Instructions for Use

Angiotensin II RIA

IVD

CE

REF **RIA-3020**

Σ **100**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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1 INTENDED USE

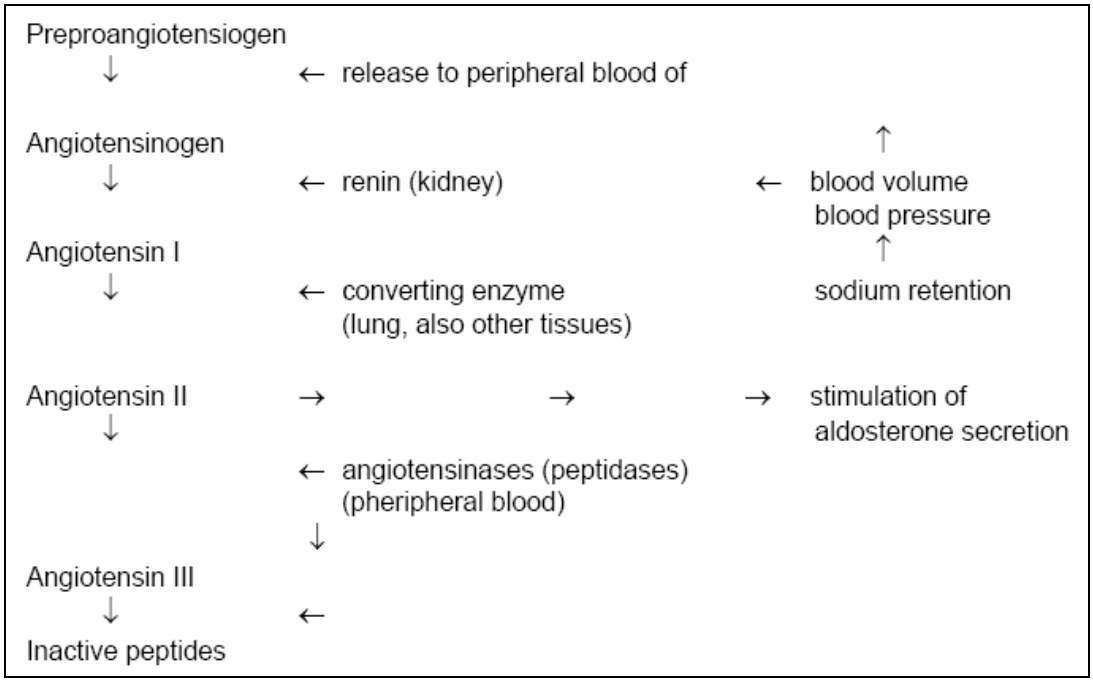
Radioimmunoassay for the *in vitro* quantitative measurement of Angiotensin II in EDTA plasma

2 CLINICAL BACKGROUND

2.1 Biological activities

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 catalysed 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:



2.2 Clinical application

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 has its established application in the treatment and monitoring of hypertension.

3 PRINCIPLES OF THE METHOD

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.

4 REAGENTS PROVIDED

	Reagents	100 Tests Kit	Reconstitution
Ab	Antiserum: Rabbit anti-angiotensin II antiserum	1 vial Lyophilised	**Add** 22 mL distilled water
Ag ^{125}J	TRACER: ^{125}Iodine labelled Angiotensin II in phosphate buffer with human serum albumin and NaN_3.	1 vial Lyophilised 56 kBq	**Add** 25 mL distilled water
DASP	Double antibody solid phase: Goat anti-rabbit Ig's bound to solid phase in phosphate buffer with human serum albumin, Tween and sodium azide. (<0.1%).	1 vial 11 mL	**Ready** for use
ASS BUF	Assay buffer: phosphate buffer containing human serum albumin and sodium azide, (<0.1%).	2 vials 50 mL	**Ready** for use
CAL	Angiotensin II Calibrator, 300 pmol/L.	1 vial Lyophilised	**Add** 5 mL distilled water
CONTROL N	Controls – N = 1 or 2	2 vials Lyophilised	**Add** 2 mL distilled water

5 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Pipettes (100 µL, 200 µL, 1.00 mL, 2.00 mL, 5.00 mL).
2. Repeating dispensers (100 µL, 200 µL).
3. Measuring cylinder 25 mL.
4. Polystyrene tubes, polypropylene or glass-tubes.
5. Vortex.
6. Refrigerated centrifuge.
7. Ethanol p.A. 98%.
8. Vac-concentrator or N_2 (nitrogen).
9. Ice bath

6 REAGENT PREPARATION

A. **Antiserum:**
Reconstitute with 22 mL distilled water. Mix gently.
Stable at -20 °C for at least 3 months after reconstitution

B. **^{125}I-Angiotensin II:**
Reconstitute with 25 mL distilled water. Mix gently. Stable at -20 °C until expiry date.

C. **Double antibody solid phase:**
Ready for use. The separation reagent should be placed on a magnetic stirrer for 10 minutes. It is possible to pipette the reagent with a repeating dispenser. Stable at 2 °C - 8 °C.

D. **Assay buffer:**
Ready for use. Stable at 2 °C - 8 °C until expiry date.

E. **Angiotensin II Calibrator 300 pmol/L:**
Reconstitute with 5 mL distilled water. Mix gently. Stable at -20°C for at least 3 months after reconstitution.
Refer to table for standard curve preparation.

F. **Controls:**
Reconstitute each vial with 2 mL distilled water. Stable at -20 °C for at least 3 months after reconstitution.
The concentration of the control is found on the label of the vials and in the QC sheet (without extraction).

PREPARE ALL REAGENTS 15 MINUTES BEFORE USE!

7 STORAGE AND EXPIRATION DATING OF REAGENTS

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 °C - 8 °C.

The reconstituted reagents should be stored according to section 6.

The reconstituted reagents are stable according to section 6, but no longer than to the expiry date.

8 SPECIMEN COLLECTION

Careful standardization of the patient 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 patient 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.

9 PROCEDURE

9.1 Extraction procedure of plasma

1. Label one extraction tube for each patient sample. Label one additional tube 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:
   - Pipette 1.0 mL of a random plasma sample into the recovery tube.
     The sample used for this recovery assay should have a protein matrix similar to the samples being tested.
   - Add 200 µL ^{125}I-angiotensin II tracer into this tube.
   - Extract this sample along with samples in step 6.
5. Prepare Total Recovery tube:
   - Pipette 200 µL ^{125}I-angiotensin II tracer into two 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.
7. Mix and vortex for 2 minutes.
8. Centrifuge all extraction tubes at 2000 *g*. for 15 minutes at 2 °C - 8 °C.
9. Decant supernatant from each extraction tube into previous prepared clean, appropriately labelled 16 × 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 by adding 1.0 mL assay buffer and vortex thoroughly.
14. Pipette 400 µL of the reconstituted recovery sample tube into two 12 × 75 mm tubes.
15. Count the total recovery and recovery 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 and multiply by 1.0／0.4:

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

9.2 Preparation of calibrator solutions

Dilution	Angiotensin II Calibrator	Concentration 300 pmol/L
1000 µL Angiotensin II Calibrator + 1000 µL assay buffer, vortex	Calibrator A	150 pmol/L
1000 µL Calibrator A + 1000 µL assay buffer, vortex	Calibrator B	75 pmol/L
1000 µL Calibrator B + 1000 µL assay buffer, vortex	Calibrator C	37.5 pmol/L
1000 µL Calibrator C + 1000 µL assay buffer, vortex	Calibrator D	18.8 pmol/L
1000 µL Calibrator D + 1000 µL assay buffer, vortex	Calibrator E	9.4 pmol/L
1000 µL Calibrator E + 1000 µL assay buffer, vortex	Calibrator F	4.7 pmol/L

9.3 Assay Procedure

1. Keep assay tubes and reagents in an ice bath 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 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 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 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 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.

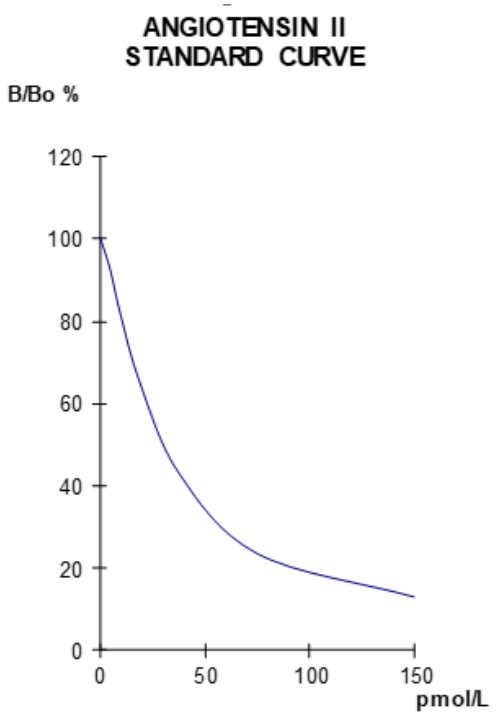
10 CALCULATION OF RESULTS

1. Subtract the mean count rate (cpm) of the NSB from the mean count rate (cpm) of the replicates of standards, controls and patient samples.
2. A standard curve can be generated by plotting cpm, % B/B_0 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 patient samples and controls, their cpm, % B/B_0 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.

Standard Curve Data

		Average cpm	Corrected cpm	% B/B_0	Results (pmol/L)
Total counts		18582			
NSB		678			
Calibrator	0 pmol/L	9559	8881	100	
Calibrator F	4.7 pmol/L	8880	8202	92.4	
Calibrator E	9.4 pmol/L	7957	7279	82.0	
Calibrator D	18.8 pmol/L	7039	5781	65.1	
Calibrator C	37.5 pmol/L	4508	3830	43.1	
Calibrator B	75 pmol/L	2770	2099	23.6	
Calibrator A	150 pmol/L	1846	1168	13.1	
Control low		7359	6681	75.2	13.1
Control high		3145	2467	27.8	63.1

Example of Standard Curve



11 PERFORMANCE AND LIMITATIONS

11.1 Sensitivity

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

11.2 Precision

Within-run				
	n	Mean pmol/L	SD	% CV
sample A	20	13.3	0.44	3.3
sample B	20	64.9	1.97	3.0

Between-run				
	n	Mean pmol/L	SD	% CV
sample A	6	11.6	0.55	4.8
sample B	6	60.9	2.4	3.9

11.3 Accuracy

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

11.4 Specificity

Angiotensin II antiserum is raisen in rabbits. The following cross-reactivities were measured at 50% B/B_0.

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

11.5 Interference

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

12 INTERNAL 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.

13 REFERENCE INTERVALS

Each laboratory should establish its own normal range of expected values.

Blood samples were drawn from 11 apparently healthy adults (09.00 - 10.00 a.m.) and Angiotensin II levels were determined.

Observed Range: 19 - 38 pmol/L

14 PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

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.

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.

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 in-vitro clinical or 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.

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16 SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrator (0)	Calibrators (A - F)	Controls	Samples
Assay buffer	-	600 µL	400 µL	-	-	-
Calibrators	-	-	-	400 µL	-	-
Controls	-	-	-		400 µL	-
Samples	-	-	-		-	400 µL
Antiserum	-	-	200 µL	200 µL	200 µL	200 µL
Vortex and incubate for 6 hours at 4 °C						
^{125}I Tracer	200 µL					
Vortex and incubate for 18-22 hours at 4 °C						
Double Ab Solid phase	-	100 µL				
Vortex and incubate for 30 - 60 min at 4 °C						
Vortex and centrifuge for 15 min (1700 g) at 4 °C						
Decant or aspirate the supernatant and count the radioactivity of the residue						

1 KOMONENTEN DES KITS

	Reagenzien	100 Tests	Rekonstitution
Ab	Antiserum: Kaninchen-anti-Angiotensin II-Antiserum	1 Fläschchen Lyophilisiert	22 mL destilliertes Wasser **hinzugeben**
Ag ^{125}J	TRACER: ^{125}Iod markiertes Angiotensin II in Phosphatpuffer mit humanem Serumalbumin und NaN_3.	1 Fläschchen Lyophilisiert 56 kBq	25 mL destilliertes Wasser **hinzugeben**
DASP	Doppel-Antikörper-Festphase: Ziege anti-Kaninchen Ig, an Festphase gebunden in Phosphatpuffer mit humanem Serumalbumin, Tween und NaN_3. (<0.1%).	1 Fläschchen 11 mL	**Gebrauchsfertig**
ASS BUF	Assaypuffer: Phosphatpuffer mit humanem Serumalbumin, Tween und NaN_3. (<0.1%).	2 Fläschchen 50 mL	**Gebrauchsfertig**
CAL	Angiotensin II-Standard, 300 pmol/L.	1 Fläschchen Lyophilisiert	5 mL destilliertes Wasser **hinzugeben**
CONTROL N	Kontrollen – N = 1 or 2	2 Fläschchen Lyophilisiert	2 mL destilliertes Wasser **hinzugeben**

2 BENÖTIGTES MATERIAL, NICHT IM KIT ENTHALTEN

Das folgende Material wird benötigt, ist aber nicht im Lieferumfang enthalten:

1. Pipetten (100 µL, 200 µL, 1,00 mL, 2,00 mL, 5,00 mL).
2. Mehrfachdispenser (100 µL, 200 µL).
3. Messzylinder 25 mL.
4. Polystyrol-Röhrchen, Polypropylen oder Glas-Röhrchen.
5. Vortex-Mixer.
6. Kühlzentrifuge).
7. Ethanol p.A. 98%.
8. Vakuum-Konzentrator oder N_2 (Stickstoffgas).
9. Eisbad

3 VORBEREITUNG DER REAGENZIEN

A. **Antiserum:**
Mit 22 mL destilliertem Wasser rekonstituieren. Vorsichtig mischen.
Haltbar bei -20 °C für mindestens 3 Monate nach Rekonstitution.

B. **^{125}I-Angiotensin II:**
Mit 25 mL destilliertem Wasser rekonstituieren. Vorsichtig mischen. Haltbar bei -20 °C bis zum Verfallsdatum.

C. **Doppel-Antikörper-Festphase:**
Gebrauchsfertig. Das Trennreagenz sollte 10 Minuten bei Raumtemperatur auf einem Magnetrührer gerührt werden. Es ist möglich, dies Reagenz mit einem Mehrfachdispenser zu pipettieren. Haltbar bei 2 °C - 8 °C.

D. **Assaypuffer:**
Gebrauchsfertig. Haltbar bei 2 °C - 8 °C bis zum Verfallsdatum.

E. **Angiotensin II-Standard 300 pmol/L:**
Mit 5 mL destilliertem Wasser rekonstituieren. Vorsichtig mischen. Haltbar bei -20 °C für mindestens 3 Monate nach Rekonstitution.
Siehe auch Tabelle: Herstellung der Standardverdünnungen.

F. **Kontrollen:**
Jedes Fläschchen mit 2 mL destilliertem Wasser rekonstituieren. Vorsichtig mischen.
Haltbar bei -20 °C für mindestens 3 Monate nach Rekonstitution.
Die Konzentration ist auf dem Etikett des Fläschchens und auf dem QC-Zertifikat angegeben (ohne Extraktion).

ALLE REAGENZIEN 15 MINUTEN VOR GEBRAUCH HERSTELLEN!

4 LAGERUNG UND HALTBARKEIT DER REAGENZIEN

Dieser Kit ist haltbar bis zum angegebenen Verfallsdatum, wenn er unter den angegebenen Bedingungen gelagert wird.

Nach dem Empfang sollten alle Reagenzien bei 2 °C - 8 °C gelagert werden.

Die rekonstituierten Reagenzien sollten gelagert werden wie im Kapitel 3 angegeben.

Die rekonstituierten Reagenzien sind haltbar wie im Kapitel 3 angegeben, aber nicht über das Verfallsdatum des Kits hinaus.

5 PROBENGEWINNUNG

Sorgfältige Standardisierung der Probenentnahme ist unbedingt erforderlich. Bedingt durch die extreme Labilität von Angiotensin II in biologischen Flüssigkeiten muss bei der Probenentnahme folgendes beachtet werden:

- Blutentnahme von ruhenden, nüchternen Patienten soll in vorgekühlte **EDTA-Röhrchen** erfolgen.
- Sofortige Zentrifugation der Proben in einer Kühlzentrifuge bei 4 °C zur Plasmagewinnung
- Proben sofort in Kunststoffröhchen bei - 20 °C bis zur Analyse tieffrieren.

6 TESTDURCHFÜHRUNG

6.1 Extraktion der Plasmaproben

1. Ein Extraktionsröhrchen für jeden Patienten kennzeichnen. Ein zusätzliches Röhrchen wird benötigt zur Bestimmung der Extraktionswiederfindung [R] und zwei für die Gesamtwiederfindung.
2. Extraktionsröhrchen und Ethanol auf Eis stellen.
3. Je 1 ml Probe in die entsprechenden Röhrchen pipettieren.
   STANDARDS UND KONTROLLEN NICHT EXTRAHIEREN.
4. Röhrchen für die Abschätzung der Wiederfindung [R]:
   - 1 ml einer zufälligen Plasmaprobe in das entsprechende Röhrchen pipettieren.
     Die Probe sollte eine ähnliche Proteinmatrix wie die zu analysieren den Proben aufweisen.
   - 200 µL ^{125}I-Angiotensin II-Tracer hinzufügen.
   - Diese Wiederfindungsprobe wie die Analysenproben extrahieren (siehe Schritt 6.).
5. Röhrchen für die Gesamtwiederfindung [TR]:
   - 200 µL ^{125}I-Angiotensin II-Tracer in die beiden Röhrchen pipettieren.
   - 200 µL Assaypuffer hinzugeben und mischen.
   - die Röhrchen verschließen und für die Messung beiseite stellen.
6. Je 4 mL kaltes Ethanol in jedes Probenröhrchen und das Wiederfindungsröhrchen [R] pipettieren (Nicht in TR).
7. 2 Minuten auf einem Vortexer mischen.
8. Alle Extraktionsröhrchen zentrifugieren bei 2000 *g* für 15 Minuten bei 2 °C - 8 °C.
9. Überstände der extrahierten Proben in saubere Röhrchen (16 × 100 mm) überführen.
10. Überstände unter Stickstoffstrom zur Trockne eindampfen (max. 37 °C).
11. Die eingedampften Proben mit 1 ml Assaypuffer rekonstituieren und vorsichtig vortexen).
12. Proben direkt im RIA weiterverarbeiten, oder bei - 20 °C bis zu zwei Wochen lagern.
13. Die getrockneten Wiederfindungsprobe [R] mit 1 mL Assaypuffer rekonstituieren und vorsichtig vortexen.
14. 400 µL der rekonstituierten Wiederfindungsprobe (R) in zwei neue 12 x 75 mm-Röhrchen pipettieren.
15. Gesamtwiederfindung [TR] und Wiederfindung [R] für mind. 2 Minuten in einem Gammacounter messen..

Berechnung der Wiederfindungsrate:

Zur Berechnung der Wiederfindung in Prozent werden die cpm der Wiederfindungsprobe [R] durch die cpm der Gesamtwiederfindung (TR) dividiert und mit 1,0／/0,4 multipliziert:

$$\% \text{ Wiederfindung} = \frac{\text{cpm Wiederfindungsprobe [R]} \times 1{,}0}{\text{cpm Gesamtwiederfindung TR] [} \times 0{,}4} \times 100$$

6.2 Herstellung der Standardverdünnungen

Verdünnung	Angiotensin II Standard	Concentration 300 pmol/L
1000 µL Angiotensin II Standard + 1000 µL Assaypuffer, vortexen	Standard A	150 pmol/L
1000 µL Standard A + 1000 µL Assaypuffer, vortexen	Standard B	75 pmol/L
1000 µL Standard B + 1000 µL Assaypuffer, vortexen	Standard C	37.5 pmol/L
1000 µL Standard C + 1000 µL Assaypuffer, vortexen	Standard D	18.8 pmol/L
1000 µL Standard D + 1000 µL Assaypuffer, vortexen	Standard E	9.4 pmol/L
1000 µL Standard E + 1000 µL Assaypuffer, vortexen	Standard F	4.7 pmol/L

6.3 Testdurchführung

1. Alle Röhrchen und Reagenzien während der Pipettierschritte auf Eis stellen.
2. 400 µL von jedem Standard, jeder Kontrollen und jeder extrahierten Proben in die entsprechend gekennzeichneten Polystyrenröhrchen pipettieren(Doppelbestimmungen).
3. 400 µL Assay Puffer in die Röhrchen für maximale Bindung (0 pmol/L) pipettieren
4. 600 µL Assay Puffer in die Röhrchen für NSB (nicht spezifische Bindung) pipettieren.
5. 200 µL Angiotensin II-Antiserum in jedes Röhrchen pipettieren, außer NSB und Totalaktivität TA).
6. Vortexen und für 6 Stunden bei 4 °C inkubieren.
7. 200 µL of ^{125}I-Angiotensin II-Tracer in alle Röhrchen geben (auch in TA).
8. Vortexen und alle Röhrchen für 18 - 22 Stunden bei 4 °C inkubieren.
9. 100 µL Doppel-Antikörper-Festphase in alle Röhrchen (Ausnahme TA) pipettieren (während des Pipettierens permanent rühren).
10. Vortexen und 30 - 60 Minuten bei 4 °C inkubieren.
11. Röhrchen für 15 Minuten bei 1700 *g* und 4 °C oder Raumtemperatur zentrifugieren.
12. Überstand dekantieren.
13. Radioaktivität des Sediments mindestens 1 Minute im Gamma-Counter messen.

7 BERECHNUNG DER ERGEBNISSE

1. Die mittleren Counts (cpm) der NSB-Röhrchen von den mittleren Counts (cpm) der Standards, Kontrollen und Patientenproben subtrahieren.
2. Die Standardkurve kann mit den cpm, % B/Bo oder %B/T des Präzipitats gegen die Konzentration der Angiotensin II Standards aufgestellt werden.
3. Die Angiotensin II-Konzentration der extrahierten Patientenproben und der Kontrollen kann anhand der Standardkurve abgelesen werden.
4. Die Standardkurve kann auch mit Computer-basierten Methoden berechnet werden. Logit/log und Spline-Methoden können verwendet werden.
5. Die Plasmawerte müssen anhand der %-Wiederfindung der Extraktion korrigiert werden.

8 NORMALWERTEBEREICH

Die aufgeführten Referenzbereiche dienen lediglich als Anhaltswerte. Jedem Labor wird empfohlen, seine eigenen Normbereiche festzulegen.

Die Blutproben stammen von 11 normalen Blutspendern.

Plasma: (9:00 - 10:00 Uhr) 19 - 38 pmol/L

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
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