MAGLUMI Aldosterone (CLIA)

**INTENDED USE**
The kit has been designed for the quantitative determination of Aldosterone (ALD) in human serum or plasma. The method can be used for samples over the range of 5-2000 pg/ml.
The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (including Maglumi 600, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 3000 and Maglumi 4000).

**SUMMARY AND EXPLANATION OF THE TEST**
Aldosterone (ALD) is a steroid hormone produced by the adrenal cortex, which controls salt and water balance in the kidney. Abnormally high levels of this hormone can cause sodium retention, high blood pressure, heart rhythm irregularities and possibly paralysis. Normally, aldosterone is mediated by renin-angiotensin system. Moreover, sodium and potassium level, ACTH, adrenal glands and dopamine also regulate the secretion of aldosterone.

Human blood aldosterone mainly binds to plasma albumin, seldom bounds to CBG. Therefore, aldosterone has a relatively short half-life (35min) and higher metabolized clearance. The non-metabolized aldosterone in human urine accounts for 6% of the secretion amount and contains hormone activity. The determination of aldosterone in human plasma or urine is of great value for diagnosis and identification of some disease.

**PRINCIPLE OF THE TEST**
Competitive immunoluminometric assay;
Use a purified ALD antigen to label FITC, and use an anti-ALD monoclonal antibody to label ABEI. Sample, Calibrator or Control, with ABEI Label, FITC Label, and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of ALD present in samples.

**KIT COMPONENTS**

<table>
<thead>
<tr>
<th>Material Supplies</th>
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<tbody>
<tr>
<td>Reagent Integral for 100 determinations</td>
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<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2% (W/V), 0.2%NaNO₃, coated with sheep anti-FITC polyclonal antibody.</td>
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<tr>
<td>Calibrator Low: bovine serum, 0.2%NaNO₃</td>
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<td>Calibrator High: bovine serum, 0.2%NaNO₃</td>
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<tr>
<td>Displacing Reagent: bovine serum, 1%ANS</td>
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<tr>
<td>FITC Label: purified ALD antigen labeled FITC, containing BSA, 0.2%NaNO₃</td>
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<tr>
<td>ABEI Label: anti-ALD polyclonal antibody labeled ABEI, containing BSA, 0.2%NaNO₃</td>
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<td>All reagents are provided ready-to-use.</td>
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**Reagent Vials in kit box**

| Internal Quality Control: containing BSA, 0.2%NaNO₃ (target value refer to Quality Control Information date sheet) | 2.0ml |

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own
Preparation of the Reagent Integral
Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation). Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability
- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it’s not going to be used on board during the next 12 hours.

Keep upright for storage.

Keep away from sunlight.

CALIBRATION AND TRACEABILITY
1) Traceability
To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance
Calibrators in the Reagent Kit are from Sigma

2) 2-Point Recalibration
Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration
- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI.
- If controls are beyond the expected range.
- The room temperature has changed more than 5°C (recommendation).

SPECIMEN COLLECTION AND PREPARATION

Serum
- Elbow vein blood 5ml in the tube, centrifugation at room temperature, serum was separated and stored at 2°C-8°C.
- Serum samples were stable for 12 hours at 2-8°C. For longer storage periods freeze to below -20°C.
- Avoid repeated freezing and thawing.

Plasma
- Elbow vein blood 5ml in the tube, then add 50ul EDTA anticoagulant (50ul 0.3M EDTA per 5ml blood), centrifuged and separated plasma, stored at 2-8°C.
- (Note: Suggest to use EDTA or heparin sodium as anticoagulants)
- Plasma was stable at 2-8°C for 24 hours. For longer storage periods freeze to below -20°C.
- Avoid repeated freezing and thawing.

Specimen Conditions
- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis
- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting.
- Specimens must be thoroughly mixed and separated plasma, stored at 2°C-8°C.
- After centrifugation, avoid the lipid layer (if present) when pipetting.
- Centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting.
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- Specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service, for a more detailed discussion of onboard sample storage constraints.

Storage
If testing will be delayed for more than 8 hours, remove serum or plasma from the serum separator, red blood cells or clot. Serum specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C. Plasma specimen removed from the separator gel, cells or clot may be stored up to 24 hours at 2-8°C.

Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping
Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

- For use in IN-VITRO diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are
any deviations from the instructions in this package insert.

Safety Precautions

**CAUTION:** This product requires the handling of human specimens.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.

- All samples, biological reagents and materials used in the assay must be considered potentially infectious. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be neutralised with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach. A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.[13]

- Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

- This product contains Sodium Azide; this material and its hazard and handled with the same safety Level 214 or other appropriate biosafety practices should be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results.

- Interfering Substances

  - No interference with test results is seen by concentrations of bilirubin<0.06mg/ml, haemoglobin<16mg/dl or triglycerides<12.5mg/ml.

- HAMA

  - Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

**RESULTS**

1) Calculation of Results

The analyzer automatically calculates the ALD concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/ml. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

2) Interpretation of Results

- Reference values of Serum:
  - Standing upright: 70-300 pg/ml
  - Lying down: 30-160 pg/ml

- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.
Inter-assay coefficient of variation was evaluated on three batches of kit. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

2) Analytical Sensitivity
The sensitivity is defined as the concentration of ALD equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 5.0 pg/ml.

3) Specificity
The specificity of the ALD assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

4) Recovery
Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratios with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90%-110%.

5) Linearity
Use ALD calibrator to prepare the six point standard curve, measuring all points’ RLU except point A, and then do four parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient (r) should be bigger than 0.9800.

6) Method comparison
A comparison of MAGLUMI ALD (y) with a commercially available ALD test (x) using clinical samples gave the following correlations (pg/ml):

<table>
<thead>
<tr>
<th>Comparator</th>
<th>Mean Measuring</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>110.26 pg/ml</td>
<td>115.78 pg/ml</td>
<td>105%</td>
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REFERENCES