DRG® Aldosterone CLIA (CLA-4650)

CE

RUO in the USA

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1 INTENDED USE

For determination of Aldosterone in human serum by chemiluminescence immunoassay (CLIA). In the United States, this kit is intended for Research Use Only.

2 PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The kit control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. When dispensing the substrate, do not use pipettes in which this liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

3 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of aldosterone in human serum. The kit is not calibrated for the determination of aldosterone in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. Only the urine diluent may be used to dilute any high urine samples. The use of any other reagents may lead to false results.

4 SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

5 SPECIMEN COLLECTION AND STORAGE

Serum: Approximately 0.2 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer.
Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Urine: Approximately 1 ml of urine is required per duplicate determination. Collect 24-hour urine into a specimen collection container. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

5.1 SERUM PRETREATMENT

No specimen pretreatment is necessary.

5.2 URINE PRETREATMENT

1. Label one glass or polypropylene tube for each urine sample.
2. Pipet 1 mL of each urine sample into an appropriate tube.
   * If the sample is cloudy, first centrifuge the urine and work with the supernatant.
3. Hydrolysis: Add 0.1 mL of 3.2 N HCl (not supplied) to every tube. Cap securely and heat for 1 hour at 60°C in the dark.
   * 3.2 N HCl can be made by adding 1 mL of concentrated HCl (1 2N) to 2.75 mL distilled water.
4. Neutralization: Add 0.1 mL of 3.2 N NaOH to every tube and mix gently and thoroughly.
   * 3.2 N NaOH can be made by dissolving 1.28 grams of NaOH pellets into 10 mL distilled water.
5. Dilution: Dilute the neutralized samples 1:50 with calibrator A.

6 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. 3.2 N HCl and 3.2 N NaOH (for urine analysis)
5. Glass or polypropylene tubes (for urine analysis)
6. Water bath (for urine analysis)
7. Plate shaker
8. Microwell plate luminometer

7 REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-Aldosterone Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (1 2x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

2. Aldosterone-Biotin : Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: Aldosterone-biotin and avidin-HRP conjugates in a protein-based buffer with a non-mercury preservative.
Volume: 200 µl/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute the aldosterone-biotin:avidin-HRP concentrate 1:100 in assay buffer before use. If the whole plate is to be used dilute 120 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

3. Aldosterone Calibrators - Ready To Use.
Contents: Six vials containing aldosterone in a protein-based buffer with a non-mercury preservative.
Prepared by spiking matrix with a defined quantity of aldosterone.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/ml	2.0 ml
Calibrator B	20 pg/ml	0.5 ml
Calibrator C	80 pg/ml	0.5 ml
Calibrator D	300 pg/ml	0.5 ml
Calibrator E	800 pg/ml	0.5 ml
Calibrator F	2000 pg/ml	0.5 ml

Storage: Refrigerate at 2-8°C

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Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Control - Ready To Use.
Contents: One vial containing aldosterone in a protein-based buffer with a non-mercury preservative.
Prepared by spiking buffer with a defined quantity of aldosterone.
Refer to vial label for expected value and acceptable range.
Volume: 0.5 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate - Requires Preparation.
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

6. Assay Buffer - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C Stability: 12 months or as indicated on label.

7. Chemiluminescence Substrate Reagent A - Requires Preparation.
Contents: One bottle containing luminol enhancer.
Volume: 1 ml/bottle
Storage: Refrigerate at 2-8°C Stability: 12 months or as indicated on label.
Preparation: See below.

8. Chemiluminescence Substrate Reagent B - Requires Preparation.
Contents: One vial containing peroxide solution. Volume: 1 ml/vial
Storage: Refrigerate at 2-8°C Stability: 12 months or as indicated on label.
Preparation: See below.

9. Chemiluminescence Substrate Reagent C - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative. Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C Stability: 12 months or as indicated on label.
Preparation: See below.

7.1 Preparation of Working Substrate Solution:

Mix **1 part** of the chemiluminescence substrate **reagent A** with **1 part of reagent B** and dilute this mixture **1:5 with reagent C**. This gives the ready to use substrate solution. Prepare fresh for each use.

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If the whole plate is to be used prepare working substrate solution as follows:

Combine 1 ml of reagent A with 1 ml of reagent B. To the 2 ml of this mixture add 10 ml of reagent C.
Total volume=12 ml of working substrate solution.

Stability: Working substrate solution is stable for 24 hours at room temperature.

8 ASSAY PROCEDURE

Specimen Pretreatment:

Serum: None.

Urine: Hydrolysis, Neutralization and Dilution (see detailed instructions under Urine Pretreatment)

Important Notes:

1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

Procedure:

1. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
2. Pipette 50 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
4. Incubate on a plate shaker (approximately 200 rpm) for 60 minutes at room temperature.
5. Wash the wells 5 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended).
6. Pipette 100 µl of chemiluminescence working substrate solution into each well.
   (We recommend using a multichannel pipette).
7. Incubate without shaking for 10 minutes at room temperature.
8. Measure the RLUs in each well on a microplate luminometer within 20 minutes after addition of the substrate.

9 CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the **serum** samples directly off the calibrator curve. If a serum sample reads more than 2000 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor
5. Read the values of the **urine** samples directly off the curve and multiply by a factor of 60 (the original urine samples are diluted 1-in-1.2 and 1 -in-50, see the urine pretreatment). Next, multiply by the volume of collected 24-hour urine (in litres). Finally, divide this figure by 1000 to obtain values in g/24 hour If a urine sample reads more than 2000 pg/ml then dilute it with the calibrator A at a dilution of no more than 1:2 (from the original 1:50 dilution). The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA**

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLUMAX (%)
A, 0 pg/ml	1561	1445	1503	100
B, 20 pg/ml	1085	1097	1091	73
C, 80 pg/ml	593	593	593	40
D, 300 pg/ml	193	194	194	13
E, 800 pg/ml	44	43	44	3
F, 2000 pg/ml	7	5	6	0.4

**- It is recommended to use the RLU/RLUMAX values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLUMAX values remain consistent.

10 REFERENCES

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