosteronism (e.g. in response to renovascular disease, salt depletion, potassium loading, cardiac failure with ascites, pregnancy, Bartter's syndrome) is characterized by increased aldosterone levels and increased plasma renin activity [4,5,8,9,10,11,13].

Condition	Serum Aldosterone	Plasma Renin
Primary Aldosteronism	High	Low
Secondary Aldosteronism	High	High

This differentiation is vital in the treatment and management of the disease. The adrenal adenomas respond well to surgery whereas hyperplastic disease of the adrenals is generally better managed medically [6].
In addition, pharmacological modulation of nuclear hormone receptors is a common strategy for the treatment of cardiovascular disease [7]. Therefore, determining the effects of such treatments on the RAAS is of increasing value in evaluating the safety and efficacy of new therapeutics.
In addition, obese subjects often exhibit hyperaldosteronism, with increased salt sensitivity of blood pressure (BP). Systemic RAS, and aldosterone/MR activation plays a key role in the development of hypertension and organ damage in obesity [14].
In summary, the precise and accurate measurement of serum aldosterone by enzyme immunoassay can be an important adjunct to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

3. PRINCIPLE OF THE TEST

The Aldosterone ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the principle of competitive binding.
The microtiter wells are coated with a monoclonal antibody (rabbit) directed towards a unique antigenic site of the Aldosterone molecule.
During the first incubation, the Aldosterone in the added sample competes with the added enzyme conjugate, which is Aldosterone conjugated to horseradish peroxidase, for binding to the coated antibody.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

4. WARNINGS AND PRECAUTIONS

- This kit is for *in vitro* diagnostic use only. For laboratory professional use only.
- Before starting the assay, read the instructions for use completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- Do not mix or use components from kits with different lot numbers. It is advised not to interchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Do not reuse microtiter wells.
- Reagents of other manufacturers must not be used together with the reagents of this test kit.

- All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact the manufacturer.
- Microbial contamination of reagents or samples may give false results.
- Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay.
- Wash Buffer may appear slightly yellow in color, however, this does not affect the performance of the assay.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into original vials as reagent contamination may occur.

General Precautions
- Follow laboratory quality assurance and laboratory safety guidelines.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
- Wear lab coats and disposable latex gloves when handling samples and reagents and where necessary safety glasses.

Biohazard Information
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, no known test method can offer total assurance that no infectious agent is present.
- The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
- Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.
- All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
- Handling must be done in accordance with the procedures defined by appropriate national biohazard and safety guideline or regulation. Waste must be discarded according to local rules and regulations.

Information to Chemical Hazards and Hazard Classification
- Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
- Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately carefully and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
- Avoid contact with Stop Solution containing < 5% H_2SO_4. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
- This product does not contain substances which have carcinogenic, mutagenic or toxic for reproduction (CMR) properties.

The following kit components are classified as hazardous: Enzyme Conjugate, Standard A – F, Control 1 & Control 2, Wash Solution.

 **Warning**	**Hazard statement(s):** H317 – May cause an allergic skin reaction. EUH071 – Corrosive to the respiratory tract.
	Precautionary statement(s): P261 – Avoid breathing dust/fume/gas/mist/vapours/spray. P280 – Wear protective gloves. P333 + P313 – If skin irritation or rash occurs: Get medical advice/attention. P362 + P364 – Take off contaminated clothing and wash it before reuse. P501 – Dispose of contents/container to an approved waste disposal plant.

For detailed information, please refer to the Safety Data Sheet, which is available upon request directly from the manufacturer.

5. MATERIALS

5.1 Materials Provided with the Kit

MS E-5231	**96**	**Microtiter plate** – Ready to use
Contents:	12 x 8 wells (break apart); Coated with anti-aldosterone antibody (monoclonal).	

Standards and **Controls** – Lyophilized

Cat. no.	Component	Concentration	Volume/ Vial
MS E-5201	**STANDARD A**	0 pg/ml	1.0 ml
MS E-5202	**STANDARD B**	20 pg/ml	1.0 ml
MS E-5203	**STANDARD C**	80 pg/ml	1.0 ml
MS E-5204	**STANDARD D**	200 pg/ml	1.0 ml
MS E-5205	**STANDARD E**	500 pg/ml	1.0 ml
MS E-5206	**STANDARD F**	1000 pg/ml	1.0 ml
MS E-5251	**CONTROL 1**	For control values and ranges please refer to vial label or QC-Report.	1.0 ml
MS E-5252	**CONTROL 2**		1.0 ml

Conversion: 1 pg/ml = 2.77 pmol/l

The standards are calibrated against the following reference material:
Certified reference material Cerilliant A-096

Contents: Contain(s) 0.0108% CMIT/ MIT (3:1).

See "Reagent Preparation"

MS E-5240 **CONJUGATE** **Enzyme Conjugate** – Ready to use

Contents: Aldosterone conjugated to horseradish, colored red.

Contain(s) 0.0108% CMIT/ MIT (3:1).

Volume: 1 x 14 ml

FR E-0055 **SUBSTRATE** **Substrate Solution** – Ready to use

Contents: Contains 3,3`,5,5`-tetramethylbenzidine (TMB).

Keep away from direct sun light.

Volume: 1 x 14 ml

FR E-0080 **STOP-SOLN** **Stop Solution** – Ready to use

Contents: Contains < 5% H_2SO_4.

Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 14 ml

FR E-0030 **WASH-CONC 40x** **Wash Solution** – 40X concentrate

Contents: Contain(s) 0.0108% CMIT/ MIT (3:1).

Volume: 1 x 30 ml

See "Reagent Preparation".

1 x Instructions for Use

1 x Certificate of Analysis (CoA)

Abbreviations:
CMIT: 5-chloro-2-methyl-4-isothiazolin-3-one
MIT: 2-methylisothiazol-3(2H)-one

5.2 Materials Required But Not Provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing microtiter plate wells
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction
- **Optional**: Reagents for determination of **Aldosterone in urine** (REF MS U-5200)
- **Contents:**
  1) ***Release Reagent***, 1 vial, 3 ml, ready to use. Containing 1 M HCl.
     Avoid contact with *Release Reagent*. It may cause skin irritation.
  2) ***Neutralization Buffer***, 1 vial, 3 ml, ready to use. Containing Tris buffer, pH 8.5.

3) ***Dilution Buffer***, 2 vials, 25 ml each, ready to use. Containing PBS.

- Optional: Plastic tubes (e.g. 0.5 – 1.5 ml) for pre-treatment of urine samples

5.3 Storage and Stability of the Kit

Unopened kits and reagents as well as **opened reagents** must be stored at 2 °C to 8 °C.
The microplate must always be stored in the resealable aluminum pouch containing a desiccant. Do not open the pouch until it has reached room temperature. The microtiter plate consists of 12 individual strips. Each strip can be divided into 8 individual wells.
Unused wells must be immediately returned to the aluminum pouch with the desiccant and stored again tightly resealed at 2 °C to 8 °C.
Once opened, reagent vials must be closed tightly again.

	Storage Temperature	**Stability**
Unopened kits and unopened reagents	2 °C to 8 °C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2 °C to 8 °C	8 weeks (For reconstituted reagents refer to "4.4 Reagent Preparation".)

5.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Standards
Reconstitute the lyophilized contents of each standard vial with 1.0 ml distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Stability after reconstitution:	at 2 °C to 8 °C	8 weeks
	at -20 °C (in aliquots)	12 months

Controls
Reconstitute the lyophilized content of each control vial with 1.0 ml distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Stability after reconstitution:	at 2 °C to 8 °C	8 weeks
	at -20 °C (in aliquots)	12 months

Wash Solution
Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml distilled water to a final volume of 1200 ml.

Stability after dilution:	at 20 °C to 26 °C	1 week

5.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

5.6 Damaged Test Kits

In case of any damage to the test kit or components, the manufacturer must be informed in writing, at the latest one week after receiving the kit. Damaged single components must not be used for a test run. They have to be stored until a final solution has been found. After this, they must be disposed of according to the official regulations.

6. SAMPLE COLLECTION, STORAGE AND PREPARATION

The following sample material can be used in this test:
Human serum or plasma (K_2 EDTA, K_3 EDTA, lithium heparin or citrate plasma 3.2%) and **urine**.

Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use hemolytic, icteric, or lipemic samples. For further information refer to chapter "*Interfering Substances*".

6.1 Human serum or plasma samples

6.1.1 Sample Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Whole blood should not be frozen before centrifugation.

Stability of whole blood [18]	at 20 °C to 26 °C	up to 4 days

6.1.2 Samples Storage

Samples must be stored tightly capped prior to performing the assay. If stored frozen, freeze only once. Thawed samples must be inverted several times prior to testing.

Stability	at 2 °C to 8 °C	5 days
	at -20 °C (in aliquots)	up to 12 months

6.1.3 Sample Preparation

Samples can be assayed without additional preparation.

6.1.4 Sample dilution

If a concentration higher than the highest standard is found in a sample in a first test run, this sample can be further diluted with Standard A and determined again.
However, the dilution must be taken into account when calculating the concentration.

Example: Dilution 1:10: 10 µl sample + 90 µl Standard A; mix carefully.

6.2 Human urine samples

Aldosterone concentration can also be determined from urine samples. However, urine samples must be pre-treated before analysis. This will need additional reagents that are not included in this kit but can be ordered separately (REF MS U-5200).

6.2.1 Sample Collection

First clean genital area with mild disinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container.
Since Aldosterone secretion follows a circadian rhythm, urine collection is recommended in a special cooled container over a full 24-hour period (24-hour urine).
Directly after collection, the urine should be centrifuged for 5 – 10 minutes (e.g. at 2,000 g) to remove cellular debris. Use supernatant for analyte quantification.

6.2.2 Sample Storage

The samples (urine supernatant) must be kept tightly sealed until the test is performed. If they are stored frozen, freeze them only once. Thawed samples must be swirled several times before testing.

Stability of the	at 2 °C to 8 °C	7 days
urine supernatant:	at -20 °C (in aliquots)	7 days

6.2.3 Protocol for Urine Sample Pre-treatment

To pre-treat urine samples, it is recommended to use the Aldosterone Urine Supplementary kit (MS U-5200).

1. Secure the desired number of vials (e.g. 0.5 – 1.5 ml plastic tubes; not included in this kit).
2. Dispense **25 µl** of **urine** with new disposable tips into appropriate tubes.
3. Dispense **25 µl *Release Reagent*** into each tube.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate overnight at 2 °C to 8 °C.
5. Add **25 µl *Neutralization Reagent*** to each tube and mix thoroughly.
6. Add **400 µl *Dilution Buffer*** to each tube and mix thoroughly
   (This pre-treatment leads to a 1:19 dilution. Therefore, the dilution factor 19 must be taken into account to calculate the final concentration of the urine sample.)
7. Transfer **100 µl of pre-treated and diluted urine samples** directly to the microtiter well and continue with step 3 of Test Procedure (Chapter 7.2).

6.2.4 Storage of pre-treated Urine Samples

Pre-treated and diluted urine samples should be capped and may be stored for up to 7 days at 2 °C to 8 °C or frozen at -20 °C prior to assaying. Samples should be frozen only once at -20 °C prior to assay.
Thawed samples should be inverted several times prior to testing.

6.2.5 Urine Sample Dilution

If in an initial assay, a urine sample is found to contain more than the highest standard, the pre-treated and diluted urine sample can be further diluted with *Dilution Buffer* and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor must be taken into account, too.
Example:
dilution 1:10: 10 µl pre-treated and diluted urine sample + 90 µl *Dilution Buffer* (mix thoroughly)
(final dilution factor = 19 x 10 = 190)

7. ASSAY PROCEDURE

7.1 Procedural Notes

- All reagents and samples must be allowed to come to room temperature (20 °C to 26 °C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid carry-over.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps must be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in chapter "Test Procedure".
- Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- **Important note to wash procedure:**
  Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:**
  Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

7.2 Test Procedure

Each run must include a standard curve.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.
The given test procedure describes manual processing.
Important note: The accuracy of this assay is markedly influenced by the correct incubation temperature.

1.	Secure the desired number of microtiter wells in the frame holder.
2.	Pipette **100 µl** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells. For urine samples dispense **100 µl** of the pre-treated and diluted urine samples (see chapter 5.2.2 *Protocol for Urine Sample Pre-treatment*, step 7).
3.	Incubate for **30 minutes** at room temperature.
4.	Add **100 µl *Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5.	Incubate for **60 minutes** at room temperature.
6.	Wash the wells as follows: If the wash step is performed manually: Briskly shake out the contents of the wells. Rinse the wells **3 times** with **300 µl** diluted *Wash Solution* per well. If an automated plate washer is used: Rinse the wells **3 times** with **300 µl** diluted *Wash Solution* per well. At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
7.	Pipette **100 µl** of ***Substrate Solution*** to each well.
8.	Incubate for **30 minutes** at room temperature.
9.	Stop the enzymatic reaction by adding **50 µl** of ***Stop Solution*** to each well.
10.	Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

7.3 Calculation of Results

1. The concentration of the **serum/plasma samples** can be read **directly** from the standard curve. For **urine samples** the concentration read from the standard curve, has to be **multiplied** with the **dilution factor 19** (see chapter 6.2.4).
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and patient sample must be taken. If the two values deviate substantially from one another, the manufacturer recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted and re-assayed as described in "Test Procedure", or must be reported as > 1000 pg/ml. For the calculation of the concentrations, this dilution factor must be considered.
4. Automated method:
   The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. Manual method:
   Using linear or semi-logarithmic graph paper, construct a standard curve by plotting the (mean) OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
   Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

7.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	**Optical Density (450 nm)**
Standard A (0 pg/ml)	2.040
Standard B (20 pg/ml)	1.634
Standard C (80 pg/ml)	0.966
Standard D (200 pg/ml)	0.516
Standard E (500 pg/ml)	0.223
Standard F (1000 pg/ml)	0.130

7.4 Final Calculation for Urine Samples

Calculate the 24 hours excretion for each urine sample: µg/24 h = **µg/l x l/24 h**

Example:
Concentration for urine sample read from the standard curve = 500 pg/ml
Result after correction with the dilution factor 19 = 9500 pg/ml
9500 pg/ml / 1000 = 9.5 µg/l

Total volume of 24 h-urine = 1.3 l (example)

9.5 µg/l × 1.3 l/24 h = **12.35 µg/24 h**

8. REFERENCE VALUES

The values are only for user's guidance.
It is strongly recommended for each laboratory to establish its own specific values that take into consideration a population indigenous to the area where the laboratory is located.
Values above or below the reference range should be considered as suspicious and require additional testing.
The results alone must not be the only reason for any therapeutic consequences. The results should be correlated with other clinical observations and diagnostic tests.

8.1 Serum/Plasma Samples

In a study conducted with K_3-**EDTA plasma samples** of apparently normal healthy adults, using the Aldosterone ELISA the following values are observed:

Healthy Adults	n	Mean (pg/ml)	Median (pg/ml)	2.5th – 97.5th Percentile (pg/ml)	Range (min. – max.) (pg/ml)
Supine position	60	56.14	39.71	14.21 – 156.47	8.58 – 272.30
Upright position	60	77.48	58.00	13.37 – 233.55	12.87 – 358.50

These values are also valid for serum, K_2-EDTA plasma, Li-Heparin plasma and citrate 3.2% plasma.
These results correspond well to published reference ranges [8, 15].

In a study conducted with apparently normal healthy adults, using the Aldosterone ELISA (MS E-5200) and the Active Renin ELISA (MS E-5300) the following ***Aldosterone-Renin Ratios*** were determined in plasma:

Ratio Aldosterone-Renin

	n	Mean (pg/ml / pg/ml)	Median (pg/ml / pg/ml)	2.5th – 97.5th Percentile (pg/ml / pg/ml)
Healthy Adults (K_3 EDTA)	89	8.68	5.30	0.52 – 37.83

These values are also valid for serum, K_2-EDTA plasma, Li-Heparin plasma and citrate 3.2% plasma.
These results correspond well to published reference ranges [16].

8.2 Urine Samples

In a study conducted with **urine samples (24-hours urine)** of apparently normal healthy adults, using the Aldosterone ELISA the following values are observed:

	n	Mean (µg/24 h)	Median (µg/24 h)	2.5th – 97.5th Percentile (µg/24 h)	Range (min. – max.) (µg/24 h)
Healthy Adults	8	11.34	9.40	3.31 – 25.09	3.06 – 27.17

These results correspond well to published reference ranges [8].

The results alone should not be the only reason for any therapeutic consequences. The results must be correlated to other clinical observations and diagnostic tests.

9. QUALITY CONTROL

Good quality assurance in the laboratory requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the Quality Control Laboratory are stated in the Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.
If available, it is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Apply appropriate statistical methods for analyzing control values and trends. If the results of the assay do not agree with the established acceptable ranges of control materials, patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above-mentioned items without finding any error contact your distributor or the manufacturer directly.

10. PERFORMANCE CHARACTERISTICS

10.1 Specificity of Antibodies (Cross-Reactivity)

The substances listed below were tested for cross-reactivity of the assay. Cross-reactivity must be < 10%:

Substance	Conc. Range of Spiked Substance	Mean Cross-Reactivity (%)
11-Deoxy Cortisol	10 – 1000 ng/ml	0.01
17-OH Progesterone	1.2 – 120 ng/ml	0.00
21-OH Progesterone	3.5 – 350 ng/ml	0.04
Androstenedione	0.2 – 22 ng/ml	0.00
Androsterone	10 – 1000 ng/ml	0.00
BSA	1 – 100 mg/ml	0.00
Cholesterol	0.5 – 50 mg/ml	0.00
Corticosterone	0.5 – 50 ng/ml	0.15
Cortisol	8 – 800 ng/ml	0.00
Cortisone	16 – 1600 ng/ml	0.01
Creatinine	50 – 5000 µg/ml	0.00

Substance	Conc. Range of Spiked Substance	Mean Cross-Reactivity (%)
DHEA	0.5 – 50 ng/ml	0.03
DHEA-S	300 – 30000 ng/ml	0.00
Estradiol	0.02 – 2 ng/ml	0.01
Estriol	1.5 – 150 ng/ml	0.00
Estrone	0.01 – 1 ng/ml	0.05
Glucose	1 – 100 mg/ml	0.00
Prednisolone	35 – 3500 ng/ml	0.00
Prednisone	35 – 3500 ng/ml	0.00
Pregnenolone	35 – 3500 ng/ml	0.00
Progesterone	42.2 – 4220 ng/ml	0.00
Testosterone	0.01 – 1 ng/ml	0.00

10.2 Detection Capability

Calculated according to CLSI guideline EP17-A2:2012.

	Serum	Urine
Limit of Blank (LoB)	5.359 pg/ml	
Limit of Detection (LoD)	7.374 pg/ml	8.902 pg/ml
Limit of Quantification (LoQ)	10.647 pg/ml	15.665 pg/ml
Lower Limit of Linear Interval (LLLI)	14.144 pg/ml	21.200 pg/ml
Measuring range	7.374 pg/ml – 1000 pg/ml	8.902 – 1000 pg/ml
Linear range	14.144 pg/ml – 1000 pg/ml	21.200 – 1000 pg/ml

10.3 Repeatability and Reproducibility

Designed on the basis of CLSI guideline EP5-A3:2014.

10.3.1 Repeatability

The repeatability was determined with 4 patient samples covering the complete measuring range within 20 days in 2 independent runs per day. CV was calculated as mean CV of 40 runs.
CV must be < 10%.

Serum	n	Mean (pg/ml)	CV (%)
1	5	55.66	6.5
2	5	88.88	6.7
3	5	325.82	3.8
4	5	709.07	4.5

Urine	n	Mean (pg/ml)	CV (%)
1	5	88.47	4.3
2	5	167.59	6.4
3	5	343.98	5.5
4	5	634.55	5.9

10.3.2 Reproducibility (Between-run Precision)

The between-run precision was determined with 4 samples covering the complete measuring range. The 4 samples were measured in 5 days with 5 replicates per run. The same procedure was performed with 4 urine samples.

Serum	n	Mean (pg/ml)	CV (%)
1	25	55.66	7.4
2	25	88.88	9.8
3	25	325.82	6.5
4	25	709.07	9.6

Urine	n	Mean (pg/ml)	CV (%)
1	25	88.47	8.2
2	25	167.59	7.3
3	25	343.98	7.5
4	25	634.55	8.3

10.3.3 Reproducibility (Between-lot Precision)

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots. CV must be < 15%.

Serum	n	Mean (pg/ml)	CV (%)
1	18	56.78	8.8
2	18	95.41	7.7
3	18	309.52	8.1
4	18	637.70	9.4

10.4 Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

Sample Type		K_3 EDTA	Li-Heparin	Citrate 3.2%	Serum	Urine	Urine	Urine	Urine
Highest concentration added [pg/ml]		80.58	80.58	80.58	94.42	168.95	168.95	121.98	69.69
Concentration [pg/ml]	19.25	49.52	298.45	589.33	589.33	79.50	153.08	178.98	233.42
Average Recovery [%]	92.0	100.0	104.5	94.5	94.5	99.9	98.8	103.0	100.6
Range of Recovery [%]	87.4	91.2	95.7	90.9	90.9	94.8	95.1	98.9	98.4
	103.4	112.8	112.4	98.2	98.2	103.0	104.3	104.6	105.3

10.5 Linearity

Samples containing different amounts of analyte were serially diluted with *Standard A.* The percentage recovery was calculated by comparing the expected and measured values for the analyte.

Sample Type		Li-Heparin	K_3 EDTA	Serum	Serum	Urine	Urine	Urine	Urine
Highest dilution		1:16	1:16	1:64	1:2	1:16	1:16	1:8	1:2
Concentration [pg/ml]		517.12	241.39	1085.81	85.90	379.70	592.90	1012.49	96.87
Average Recovery [%]		98.4	102.5	100.8	111.0	90.5	108.9	76.24	100.28
Range of Recovery [%]	from	92.7	93.8	88.3	108.5	85.8	106.5	86.2	90.7
	to	102.9	110.2	96.5	112.3	93.5	110.2	105.8	110.8

11. LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use and in compliance with the laboratory quality assurance guidelines.
Any improper handling of samples or modification of this test might influence the results.

11.1 Interfering Substances

11.1.1 Matrix Interference

No interference (bias < ± 20%) was found for addition of interferent up to concentration stated in the table below.

Bilirubin unconjugated	up to 0.3 mg/ml
Bilirubin conjugated	up to 0.1 mg/ml
Hemoglobin	up to 2.0 mg/ml
Triglyceride	up to 7.5 mg/ml
Cholesterol	up to 4.0 mg/ml
Ethanol	up to 0.5 mg/ml
Glucose	up to 12.5 mg/ml

11.1.2 Heterophilic Antibody Interference

Patient samples may contain heterophilic antibodies, including endogenous, polyclonal, weakly poly-specific human anti-mouse antibodies (HAMA) and endogenous, monospecific, high-affinity human anti-animal antibodies (HAAA). Heterophilic antibodies in a sample may cause false positive (mostly) or false negative results in immunoassays [17].
However, it is generally accepted that heterophile antibodies do not interfere in competitive binding, immunenephelo-metric or immunoturbidimetric assays [19]. Since the Aldosterone ELISA has a competitive assay format, no interference testing for heterophilic antibodies was done.

11.1.3 Autoantibody Interference

Patient samples may contain autoantibodies including Rheumatoid Factors (RFs) which are directed against endogenous substances of the patient. Autoantibodies may cause false positive (mostly) or false negative results in immunoassays [17]. In immunoassays, they react similar to heterophilic antibodies.
Heterophilic antibodies in a sample may cause false positive (mostly) or false negative results in immunoassays[9].
However, it is generally accepted that heterophile antibodies do not interfere in competitive binding, immunonephelometric or immunoturbidimetric assays [19]. Since the Aldosterone ELISA has a competitive assay format, no interference testing for autoimmune antibodies and RFs was done.

11.1.4 Drug Interferences

The following drugs were tested. Bias must be < 10%.

Substance	Concentration Range of spiked Substance (ng/ml)	Mean Bias (%)
Verapamil	21.6 – 2160	-1.91
Enalapril	4.24 – 424	-2.53
Acetylsalicylic Acid	652 – 65200	-0.38
Paracetamol	2000 – 200000	-3.47
Spironolacton	6 – 600	-1.93
Prazosine HCl	120 – 12000	-0.99
Fludrocortisone	20 – 2000	6.59
L-Ascorbic Acid	600 – 60000	1.39
Furosemide	60 – 6000	4.70
Dexamethasone	20 – 2000	4.93

11.2 High-Dose Hook Effect

"High-Dose Hook Effect" is not detected up to 20,000 pg/ml of Aldosterone.

11.3 Trueness (Bias)

The assay is calibrated in the range of the reference material (Certified reference material Cerilliant A-096). The difference from the expected value was below ± 20%.

Concentration	Bias	Uncertainty of the reference material
50.00 pg/ml	-6.7%	5.0%
150 pg/ml	-7.3%	2.7%
700 pg/ml	-8.4%	1.6%

12. LEGAL ASPECTS

12.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the laboratory quality assurance guidelines and applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. If there is any doubt or concern regarding a result, please contact the manufacturer.

12.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 12.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

12.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 12.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12.4 Reporting of Serious Incident

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

13. LITERATURE

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE marking of conformity
	Caution	REF	Catalogue number		Distributor
	Date of manufacture				