

Renin From a Laboratory Perspective

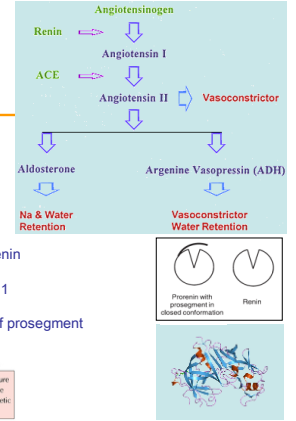
Anneke Muller Kobold

Clinical Chemist Endocrinology
Laboratory of Binding Analysis

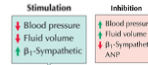
University Medical Center Groningen
The Netherlands



Renin



- Proteolytic enzyme, 40kD MW
- Synthesized as prorenin
- Stored in granules as prorenin or renin
- Juxtaglomerular cells of the kidney
- Blood: prorenin to renin ratio: 10 to 1
- Prorenin is inactive
- Activation by enzymatic cleavage of prosegment
- Renin secretion tightly controlled



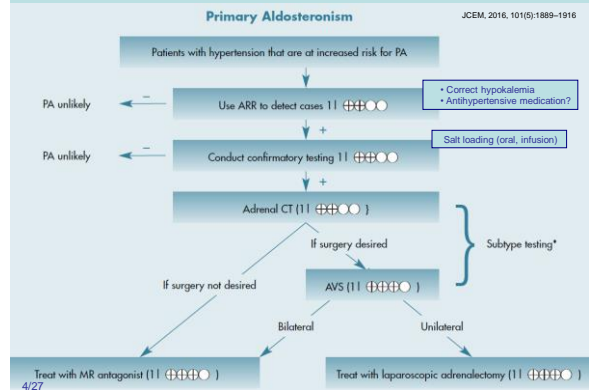
2/27

Use of renin and aldosterone measurements

Disorder	Expected findings	
	Renin	Aldosterone
PA	↓	↑
Renal artery stenosis	↑	↑
Bartter's syndrome	↑	↑
Renin secreting tumours	↑	↑
Pseudohypoaldosteronism	↑	↑
Primary aldosteron deficiency	↑	↓
Secondary aldosteron deficiency	↑	↓
Congenital adrenal hyperplasia	Renin to monitor fludrocortisone treatment	

3/27

Guideline PA



4/27

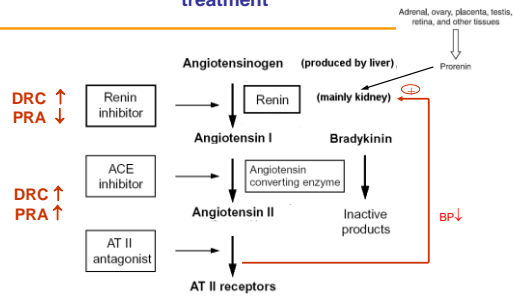
Effect of medication on renin levels

TABLE 4. Factors that may affect the aldosterone-renin ratio and thus lead to false positive or false negative results

Factor	Effect on aldosterone levels	Effect on renin levels	Effect on ARR
Medications			
Beta-adrenergic blockers	↓	↓ ↓	↑ (FP)
Central alpha-2 agonists (e.g., clonidine, alpha-methyl dopa)	↓	↓ ↓	↑ (FP)
NSAIDs	↓	↓ ↓	↑ (FP)
K ⁺ -wasting diuretics	→ ↑	↑ ↑	↓ (FN)
K ⁺ -sparing diuretics	↑	↑ ↑	↓ (FN)
ACE inhibitors	↓	↑ ↑	↓ (FN)
ARBs	↓	↑ ↑	↓ (FN)
Ca ²⁺ blockers (DHPs)	→ ↓	↑	↓ (FN)
Renin inhibitors	↓	↓ ↓ *	↑ (FP)*
			↓ (FN)*

6/27 Clin Endocrinol Metab 93: 3266-3281, 2008

PRA vs PRC Interpretation of lab results & antihypertensive treatment



5/27

Preanalytical conditions affecting renin levels

		effect on renin level	Effect on ARR
Time of day, diurnal rhythm	early morning	↑	
	evening		↓
Posture	standing	↑	↓ (FN)
	supine		↑ (FP)
Age	old age	↓	↑ (FP)
Pregnancy, luteal phase		↑	↓ (FN)
Ethnicity	black subjects	↓	↑ (FP)
Male/female	Female (follicular)		↑ (FP)
Potassium status	hypokalemia	↑	↓ (FN)
Dietary salt intake	loading		↑ (FP)
Chronic kidney disease			↑ (FP)

7/27

Renin assays

Activity or Mass?



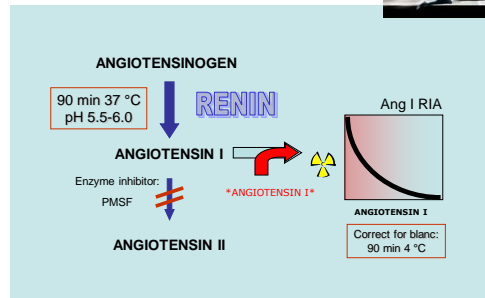
8/27

Plasma Renin Activity



9/27

PRA assay



10/27

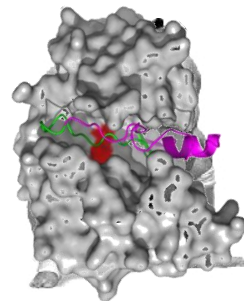
PRA: remarks



- Endogeneous angiotensinogen as substrate
- Low renin concentrations difficult?
- Prolongation incubation time (37°C) may increase sensitivity
- Lack of standardization
 - Preanalytical conditions
 - Incubation time
 - pH
 - Differences in assay sensitivity
 - Precision/accuracy/reproducibility

11/27

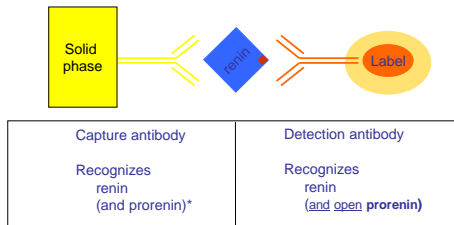
Renin mass assay Direct Renin concentration (DRC)



12/27



Direct Renin assay



* Some kit-inserts: Active and 'inactive' renin'...

13/27

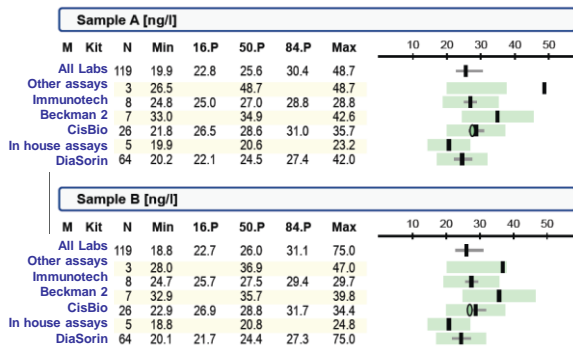


Direct Renin assay

- Independent of plasma angiotensinogen levels
- Sample processing at RT
- Assay can be automated
- Relatively large sample volume required
- Calibrated against WHO IR 68/356
- Interlaboratory CVs are lower than for PRA
- Analytical sensitivity?

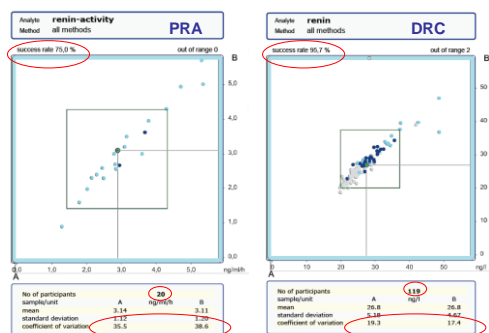
14/27

EQAS (DRV) results



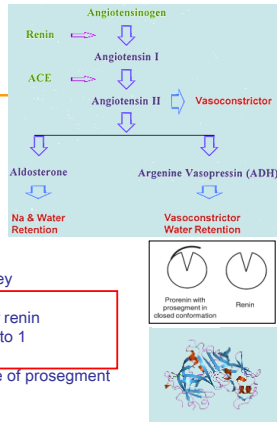
15/27

EQAS (DRV) results



16/27

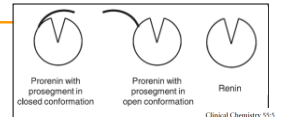
Preanalytical concerns II



- Proteolytic enzyme, 40kD MW
- Juxtaglomerular cells of the kidney
- Synthesized as prorenin
- Stored in granules as prorenin or renin
- Blood: prorenin to renin ratio: 10 to 1
- Prorenin is inactive.....??
- Activation by enzymatic cleavage of prosegment
- Renin secretion tightly controlled

17/27

activation of prorenin

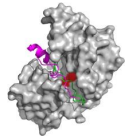


Proteolytic activation of prorenin

- Irreversible
- Proteolytic (serine proteases) removal of the prosegment
- secretory granules of the juxtaglomerular cells
- Neutral or low pH (depending on proteases)
- At low (4°C) temperature and neutral pH (cryoactivation)

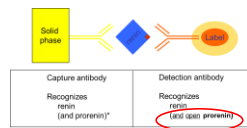
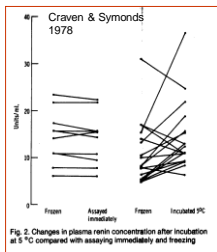
Nonproteolytic activation of prorenin

- Reversible
- conformational changes (closed to open conformation)
- At low (4°C) temperature (cryoactivation)



Schalekamp et al.
18/27 J Hypertens 26:928-937 © 2008

Effect of low temperature on PRA and DRC



14.5 Pro-Renin

A study was performed to evaluate the effect of Pro-Renin on the Renin assay. The following results were obtained. It is determined that Cross Reaction with pro-renin is below 0.4 %.

Samples	Renin measured pg/mL	Pro-renin measured pg/mL	% CR
Sample A	24		
A + pro-renin (7.1ng/mL)	46	22	0.31
A + pro-renin (5.9ng/mL)	42	18	0.30
A + pro-renin (4.7ng/mL)	37	13	0.27
A + pro-renin (3.5ng/mL)	35	10	0.30
Sample B	177		
B + pro-renin (8.3ng/mL)	209	32	0.39
B + pro-renin (7.1ng/mL)	204	27	0.38
B + pro-renin (5.9ng/mL)	199	22	0.37
B + pro-renin (4.7ng/mL)	187	11	0.23
B + pro-renin (3.5ng/mL)	190	13	0.37

19/27

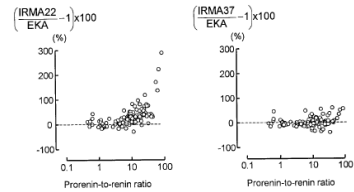


Low renin - high prorenin!



Effect of Prorenin

- Blood: prorenin to renin: 10 to 1
- The lower the renin concentration the higher the proportion of plasma prorenin concentration
- Patients with low-renin hypertension or diabetich nephropathy have 100 fold more prorenin than renin levels
- **Cryoactivation**: falsely elevated renin values!
 - 4 °C or lower, not at 37 °C, serum > EDTA plasma
 - mainly due to conformational change of epitope
 - **Both PRA and DRC may be influenced**



Effect of incubation temperature (22 °C, left and 37 °C, right) on assay (IRMA) performance

20/27

21/27

Activity or Mass?

- Which assay is more elegant?
- Which assay is the fastest?
- Which assay is the most solid one?
- Which assay gives the best information?



Reference Values for Aldosterone–Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption

Michiel N. Kerstens,^{1*} Anneke C. Muller Kobold,² Marcel Volmer,² Jan Koerts,² Wim J. Sluiter,¹ and Robin P.F. Dullaart¹

- 100 normotensive individuals
- 50 /50 m/f, age 20-70
- 10/10 per age decade
- PRA vs DRC (Cisbio)
- Aldosterone (Siemens RIA)
- Aldosterone-renin ratios
- Before & after 3 day oral salt loading test (additional 9 g NaCl/day)

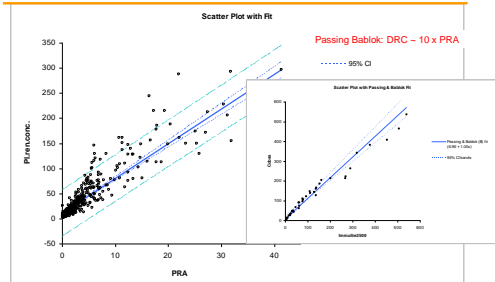
22/27

23/27

Clinical Chemistry 57:11 (2011)

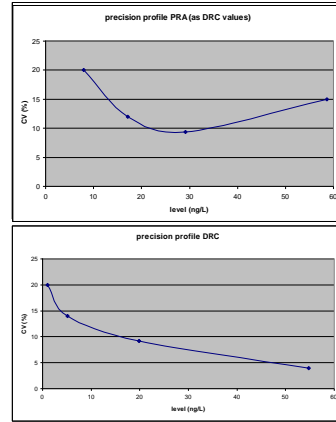


PRA vs DRC



24/27

Precision profile, functional sensitivity and LOD



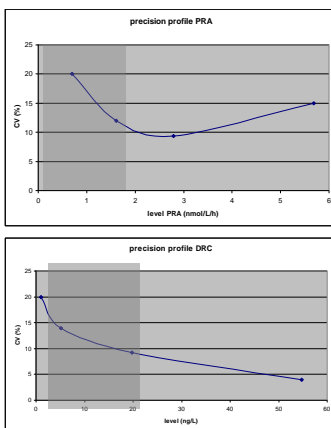
Passing Bablok:
DRC = 10 x PRA

LOD = 6,86 ng/b/L
FS = 0,9 ng/b/L/h

LOD = 0,7 ng/L
FS = 1,0 ng/L

25/27

Precision profile, functional sensitivity and LOD



LOD = 0.45 nmol/L/h
FS = 0.7 nmol/L/h

After SLT: 0.09-1.84 nmol/L/h

LOD = 0.7 ng/L
FS = 1.0 ng/L

After SLT: 1.8-20.0 ng/L

26/27

Reference Values for Aldosterone-Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption

Michiel N. Kerstens,^{1*} Anneke C. Muller Kobold,² Marcel Volmer,² Jan Koerts,² Wim J. Sluiter,¹ and Robin P.F. Dullaart¹

		Reference values		units
		Before SLT	After SLT	
DRC	plasma	3.4 - 29.6	1.8 - 20.0	ng/L
PRA	plasma	0.1 - 2.35	0.09 - 1.84	nmol/L/hr
Aldosterone	plasma	35 - 827	15 - 408	pmol/L
ARRdrc	plasma	4.1 - 81.3	3.9 - 74.8	pmol/ng
ARRpra	plasma	0.07 - 1.45	0.06 - 1.84	/hr
Aldosterone	urine	6.7 - 84.3	6.4 - 37.6	nmol/24 hr
		Screeningtest	Confirmation test	

27/27

Clinical Chemistry 57:11 (2011)

In conclusion

- Standardize preanalytical conditions
- Stop, if possible, antihypertensives
- Both PRA and DRC measure renin
- But PRA and DRC measure different things
- Prevent cryoactivation of prorenin
- PRA and DRC can both be influenced by cryoactivation
- Take caution when interpreting PRA or DRC results under antihypertensive medication (especially renin inhibitors)
- Reference values for DRC, Aldosterone, ARRdrc

Renin and units: pmol/L/min, ng/ml/h, mU/L, ng/L.....????

TABLE 5. ARR cut-off values, depending on assay and based on whether PAC, PRA, and DRC are measured in conventional or SI units

	PRA (measured in ng/mL/h)	PRA (measured in pmol/L/min)	DRC* (measured in mU/L)	DRC* (measured in ng/L)
PAC (as ng/dL)	20	1.6	2.4	3.8
	30^a	2.5	3.7	5.7
	40	3.1	4.9	7.7
PAC (as pmol/L)	750^a	60	91	144
	1000	80	122	192

ARR, Aldosterone-renin ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity; DRC, direct renin concentration; SI, Systeme International
^a Values shown are on the basis of a conversion factor of PRA (ng/mL/h) to DRC (mU/L) of 8.2. DRC assays are still in evolution, and in a recently introduced and already commonly used automated DRC assay, the conversion factor is 12 (see text).
^b The most commonly adopted cut-off values are shown in bold: 30 for PAC and PRA in conventional units [equivalent to 830 when PAC is in SI units] and 750 when PAC is expressed in SI units [equivalent to 27 in conventional units].

Activity assays

Mass assays

M. Fischer et al. / Cardiovascular Research 53 (2002) 672–677

673

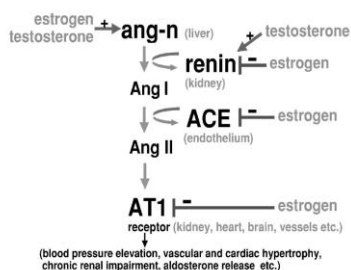
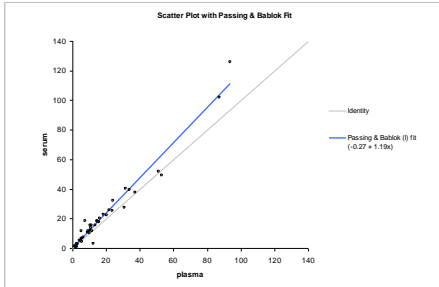
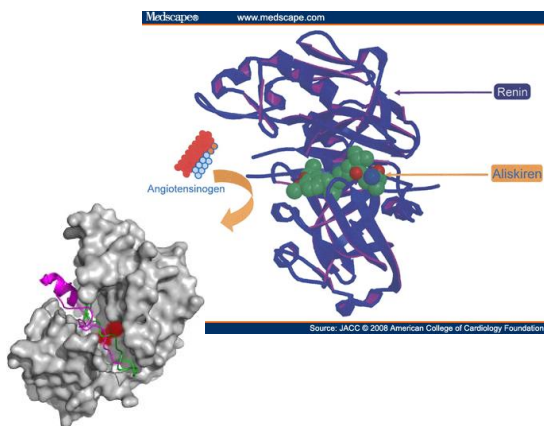
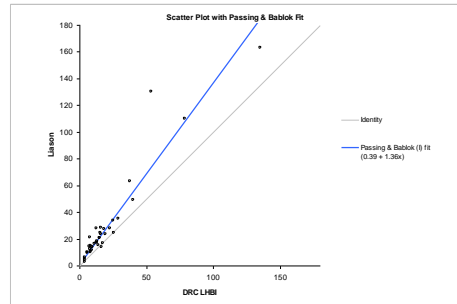


Fig. 1. The figure displays the cascade leading to angiotensin II formation and subsequent receptor activation and the influence of estrogen and testosterone on various components.

Serum vs EDTA plasma effect of prorenin activation?



DiaSorin vs CisBio



Renin assay confusion...?

- Enzyme kinetic (indirect) assay
 - Production Ang I
 - Plasma renin activity assay **PRA**
 - Endogenous substrate (angiotensinogen)
 - Plasma renin concentration assay **PRC**
 - Exogenous substrate (angiotensinogen)
 - Total renin concentration (activity of prorenin & renin) **acTRC**
- Immunosorbent (direct assays) **DRC**
 - ELISA's & IRMA's
 - Immunoreactive renin
 - Total renin concentration (concentration of prorenin & renin) **irTRC**

Schalekamp et al.
J Hypertens 26:928-937 © 2008

Campbell et al.
Clinical Chemistry 55:5
867-877 (2009)

Renin assay confusion...?

- Enzyme kinetic (indirect) assay Production Ang I

Plasma renin activity assay **PRA**

Endogenous substrate (angiotensinogen)

Plasma renin concentration assay **PRC**

Exogenous substrate (angiotensinogen)

Total renin concentration (activity of prorenin & renin) **acTRC**

- Immunosorbent (direct assays) **DRG**

ELISA's & IRMA's

Immunoreactive renin assay

Total renin concentration (concentration of prorenin & renin) **irTRC**

Schalekamp et al.
J Hypertens 26:928-937 © 2008

Campbell et al.
Clinical Chemistry 55:5
867-877 (2009)