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User's Manual

Aldosterone RIA

Radioimmunoassay for the determination of Aldosterone in human serum and urine.

REF

IB78247



100

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

1. BACKGROUND

Aldosterone is a steroid hormone with molecular weight of 360 produced by the zona glomerulosa of the adrenal cortex. Blood levels of aldosterone follow also a circadian rhythm and are strictly related to states of rest or exercise, it is necessary to study blood aldosterone levels by means of stimulation and/or suppression dynamic tests.

2. PRINCIPLE OF THE ASSAY

The present method is based on a competitive radioimmunoassay (RIA). During the incubation, the sample/calibrator aldosterone competes with the aldosterone labeled with Iodine 125 (tracer) for the specific sites of the antiserum coated on the tubes. 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.
For research use only – Not for use in diagnostic procedures.

3. REAGENTS PROVIDED WITH THE KIT

- The reagents are sufficient for 100 determinations.
 - Store the kit and reagents at 2-8°C.
 - The expiration date of each reagent is shown on the label.
1. **Aldosterone ¹²⁵I-125 Radioactive Tracer:** 1 vial (50 ml) of ¹²⁵I-Aldosterone in phosphate buffer. Radioactivity contents: 85 KBq. Preservative: NaN₃ (<0.1%).
 2. **CAL 0 and CAL 1-6 Calibrators:** 8 vials of Aldosterone in human serum. Preservative: NaN₃ (<0.1%). Lyophilized. Reconstitute the Zero Calibrator with 3 ml of distilled water and the Calibrators 1-6 with 2 ml of distilled water. After reconstitution, aliquot and freeze unused calibrators and control at -20°C. For the exact value, refer to the Quality Control data sheet.
 3. **CONTROL Control:** 1 vial of Aldosterone in human serum. Preservative: NaN₃ (<0.1%). Lyophilized. Reconstitute with 2 ml of distilled water. After reconstitution, aliquot and freeze unused control at -20°C. For the exact value, refer to the Quality Control data sheet.
 4. **SORB CT Coated Tubes:** 100 tubes coated with anti-Aldosterone antibody. Unused tubes must be stored at 2-8°C, protected from moisture.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Plastic test tubes
- Test tube racks.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Graduated cylinder.
- Aspiration pump or automated washing device.
- Scintillation gamma counter.
- Distilled water.
- Orbital shaker adjustable at 150 rpm.
- Hydrochloric acid.

5. WARNINGS AND PRECAUTIONS FOR USERS

For research use only - Not for use in diagnostic procedures.

Only experienced laboratory personnel should use this test and handling should be in agreement with GLP.

Radioactive Material - Not for Internal or External Use in Humans or Animals.

This radioactive material may be received, acquired, possessed, and used only by physicians, laboratories or hospitals and only for laboratory tests not involving internal or external administration of the material, or the radiation there from to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of each country.

Physical characteristics of ¹²⁵I: see end of instructions

A. Safety Precautions: The following precautions should be observed in handling radioactive material:

- o Store radioactive materials in a designated area.
- o Do not eat, drink, smoke or apply cosmetics where radioactive materials are being handled.
- o Do not pipette by mouth.
- o Wear gloves when handling radioactive materials and wash hands thoroughly afterwards.
- o Cover working area with disposable absorbent paper.
- o Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste.
- o Dispose of the liquid radioactive waste into the sanitary sewage system if permitted by the local regulations.

B. CHEMICAL HAZARD Sodium Azide (NaN₃) Warning: Some of the reagents in this kit contain sodium azide as a preservative. For all such reagents, the concentration of sodium azide is <0.1% w/w. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Dispose of all non radioactive reagents by flushing with large amounts of water through the plumbing system.

H370 : very toxic if swallowed

EUH 031: contact with acids liberates very toxic gas

C. POTENTIAL BIOHAZARDOUS MATERIAL Warning: This kit may contain some reagents made with human serum or plasma. The serum or plasma used has been tested by an FDA-approved method and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. Because no method can offer complete assurance that HIV-1/2, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 3rd Edition 1993.

6. SAMPLE COLLECTION

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.

7. ASSAY PROCEDURE

- Bring all reagents and samples to room temperature prior to use.
 - Before use, mix the samples by inversion.
 - For all calibrators, a duplicated measure is recommended.
1. Prepare plain tubes for Total Activity (T) and Non-specific Binding (NSB) as well as coated tubes for Zero Calibrator (Bo), Calibrators (1-6), Control and Samples.
 2. Pipette **200 µl** of each Calibrator, Control and Sample into each 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 set at 150 rpm.
 7. Carefully aspirate the incubation mixture from all tubes, except those for total activity, with an aspiration 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 | Total Activity | NSB | Cali-brators | Control | Samples | Urine samples |
|---|----------------|--------|--------------|---------|---------|---------------|
| Reagent | | | | | | |
| 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 |
| <ul style="list-style-type: none"> • Incubate: 18 – 24 h R.T. or 3 h R.T. shaking (150 rpm) • Aspirate • Count | | | | | | |

8. RESULTS

Draw the calibration curve on logit-log or semi-log paper, with the calibration 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 calibration curve to obtain the Aldosterone concentration in the tested samples, expressed in pg/ml.

Urine Samples.

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

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

EXAMPLE OF CALCULATION

The values reported below must be considered as an example and may not be used in place of experimental data.

| Description | Average cpm | B/B0 (%) | 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 |

9. 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 |

9.1 Other sample type:

| | |
|----------------------|------------------------------|
| Urinary Aldosterone: | <18µg/24h (95th percentile) |
| Plasma EDTA : | <122 pg/ml (95th percentile) |

10. PERFORMANCES OF THE ASSAY**SPECIFICITY**

The percentage of cross-reactivity is calculated by using the EP7-A2 method.

% Cross reactivity = $100 \times ((\text{measured value} - \text{true value}) / \text{interfering concentration})$.

The present method has shown the following cross-reactions:

| Substance | % cross reactivity |
|------------------------|--------------------|
| Cortisol | 0.00 |
| 17 Hydroxyprogesterone | 0.33 |
| Progesterone | 0.06 |
| Androstenedione | 0.07 |
| Prednisolone | 0.001 |
| Corticosterone | 0.015 |
| 11-Deoxycortisol | 0.380 |
| 11-Deoxycorticosterone | 7.952 |
| Prednisone | 0.001 |
| Testosterone | 0.238 |

SENSITIVITY**Analytical sensitivity**

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value - 2 S.D.). This dose is 1.4 pg/ml.

Functional sensitivity

The functional assay sensitivity is the lowest value which is measured with a precision of maximum 20% inter-assay variance. For the Aldosterone, this value is 25 pg/ml.

PRECISION

Precision was evaluated upon intra- and inter-assay variability at different analyte concentrations.

Intra-assay

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

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 | 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 |

Physical characteristics of ¹²⁵I

$t_{1/2} = 59.9$ days,

Main emissions

| | E (MeV) | % |
|----------|---------|-----|
| γ | 0.035 | |
| X | 0.027 | 114 |
| | 0.032 | 25 |












11. BIBLIOGRAPHY

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|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las Instrucciones | Consultare le istruzioni per l'uso |
|  | In vitro diagnostic device | In-vitro-Diagnostikum | Ussage Diagnostic in vitro | Diagnóstico in vitro | Per uso Diagnostica in vitro |
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