MAGLUMI A Ⅱ (CLIA)

REF 130206006M Σ 100

EC REP

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CE

FOR PROFESSIONAL USE ONLY
Store at 2...8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

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SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in Europe
	Manufacturer
	Attention. See Instructions For Use
CONT	Contents of kit
IVD	In vitro diagnostic medical device (In vitro diagnostic use)
LOT	Lot number
REF	Catalogue Code
	Expiry date (Use by…)
8°C 2°C	Temperature limitation (store at 2...8 °C)
Σ	Number of tests
	Keep away from direct sunlight
	Keep upright for storage

INTENDED USE

The kit has been designed for the quantitative determination of Angiotensin Ⅱ (A Ⅱ) in human plasma.

The method can be used for samples over the range of 0-1000 pg/ml.

The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

SUMMARY AND EXPLANATION OF THE TEST

Angiotensin is a polypeptide existing in two forms in the blood, which is associated with high blood pressure. Angiotensin I is an inactive decapeptide produced by the action of rennin，a kidney enzyme released when blood pressure is low，on a globulin in the blood. The active form， angiotensin Ⅱ, is formed by the enzyme-catalyzed removal of two terminal amino acids from angiotensin I. Angiotensin Ⅱ raises blood pressure by stimulating the constriction of arterioles and the secretion of the hormone aldosterone. High blood pressure may be treated by inhibiting the enzyme responsible for converting inactive angiotensin I to active angiotensin Ⅱ.

Angiotensin I is formed by the action of rennin on angiotensinogen. Renin is produced in the kidneys in response to both decreased intra-renal blood pressure at the juxtaglomerular cells, or decreased delivery of Na^+ and Cl^- to the macula densa. If more Na+ is sensed, rennin release is decreased.

Renin cleaves the peptide bond between the leucine (Leu) and valine (Val) residues on angiotensinogen, creating the ten amino acid peptide (des-Asp) angiotensin I. Angiotensin I appears to have no biological activity and exists solely as a precursor to angiotensin Ⅱ.

Angiotensin I is converted to angiotensin Ⅱ through removal of two C-terminal residues by the enzyme angiotensin-converting enzyme (ACE, or kinase), which is found predominantly in the capillaries of the lung.[3] ACE is actually found all over the body, but has its highest density in the lung due to the high density of capillary beds there. Angiotensin Ⅱ acts as an endocrine, autocrine/paracrine, and intracrine hormone.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay:

Use purified A Ⅱ antigen to label ABEI, and use anti-A Ⅱ polyclonal antibody to coat nano magnetic microbeads. Sample, Calibrators or Control with ABEI Label nano magnetic microbeads coated with sheep anti- A Ⅱ are mixed thoroughly and incubated at 37℃, forming 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 A Ⅱ present in controls or samples.

CONT KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations

Nano magnetic microbeads: TRIS buffer, 1.2% (W/V), 0.2%NaN_3, coated with sheep anti-A II polyclonal antibody.	2.5ml
Calibrator low: bovine serum, 0.2%NaN_3.	3.0ml
Calibrator high: bovine serum, 0.2%NaN_3.	3.0ml
ABEI Label: A II purified antigen labeled ABEI, containing BSA, 0.2% NaN_3.	7.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml
Anticoagulant: 0.3mol/L EDTA-2K buffer	5.5ml
Enzyme Inhibitor: 0.34mol/L 8-Hydroxyquin-oline sulfate solution	5.5ml
Dimercaprol：0.32 mol/L	3.0ml

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

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 color 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 ℃ 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 direct 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 lots (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

SPECIMEN COLLECTION AND PREPARATION

Test process:

1**.** Draw elbow vein blood 5ml and then add the anticoagulants and enzyme inhibitors by the ratios below:

5ml	Whole blood in one tube
+50μl	Anticoagulants (if there're any precipitation of crystals, please use water bath to redissolve them before using)
+25μl	0.32 mol/L Dimercaprol
+50ul	Enzyme Inhibitors

Note :

- The anticoagulants and Enzyme Inhibitors could not be mixed together before added into the blood! (The correct procedure is anticoagulants firstly and the enzyme inhibitors secondly)
- If using the Vacuum tubes with anticoagulants additives, then please make sure that use EDTA (EDTA-2K or EDTA-4Na) as anticoagulant. In this situation, there is no need to add anticoagulant, only adding the Enzyme Inhibitor directly.
- If the volume of sample is different, please increase or decrease the reagents to the same proportion. For example, 2ml blood, with 20ul Anticoagulants, 10ul Dimercaprol and 20ul Enzyme Inhibitors.

2. After sealing up the tube with cap, turn tube upside down several times to mix the sample.

And place it in ice-water bath or 4℃ refrigerator for 1-2 hours right now, then centrifuge the tube for 7 minutes with 4020 r/min, (preferably centrifuged at 4℃) to separate the plasma. If the plasma need to be stored for long time, please seal and stored in the refrigerator (below -15℃).

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.
(b) If plasma sample is needed, EDTA tube is conformed has no effect on the results RLUs.
(c) Liquaemin Sodium tube is found to increase the sample RLU and cause test results deviation.
(d) Please ask SNIBE for advice if special additive must be used in the sample blood.

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 and plasma 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 the specimen into a sample cup or secondary tube.
- 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 or plasma separator, red blood cells or clot. Specimens 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

IVD

- 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 able to transmit infectious agents. 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 decontaminated 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 (USP 24, 2000, p.2143). A minimum of one hour at 121℃ 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 container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

100µl +50µl +20µl	Sample, calibrator or controls ABEI label Nano magnetic microbeads
10min	Incubation
400µl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.
Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

(1) β-blockers, vasodilators, diuretics and steroids, licorice and other material interfere the renin, the PRA should be measured after stopping using them for two weeks. For reserpine and other drugs with slower metabolism, the PRA should be measured after three weeks. Patients who shouldn't stop using drug, can change to use drugs like guanethidine.
(2) PRA levels can be interfered by the intake of sodium, so the patient should decrease the intake of salt three days before the determination of PRA. Patients should be measured 24-hours-urinary sodium, before drawing blood and the results can be used for the analysis of reference.
(3) Provocation test: the patient does not get up in the morning, or lie down for two hours. Drawing blood between 6:00-8:00, and then inject furosemide with 0.7mg/kg of body weight, but the total dose should less than 50mg. Keep upright for two hours (the patient can walk), then draw blood samples in excited state.
(4) During the two hours after injection of furosemide, a large amount of water and electrolytes lost with the urine. If the patients have low serum potassium, it's better to supply that before drawing

blood. Patients may feel thirsty, weakness, sweating, etc. during the test, generally not heavy. If the symptom is overweight, the test should be terminated.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<0.125mg/ml, haemoglobin<16mg/dl or triglycerides< 12.5mg/ml.

3) HAMA

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

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the A Ⅱconcentration 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 MAGLUMI Chemiluminescence Analyzer Operating Instructions.

2) Interpretation of Results

- Reference values:

Diet	Status	Reference values
Normal	Lying position	25 pg/ml -60 pg/ml
	Standing position	50 pg/ml -120 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.

Intra-assay precision			
Control	Mean(pg/ml)	SD(ng/ml)	CV%
Level 1	69.05	5.31	8.01
Level 2	95.34	6.84	7.20
Level 3	118.23	7.91	6.70

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(pg/ml)	SD(ng/ml)	CV%
Level 1	70.45	5.78	8.20
Level 2	94.92	6.76	7.12
Level 3	118.63	7.43	6.30

2) Analytical Sensitivity

The sensitivity is defined as the concentration of A Ⅱ 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 pg/ml.

3) Specificity

The specificity of the A Ⅱ assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
A I	10000pg/ml	0.2%

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio 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%.

Expected	Mean Measuring	Recovery
306.619 pg/ml	311.624 ng/ml	102%

5) Linearity

Use A Ⅱ calibrator to prepare the sixpoint 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.

Calibrator Point	Concentration pg/ml	Absolute linear correlation coefficient (r)
A	0	
B	20.0	r=0.9850
C	100.0	
D	250.0	
E	500.0	
F	1000.0	

(6)Method comparison

A comparison of MAGLUMI AII(y) with a commercially available AII(x) using clinical samples gave the following correlations(ng/ml):

Linear regression

y=0.95x+100

r=0.955

Sy.x=202

Number of samples measured: 200

The sample concentrations were between 15-900ng/ml.

REFERENCES

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