Instruction

EURIA-Angiotensin II

Angiotensin II radioimmunoassay

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For Research Use Only. Not for use in diagnostic procedures.

REF **RB 320 RUO**

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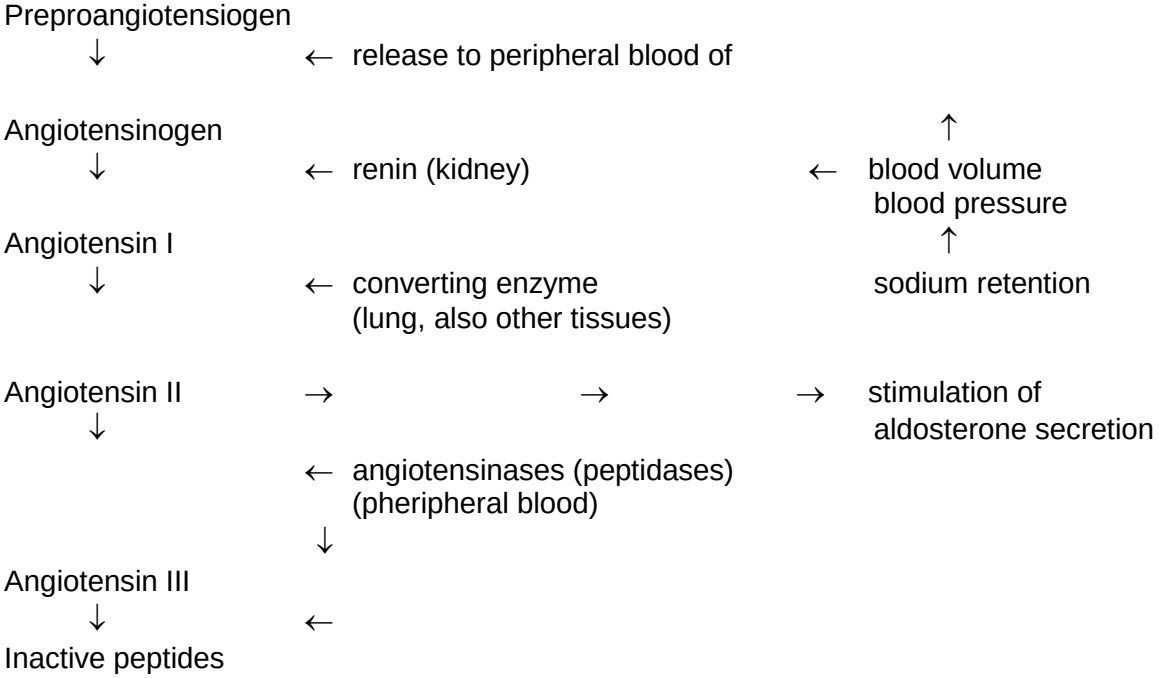
PURPOSE OF RESEARCH PRODUCT

The Euro-Diagnostica angiotensin II kit contains reagents and instructions for the measurement of angiotensin II in plasma. After extraction the angiotensin II concentrations are measured by radioimmunoassay (RIA).
The result shall not be used for clinical diagnosis or patient management.

RESEARCH APPLICATION

Angiotensin II is the biologically active product of the renin-angiotensin system (1,2).
The octapeptide angiotensin II (molecular weight 1046) is the strongest physiological vasoconstrictor known. From a large protein precursor (pre-proangiontesinogen) synthesized in the liver it is liberated in a series of proteolytic steps catalyzed by enzymes from various tissues (1, 2 4). Angiotensin II is very short-lived in the plasma: Once generated from angiotensin I, it is degraded further into physiologically inactive peptides by various plasma peptidases, at a plasma half life of less than a minute (5). The scheme below gives an outline of the so-called renin-angiotensin system:



Since the generation of angiotensin II from angiotensinogen via angiotensin I is strongly affected by changes of the renin activity, all external factors influencing renin activity are to be carefully considered: renin activity is elevated during pregnancy, after sodium depletion, in upright position, and under the influence of a range of drugs, e.g. oral contraceptives, adrenalin, antihypertensive vasodilatators, diuretics, high doses of spironalactone and progesterone. Factors decreasing renin activity are: horizontal position, increased sodium uptake, a-methyl-DOPA, L-DOPA, propranolol, reserpin, clonidin and old age. Renin activity is also subject to a diurnal rhythm with peak values in the morning.

The angiotensin II radioimmunoassay is useful in the study of hypertension monitoring and treatment.

PRINCIPLE OF THE TEST

After extraction of the plasma samples, angiotensin II is assayed by a competitive radioimmunoassay. This radioimmunoassay is using a rabbit anti-angiotensin II antiserum and a radio-iodinated angiotensin II tracer. Bound and free phases are separated by a second antibody bound to solid phase particles, followed by a centrifugation step. The radioactivity in the bound fractions is measured and a typical standard curve can be generated.

PRECAUTIONS

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

1. Materials derived from human blood and used in the preparation of this kit were tested and found negative for hepatitis B surface antigen (HBsAg), antibodies to HCV and for antibodies to HIV-1 and HIV-2. However, handle all components as a possible source of infection.

2. The reagents in this kit contain sodium azide. Contact with copper or lead drain pipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbling suspected of being contaminated with these explosive deposits should be cleaned thoroughly with 10% sodium hydroxide solution.

3. This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. The radioactive material included may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals for laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulation of each country.

Adherence to the basic rules of radiation safety should provide adequate protection.

- Do not eat, drink, smoke or apply cosmetics where radioactive materials are used.
- Do not pipette radioactive solutions by mouth.
- Avoid direct contact with all radioactive materials by using protective articles such as lab coats and disposable gloves.
- All radiological work should be done in a designated area.
- Radioactive materials should be stored in original containers in a designated area.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radio-isotopes.
- Any radioactive spills should be taken care of immediately in accordance with established procedures.
- All radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies jurisdiction over the laboratory.

SPECIMEN COLLECTION

Careful standardization of the subject preparation and sampling conditions is recommended. Due to the extreme lability of angiotensin II in biological fluid much care must be taken to ensure that the blood sample is collected properly:

- draw blood from fasting subject in recumbent position into cold tube containing EDTA;
- centrifuge immediately at 4° C to separate the plasma;
- freeze the sample immediately in plastic tubes at -20° C until assayed.

MATERIALS AND EQUIPMENT REQUIRED

Pipettes (100 µL, 200 µL, 400 µL, 1.00 mL, 2.00 mL, 5.00 mL)
Repeating dispensers (100 µL, 200 µL)
Measuring cylinder 25 mL
Polystyrene tubes, polypropylene or glass-tubes
Vortex
Refrigerated centrifuge
Ethanol p.A. 98%
Vac-concentrator or N_2 (nitrogen)
Icebath

QUALITY CONTROL

Controls should be carried out in each assay run. Two controls are included in the kit, the value (without extraction procedure) is indicated on the Control sheet and the labels of the vials. Use controls as recommended by the control plasma manufacturer and in accordance with reference laboratories practice to monitor the accuracy and precision of reagents and techniques.

SHELFLIFE AND STORAGE

This kit is stable until the stated expiry date if stored as specified.
Upon receipt of the kit, all reagents should be stored at 2-8°C.
The reconstituted reagents should be stored according to table on page 6.
The reconstituted reagents are stable according to table on page 6, but no longer than to the expiry date.

CONTENTS OF THE KIT

Item	Nr. of Vials	Containing
Anti-angiotensin II (Reagent A)	1	Lyophilized anti-angiotensin II (Rabbit) for 100 tubes. Colour: Yellow.
^{125}I-angiotensin II (Reagent B)	1	56 KBq or 1.5 μCi. Lyophilized. Specific activity: 62-77 MBq/nmol (1700-2100 μCi/nmol). Colour: Blue.
Double antibody solid phase (Reagent C)	1	Goat anti-rabbit IgG's bound to solid phase in 0.01 M phosphate buffer pH 6.8 with 0.25% Human serum albumin, 0.045% NaCl, 0.05% NaN3, 0.185% EDTA and 0.05% Tween 80. 11 mL suspension.
Assay buffer (Reagent D)	2	0.05 M phosphate buffer with 0.25% HSA, 0.25% EDTA disodium salt, 0.05% NaN_3, 500 KIU Trasylol/mL, pH 7.4. 2 x 50 mL (liquid).
Angiotensin II standard 300 pmol/L (Reagent E)	1	5.0 mL angiotensin II standard, 300 pmol/L. Lyophilized in assay buffer.
Angiotensin II, low control (Reagent F)	1	2.0 mL lyophilized angiotensin II control, low level.
Angiotensin II, high control (Reagent G)	1	2.0 mL lyophilized angiotensin II control, high level.

PREPARATION OF REAGENTS

(reconstitute 15 minutes before use)

Item	**Reconstitute each vial with**		**Stable at**	**Special remarks**
Anti-angiotensin II (Reagent A)	22 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	
^{125}I-angiotensin II (Reagent B)	25 mL distilled water	Mix gently	-20° C until expiry date	
Double antibody solid phase (Reagent C)	Ready for use. The separation reagent should be placed on a magnetic stirrer for 10 minutes at room temp		2-8° C until expiry date	It is possible to pipette the reagent with a repeating dispenser
Assay buffer (Reagent D)	Ready for use		2-8° C until expiry date	
Angiotensin II standard 300 pmol/L (Reagent E)	5.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	Refer to table for standard curve preparation
Angiotensin II low control (Reagent F)	2.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	The concentration of the control is found on the lable of the vial and in the QC sheet (without extraction)
Angiotensin II high control (Reagent G)	2.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	The concentration of the control is found on the lable of the vial and in the QC sheet (without extraction)

PERFORMANCE

A. Extraction procedure of plasma

1. Label one extraction tube for each sample. Label one additional tube (R) in order to estimate the extraction recovery.
2. Place the extraction tubes and ethanol on ice.
3. Pipette 1.0 mL of each sample into the appropriately labelled extraction tubes. DO NOT EXTRACT STANDARDS AND CONTROLS.
4. Prepare a recovery estimation tube (R):

- Pipette 1.0 mL of a random plasma sample into the recovery tube (R). The sample used for this this recovery assay should have a protein matrix similar to the samples being tested.
- Add 200 µL ^{125}I-angiotensin II tracer into two R tubes.
- Extract this sample along with samples in step 6.

5. Prepare Total Recovery tube (TR):

- Pipette 200 µL ^{125}I-angiotensin II tracer into two TR tubes.
- Add 200 µL assay buffer and mix.
- Cap and set aside this tube to be counted for recovery calculation.

6. Add 4 mL chilled ethanol to each sample and Recovery tube (R).
7. Mix and vortex for 2 minutes.
8. Centrifuge all extraction tubes at 2000 g. for 15 minues at 2-8°C.
9. Decant supernatant from each extraction tube into previous prepared clean, appropriately labelled 16 x 100 mm tubes.
10. Evaporate the supernatants under a stream of nitrogen to dryness (at max. 37°C).
11. Reconstitute the dried samples by adding 1.0 mL assay buffer and vortex thoroughly.
12. Proceed RIA procedure immediately or store the extracted samples at -20°C up to two weeks before using it in the assay.
13. Reconstitute the dried recovery sample (R) by adding 1.0 mL assay buffer and vortex thoroughly.
14. Pipette 400 µL of the reconstituted recovery sample tube (R) into two 12 x 75 mm tubes.
15. Count the total recovery (TR) and recovery (R) tubes for at least two minutes in a gamma counter.

Recovery calculation:

Calculate % recovery by dividing the cpm in the recovery tubes (R) by cpm in the total recovery tubes (TR) and multiply by 1.0/0.4:

$$\% \text{ Recovery} : \frac{\text{cpm recovery tube} \quad 1.0}{\text{cpm total recovery tube } 0.4} \times 100$$

B. Preparation of standard solutions

TABLE FOR PREPARATION OF STANDARD SOLUTIONS		
Dilution	**Reagent E**	**Concentration 300 pmol/L**
1000 µL of Reagent E + 1000 µL assay buffer vortex	standard a	150 pmol/L
1000 µL of standard a + 1000 µL assay buffer vortex	standard b	75 pmol/L
1000 µL of standard b + 1000 µL assay buffer vortex	standard c	37.5 pmol/L
1000 µL of standard c + 1000 µL assay buffer vortex	standard d	18.8 pmol/L
1000 µL of standard d + 1000 µL assay buffer vortex	standard e	9.4 pmol/L
1000 µL of standard e + 1000 µL assay buffer vortex	standard f	4.7 pmol/L

C. Assay Procedure

1. Keep assay tubes and reagents in an icebath during all pipetting steps.
2. Pipette 400 µL of each standard, 400 µL of controls and 400 µL of each plasma extract in duplicate into the corresponding labelled polystyrene tubes.
3. Add 400 µL of assay buffer (Reagent D) to the max. binding tubes (0 pmol/L).
4. Add 600 µL of assay buffer to the NSB (blank) tubes.
5. Add 200 µL of angiotensin II antiserum (Reagent A) to each tube, except blank and TC-tubes.
6. Vortex and incubate for 6 hours at 4° C.
7. Add 200 µL of ^{125}I-Angiotensin II tracer (Reagent B) to all tubes.
8. Vortex all tubes and incubate at 4° C for 18-22 hours.
9. While stirring continuously add 100 µL of the double antibody solid phase (Reagent C) to all tubes, except TC- tubes.
10. Vortex and incubate 30-60 minutes. at 4° C.
11. Centrifuge all tubes for 15 minutes at 1700 g at 4° C or room temperature.
12. Decant the supernatants carefully.
13. Count residue for 1-2 minutes.

	Tubes No.	Assay buffer (D)	Standard or sample or contr.	Anti-angiotensin II antiserum (A)		^{125}I-angiotensin II (B)		Separation reagent (C)		
TOT	1-2	-	-	-	Vortex	200 µL	Vortex	-	Vortex	Vortex and
Blank (NSB)	3-4	600 µL	-	-	and	200 µL	and	100 µL	and	centrifuge
St. 0 pmol/L	5-6	400 µL	-	200 µL	incubate	200 µL	incubate	100 µL	incubate	for 15
St. 4.7 pmol/L	7-8	-	400 µL	200 µL	for 6 hrs	200 µL	for 18-22	100 µL	for 30-60	minutes at
St. 9.4 pmol/L	9-10	-	400 µL	200 µL	at +4° C.	200 µL	hrs	100 µL	minutes	1700 g at
St. 18.8 pmol/L	11-12	-	400 µL	200 µL		200 µL	at +4° C	100 µL	at +4° C.	+4° C.
St. 37.5 pmol/L	13-14	-	400 µL	200 µL		200 µL		100 µL		Aspirate or
St. 75 pmol/L	15-16	-	400 µL	200 µL		200 µL		100 µL		decant the
St. 150 pmol/L	17-18	-	400 µL	200 µL		200 µL		100 µL		supernatant.
Control (F)	19-20	-	400 µL	200 µL		200 µL		100 µL		Count the
Control (G)	21-22	-	400 µL	200 µL		200 µL		100 µL		residue for
Unknown sample	23-24									1-2 minutes.

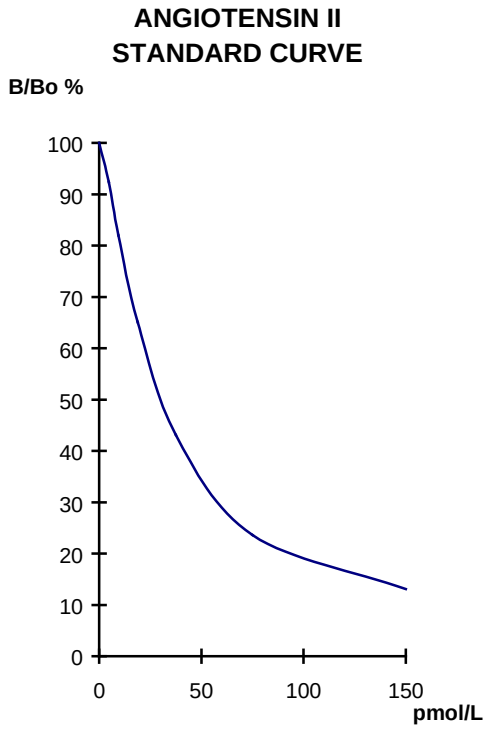
D. Calculation of testresults

1. Subtract the mean count rate (cpm) of the NSB from the mean count rate (cpm) of the replicates of standards, controls and samples.
2. A standard curve can be generated by plotting cpm, % B/Bo or %B/T of precipitated bound fraction, against the concentration of the angiotensin II standards.
3. To obtain the angiotensin II concentration in the extracted samples and controls, their cpm, % B/Bo or B/T of precipitated bound fractions are interpolated now from generated standard curve.
4. The standard curve can also be constructed by computer methods. For automated data reduction, both logit/log and Spline methods can be used.
5. Correct plasma values for % extraction recovery.

E. Standard Curve Data

	Average cpm	Corrected cpm	% B/Bo	Results (pmol/L)
Total counts	18582			
NSB	678			
Standard 0 pmol/L	9559	8881	100	
Standard f 4.7 pmol/L	8880	8202	92.4	
Standard e 9.4 pmol/L	7957	7279	82.0	
Standard d 18.8 pmol/L	7039	5781	65.1	
Standard c 37.5 pmol/L	4508	3830	43.1	
Standard b 75 pmol/L	2770	2099	23.6	
Standard a 150 pmol/L	1846	1168	13.1	
Control low	7359	6681	75.2	13.1
Control high	3145	2467	27.8	63.1

F. Example of Standard Curve



ASSAY CHARACTERISTICS

Sensitivity
The sensitivity judged as 3 standard deviations change from zero calibrator is 2.0 pmol/L

Precision										
Within-run						**Between-run**				
	n	**mean pmol/L**	**SD**	**% c.v.**			**n**	**mean pmol/L**	**SD**	**% c.v.**
sample A	20	13.3	0.44	3.3		sample A	6	11.6	0.55	4.8
sample B	20	64.9	1.97	3.0		sample B	6	60.9	2.4	3.9

Recovery
Four different samples are spiked with different amounts of angiotensin II standard

Sample	Expected conc. (pmol/L)	Observed conc. (pmol/L)	% Recovery
A1	12.4	12.3	99.2
A2	23.9	23.5	96.8
A3	27.2	22.0	103.0
A4	46.0	51.1	111.0

Specificity
Angiotensin II antiserum is raisen in rabbits. The following cross-reactivities were measured at 50% B/Bo.

Peptide	Cross-reaction
Angiotensin II	100
Angiotensin I	<0.1
Leu-Heptapeptide	100
Asn^{1}-Val^{5} Angiotensin II	30
Sar^{1} Ile^{8} Angiotensin II	100
Angiotensin III	80

Normal Range
Each laboratory should establish its own normal range of expected values. Bloodsamples were drawn from11 apparently healthy adults (09.00 - 10.00 a.m.) and Angiotensin II levels were determined.

Observed Range: 19 - 38 pmol/L

Interference
Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

LITERATURE / REFERENCES / REFERENCIAS / LITERATUR / BIBLIOGRAFIA / REFERENSER

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SYMBOLS USED ON LABELS

	Batch code.
	Catalogue number.
	Use by date.
	Temperature limit.
	Date of manufacture.
	Contains radioactive substances.
	Biological risks.
	Consult instructions for use.
	Manufacturer.
 **100**	Contains sufficient for 100 tests.

REAG A Ab	Anti-angiotensin II.
REAG B Ag ^{125}I	^{125}I-angiotensin II.
REAG C DASP	Double antibody solid phase.
REAG D BUF AS	Assay buffer.
REAG E CAL 300	Angiotensin II standard 300 pmol/L.
REAG F CONTROL	Control, level 1 (low).
REAG G CONTROL	Control, level 2 (high).

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