Aldosterone (Plasma) ELISA

For the quantitative determination of aldosterone in serum, plasma, and urine

Please read carefully due to Critical Changes, e.g., Reagents and Assay Procedure

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 11-ALDHU-E01-PLAS
Size: 96 wells
Version: 8.0 May 25, 2015 - ALPCO June 10, 2015
INTENDED USE
For the direct quantitative determination of Aldosterone in human serum, plasma and urine by an enzyme linked immunosorbent assay. For Research Use Only. Not For Use In Diagnostic Procedures.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. 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 stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the color 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 samples and controls can be directly read.

APPLICATIONS
Aldosterone is a potent mineral corticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the re-absorption 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.

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 controls 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 controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
11. When dispensing the substrate and stop solution, do not use pipettes in which these 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.

**LIMITATIONS**

1. All the reagents within the kit are calibrated for the determination of aldosterone in human serum, plasma and urine. The kit is not calibrated for the determination of aldosterone in saliva or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum or plasma.
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 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.
5. This kit is for research use only.

**SAFETY CAUTIONS AND WARNINGS POTENTIAL BIOHAZARDOUS MATERIAL**

Human serum that may be used in the preparation of the standards and controls 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.

**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 labeled 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.

**Plasma:** Approximately 0.2 ml of plasma is required per duplicate determination. Collect 4-5 ml of blood into EDTA plasma tubes. 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 0.2 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.

**SPECIMEN PRETREATMENT**

**Serum and plasma:** This assay is a direct system; no specimen pretreatment is necessary.

**Urine:** Dilute urine samples 1:50 in urine diluent before use.

**Example:** To 1 ml of urine diluent, add 20 μl of urine sample.

**REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Precision pipettes to dispense 10-1000 μl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).
6. Urine Diluent - Required if urine samples are to be analyzed. Used for dilution of urine specimens before assaying. Available in any quantity.

**REAGENTS PROVIDED**

1. **Rabbit Anti-Aldosterone Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.
   Contents: One 96 well (12x8) 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-Horse Radish Peroxidase (HRP) Conjugate Concentrate** - Ready to use
   Contents: Aldosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

3. **Aldosterone Calibrators** - Ready To Use.
   Contents: Six vials containing aldosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of aldosterone.

   *Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0 pg/ml</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>15 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>50 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>200 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>500 pg/ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>1000 pg/ml</td>
<td>0.6 ml</td>
</tr>
</tbody>
</table>

   Storage: Refrigerate at 2-8°C
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquotted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** - Ready To Use.
   Contents: Two vials containing aldosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of aldosterone. Refer to vial labels the acceptable range.
   Volume: 0.6 ml/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquotted 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. **TMB Substrate** - Ready To Use.
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

7. **Stop Solution** - Ready To Use.
Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

**ASSAY PROCEDURE**

**Specimen Pretreatment:**

*Serum and plasma: None.*

*Urine: Dilute 1:50 in Urine Diluent Before Use.*

All reagents must reach room temperature before use. Calibrators, controls and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the wash buffer. Dilute any urine samples if they are to be analyzed.

2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.

3. Pipette 50 μl of each calibrator, control and sample (serum or diluted urine) into correspondingly labeled wells in duplicate.

4. Pipette 100 μl of the aldosterone-HRP conjugate into each well (The use of a multichannel pipette is recommended).

5. Incubate on a plate shaker (approximately 200 rpm) for one hour at room temperature.

6. Wash the wells three 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).

7. Pipette 150 μl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 15-20 minutes at room temperature (or until calibrator A attains dark blue color for desired OD).

9. Pipette 50 μl of stop solution into each well at the same timed intervals as in step 7.

10. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower; however, this will not affect the results of the samples and control.

**CALCULATIONS**
1. Calculate the mean optical density of each calibrator duplicate.

2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.

3. Calculate the mean optical density of each unknown duplicate.

4. Read the values of the serum and plasma samples directly off the calibrator curve.

5. Read the values of the urine samples directly off the curve and multiply by a factor of 50. Next, multiply by the volume of collected 24-hour urine (in mL) to obtain values in pg/24 hour. Finally, divide the pg/24 hour values by 1x10⁶ to obtain values in μg/24 hour.

6. If a serum or plasma sample reads more than 1000 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. If a urine sample reads more than 1000 pg/ml then dilute it with the urine diluent 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**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Mean OD</th>
<th>Value (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.278</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2.167</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>1.798</td>
<td>50</td>
</tr>
<tr>
<td>D</td>
<td>0.950</td>
<td>200</td>
</tr>
<tr>
<td>E</td>
<td>0.485</td>
<td>500</td>
</tr>
<tr>
<td>F</td>
<td>0.322</td>
<td>1000</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.383</td>
<td>103</td>
</tr>
</tbody>
</table>

**TYPICAL CALIBRATOR CURVE**
Sample curve only. **Do not** use to calculate results.
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:
\[
\text{LoD} = \mu_B + 1.645\sigma_B + 1.645\sigma_S,
\]
where \(\sigma_B\) and \(\sigma_S\) are the standard deviation of the blank and low value sample and \(\mu_B\) is the mean value of the blank.
The Limit of Detection (LoD) was determined to be 14 pg/mL.

SPECIFICITY (CROSS-REACTIVITY)
The following compounds were tested for cross-reactivity with the Aldosterone ELISA kit with aldosterone cross-reacting at 100%.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>%Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>100</td>
</tr>
<tr>
<td>11-Deoxycorticosterone</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The following steroids were tested but cross-reacted at less than 0.001%: Androsterone, Cortisone, 11-Deoxycortisol, 21-Deoxycortisol, Dihydrotestosterone, Estradiol, Estriol, Estrone and Testosterone.

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in pg/ml) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78.6</td>
<td>5.89</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>204</td>
<td>7.75</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>386</td>
<td>15.05</td>
<td>3.9</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in pg/ml) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.36</td>
<td>1.72</td>
<td>9.4</td>
</tr>
<tr>
<td>2</td>
<td>128.52</td>
<td>12.50</td>
<td>9.7</td>
</tr>
<tr>
<td>3</td>
<td>505.77</td>
<td>48.55</td>
<td>9.6</td>
</tr>
</tbody>
</table>
**RECOVERY**
Spiked samples were prepared by adding defined amounts of aldosterone to three serum samples. The results (in pg/ml) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs.Result</th>
<th>Exp.Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+51.0</td>
<td>45.30</td>
<td>96.3</td>
<td>123.7</td>
</tr>
<tr>
<td>+101.90</td>
<td>119.1</td>
<td>147.2</td>
<td>97.6</td>
</tr>
<tr>
<td>+203.80</td>
<td>143.8</td>
<td>249.1</td>
<td>91.3</td>
</tr>
<tr>
<td>2 Unspiked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+51.0</td>
<td>130.0</td>
<td>181.0</td>
<td>115.7</td>
</tr>
<tr>
<td>+101.90</td>
<td>209.4</td>
<td>231.9</td>
<td>104.8</td>
</tr>
<tr>
<td>+203.80</td>
<td>243.1</td>
<td>333.8</td>
<td>92.1</td>
</tr>
<tr>
<td>3 Unspiked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+51.0</td>
<td>208.4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>+101.90</td>
<td>289.3</td>
<td>259.4</td>
<td>111.5</td>
</tr>
<tr>
<td>+203.80</td>
<td>341.6</td>
<td>310.3</td>
<td>110.1</td>
</tr>
</tbody>
</table>

**LINEARITY**
Two serum samples were diluted with calibrator A. The results (in pg/ml) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs.Result</th>
<th>Exp.Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>395.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>198.6</td>
<td>197.6</td>
<td>100.5</td>
</tr>
<tr>
<td>1:4</td>
<td>80.7</td>
<td>98.8</td>
<td>81.7</td>
</tr>
<tr>
<td>1:8</td>
<td>44.2</td>
<td>49.5</td>
<td>89.5</td>
</tr>
<tr>
<td>2</td>
<td>414.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>206.7</td>
<td>207.1</td>
<td>99.8</td>
</tr>
<tr>
<td>1:4</td>
<td>103.9</td>
<td>103.6</td>
<td>100.3</td>
</tr>
<tr>
<td>1:8</td>
<td>56.7</td>
<td>51.8</td>
<td>109.5</td>
</tr>
</tbody>
</table>

**EXPECTED NORMAL VALUES-SERUM / PLASMA**
Each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Range (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Salt Intake, Recumbent</td>
<td>120</td>
<td>15 – 133</td>
</tr>
</tbody>
</table>

**REFERENCE NORMAL VALUES-URINE**
Each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (μg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Salt Intake</td>
<td>5-19</td>
</tr>
</tbody>
</table>

REFERENCES