1. NAME AND INTENDED USE

RENIN-ELISA kit is an immuno-assay for the quantitative in vitro diagnostic measurement of the level of active renin in plasma EDTA. Renin measurements are used in the diagnosis and treatment of certain types of hypertension.

2. SUMMARY AND EXPLANATION

Renin is a proteolytic acidic enzyme produced and secreted by the juxtaglomerular cells. It cleaves angiotensinogen into angiotensin I (inactive), which ultimately leads to the production of angiotensin II (active). Therefore, renin, which has a limiting effect on the production of angiotensin, is a key-factor in the regulation of arterial pressure and hydrosodic metabolism.

As most enzymes which act outside of the cells in which they are synthesized, renin exists in both inactive and active forms. Inactive renin (prorenin) which is found in plasma, amniotic fluid and in the kidney, can be activated in different ways (cryoactivation, acidification or partial proteolysis) which expose the active site of the enzyme. Inactive renin can account for up to 90 % of the total renin in the circulation.

However, it is the active renin which provides the medium through which biological activity takes place. Now, human active renin is well known: it is a polypeptidic chain of 345 amino acids, with a molecular weight of about 40,000. Angiotensinogen, the substrate of renin, is a liver protein from which angiotensin I is produced. It must be noted that the concentration of angiotensinogen in circulation influences the level of plasmatic renin activity and so, ultimately, the level of angiotensin II. This shows the importance, for the synthesis of angiotensinogen, of those factors directly active at the liver level.

It has been proved that the in vivo hypertensive action of angiotensin I is due to its conversion into angiotensin II by a carboxypeptidase (converting enzyme). The converting enzyme is regulated by the glucocorticoids and the thyroid hormones. Angiotensin II is the major effector in the renin-angiotensin system, maintaining circulatory homeostasis through its direct effect on the smooth vascular muscle and on the stimulation of aldosterone, and by its stimulatory effect on the sympathetic nervous system.

In the kidney, angiotensin II is involved in the control of glomerular filtration and in renal blood flux. Renin is secreted by the kidneys in response to a reduction in the perfusion of the renal artery (intrarenal baroceptor), a reduction of distal tubular reabsorption of Na+ (sodium leakage), an hypokaliemia or a B-adrenergic stimulation. In addition renin secretion is reduced (negative feedback) when there is a high plasmatic concentration of angiotensin II.

Renin assay is necessary in hypertensive patients and in the therapeutic follow up of high blood pressure. Renin should be measured:

- Whenever diastolic blood exceeds 110 mm Hg (to trace an hypertension of renal origin).
- Whenever there is an hypokaliemia (< 3.8 mmol/l): to try to find a secondary hyperaldosteronism or a primary hypermineralocorticism.
- Whenever the response to antihypertensive treatment is insufficient.
- In order to determine the functional character of a renal artery stenosis (by measurement of renin in the renal veins during acute inhibition of the converting enzyme).
- Whenever a cancer is linked to an increase in blood pressure (to look for ectopic production of renin).

3. PRINCIPLE

The RENIN-ELISA kit is a sandwich ELISA-type immunoassay. A first monoclonal antibody, immobilized on the microplate, captures the renin proteins contained in the calibrators and samples. After washing, the bound proteins are then recognized by a second monoclonal antibody conjugated to HRP (Horseradish Peroxidase). After a second incubation, the unbound reagents are eliminated by washing. Then, the colorimetric reaction is started by the addition of an HRP substrate, TMB (3,3',5,5' Tetramethyl-benzidine). After, the reaction is stopped by addition of an acid solution, the optical density (OD) of each well is read first at 405 nm (extended range) and/or 450 nm (high sensitivity range). The OD values are proportional to the renin protein concentrations contained in the calibrators and samples.

The assay kit allows to perform 96 tests:
- 7 calibrators + 2 controls + 78 tests or 39 unknown samples in duplicate if using the high sensitivity + extended range mode
- 5 calibrators + 1 control + 84 tests or 42 unknown samples in duplicate if using the high sensitivity mode only
### 4. REAGENTS

Each kit contains enough reagents for 96 tests. The expiry date is marked on the external label.

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>SYMBOLS</th>
<th>QUANTITY</th>
<th>STORAGE</th>
</tr>
</thead>
</table>
| MICROPLATE: Ready for use.  
Anti-renin mouse monoclonal antibody immobilized onto the surface of the wells. | MICROPLATE | 1 plate (96 wells)  
6 strips of 16 wells (foil pouch with dessicant) | 2-8°C until the expiry date.  
After opening, any unused strips may be stored for 6 weeks at 2-8°C in the plastic bag supplied, with dessicant, properly sealed. |
| INCUBATION BUFFER: Ready for use.  
This reagent is used as an incubation buffer. Buffered solution containing bovine proteins and preservative. | INCBUF | 1 x 12 mL vial | 2-8°C until the expiry date.  
After opening, the solution should be stored at 2-8°C and used within 6 weeks. |
| CONJUGATE: Ready for use.  
Buffered solution containing mouse monoclonal anti-renin antibody linked to HRP, bovine proteins, stabilizers, green dye and preservative. | CONJ | 1 x 22 mL vial | 2-8°C until the expiry date.  
After opening, the solution should be stored at 2-8°C and used within 6 weeks. |
| DILUENT – CALIBRATOR 0 (CAL 0): Ready for use.  
Buffered solution containing bovine proteins, yellow dye and preservative. | DILCAL | 1 x 20 mL vial | 2-8°C until the expiry date.  
After opening, the solution should be stored at 2-8°C and used within 6 weeks. |
| CALIBRATORS (CAL 1 – CAL 6): lyophilized.  
Lyophilized buffered solution containing human recombinant renin, bovine proteins, yellow dye and preservative.  
5 – 25 – 50 – 100 – 175 – 250 µIU/mL * / **  
Reconstitute with 1 ml distilled water, replace the cap, invert several times and vortex to ensure complete reconstitution. | CAL | 6 vials  
qs  
1 mL | 2-8°C until the expiry date.  
After reconstitution, do not store for more than 2 hours at room temperature. Store at 2-8°C for 2 weeks or divide into aliquots and freeze at < -16°C for a period of 6 weeks (maximum of 2 freezing steps). |
| CONTROLS 1 & 2 (Low and High): lyophilized.  
Lyophilized human plasma containing endogeneous renin and 0.1% sodium azide.  
Reconstitute with 1 ml distilled water, replace the cap, invert several times and vortex to ensure complete reconstitution. The actual acceptance limit values are printed on the vial label. | CONTROL | 1 vial each  
qs  
1 mL | 2-8°C until the expiry date.  
After reconstitution, do not store for more than 2 hours at room temperature. Divide into aliquots and freeze at < -16°C for a period of 6 weeks (maximum of 2 freezing steps). |
| PBS BUFFER: tablets.  
Phosphate buffered saline.  
Solubilize 1 tablet into distilled water to prepare 100 ml PBS buffer.  
WARNING! To prepare the wash buffer solution, add 0.3 mL of TWEEN20 reagent to each 100 mL of PBS buffer and mix slowly. | BUFWASH | 4 blisters of 5 tablets each  
(quantity sufficient to prepare 2 liters of wash buffer solution) | 2-8°C until the expiry date. |
| TWEEN20: Tween-20 solution. | TWEEN 20 | 1 x 10 mL vial | 2-8°C until the expiry date. |
| SUBSTRATE: Ready for use.  
Reagent containing 3, 3', 5, 5' tetramethylbenzidine (TMB). | SUBSTMB | 1 x 12 mL vial | 2-8°C until the expiry date. |
| STOP SOLUTION: Ready for use.  
0.5 M sulphuric acid solution. | STOPSOLN | 1 x 22 mL vial | 2-8°C until the expiry date. |
| ADHESIVE FILM FOR MICROPLATE | | 3 | |
| PLASTIC BAG | | 1 | |

Opened kits retain activity for 6 weeks if stored as described above.  
*The values indicated above are only target values. The true value of each calibrator is shown on its label.  
** The assigned renin values of the RENIN-ELISA kit are expressed in International Units and are traceable to the WHO reference materials (68/356).
5. PRECAUTIONS FOR USE

5.1. Safety measures
The raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and have been found to be negative for anti-HIV 1, anti-HIV 2 and anti-HCV antibodies and the HBs antigen. However, as it is still impossible to strictly guarantee that such products are incapable of transmitting hepatitis, the HIV virus or any other viral infection, **all raw materials of human origin, including the samples to be assayed, must be treated as potentially infectious.**

Do not pipette by mouth.
Do not smoke, eat or drink in areas in which samples or kit reagents are handled.
Wear disposable gloves while handling kit reagents or samples and wash hands thoroughly afterwards. Avoid splashing.
Decontaminate and dispose of samples and all potentially contaminated materials as if they contained infectious agents. The best decontamination method is autoclaving for a minimum of one hour at 121.5°C.
Sodium azide may react with lead or copper piping to form highly explosive metal azides. When disposing of waste, dilute thoroughly to prevent the formation of such products.
Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.

5.2. Handling precautions
Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbial contamination of the reagents and water. Comply with the incubation times.

6. SAMPLE COLLECTION AND PREPARATION

The assay is performed directly on EDTA plasma.

- If samples can be tested within 4 hours of primary collection, specimens should be capped and stored at room temperature (never at 2-8°C) prior to processing.
- If the test is not run within 4 hours following sampling, samples must be aliquoted and stored frozen at -20°C. Under these conditions samples can be stored 10 months.

After thawing, plasma must be shaken and carefully centrifuged to eliminate any trace of fibrin. Avoid successive freezing and thawing.

**WARNING!** Samples must NEVER be stored or thawed at 2-8°C as cryoactivation of prorenin may occur at these temperatures, giving higher and potentially false positive active renin concentrations.

Dilutions
Should elevated renin levels be suspected (>~250 µIU/mL or >~100 µIU/mL if the extended range is not employed), the Diluent - Calibrator 0 reagent supplied in the kit is used for dilution.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1 Equipment required

- Precision micropipettes or similar equipment with disposable tips for distribution of 20, 50, 100, 200 and 1000 µL. Calibration of these must be regularly checked.
- Distilled water.
- Disposable plastic tubes.
- Vortex mixer.
- Circular horizontal plate shaker (700 rpm).
- Microplate washer (optional).
- Microplate reader, capable of measuring absorbance at 450 nm. If the extended range is employed, the reader must be fitted with a filter at 405 nm. As an option, the reader may be fitted with a filter capable of reading the absorbance at a wavelength of between 610 nm and 650 nm. This second reading makes it possible to correct the microplate's imperfections.

7.2 Protocol

All the reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. The reagents are taken up and distributed into wells at room temperature (18-25°C). Each calibrator, control or sample must be tested in duplicate.
Determine the number of wells required for the assay and remove any unused strips. Store at 2-8°C in the plastic bag supplied for this purpose, with dessicant, and properly sealed.
Reconstitute the vials of calibrators and control. Carefully check that all the lyophilisate is dissolved, and use within an hour following reconstitution.
To obtain reliable and reproducible results, it is recommended that the washing stages be performed as indicated; the residual washing solution volume must be as low as possible. The use of a microplate washer is recommended.

**CAUTION!** The BUF.WASH tablets are intended to prepare a phosphate buffered saline solution. It is mandatory to add 0.3 mL TWEEN solution for each 100 mL of phosphate buffered saline solution to constitute the wash buffer solution mentioned in the protocol during the washing steps.

Preparation of the Wash buffer solution

- Solubilize 1 BUF.WASH tablet into distilled water to prepare 100 mL PBS buffer
- Add 0.3 mL of TWEEN reagent to each 100 mL of solution and mix slowly
- The wash solution is stable for 1 week at 2-8°C.
PROTOCOL - Comply with the order for addition of reagents:

If applicable, predilute samples using the diluent DIL CAL reagent supplied in the kit.

1 - Place a sufficient number of microtiter well strips in the frame holder.

2 - Dispense 100 µL of incubation buffer INC BUF in all wells

3 - Add 100 µL of calibrators CAL controls CONTROL and samples to the appropriate wells in duplicate. Note that calibrators are colored in yellow.
   - If the extended range mode is not used, only dispense control CONTROL 1 calibrators CAL to CAL4 corresponding to the sensitive range (~0-100 µU/mL).
   - If a 405 nm filter is available on the microplate reader and the extended range mode is used (~100-250 µU/mL), dispense also CONTROL 2 and calibrators CAL5 and CAL6.

4 – Cover with the adhesive film and incubate for 1h +/- 5 min at room temperature (18-25°C) under agitation on a plate shaker at 700 rpm (700 rpm)

5 – Wash the wells as follows:
   a. Remove the content of the wells
   b. Distribute 300 µL of wash solution
   c. Repeat this operation another four times for a total of 5 washing cycles.
   d. Finish by aspirating. The residual washing solution volume must be as low as possible.

6 - Dispense 200 µL of antibody-HRP conjugate CONJ in wells. Note that the color of this liquid reagent is green.

7 - Cover with the adhesive film and incubate for 1h30 +/- 5 min at room temperature (18-25°C) under agitation on a plate shaker at 700 rpm

8 - Wash the wells as follows:
   a. Remove the content of the wells
   b. Distribute 300 µL of wash solution
   c. Repeat this operation another four times for a total of 5 washing cycles.
   d. Finish by aspirating. The residual washing solution volume must be as low as possible.

9 - Dispense 100 µL of TMB substrate SUBS TMB in all wells.
10 – Cover with the adhesive film and incubate for **20 min** at room temperature (18-25°C) under agitation on a plate shaker at 700 rpm.

11 - Stop the reaction by adding **100 µL** stop solution **STOP SOLN** to all wells.

12 - It is recommended to clean the outer bottom of the wells with a lint-free soft tissue to eliminate possible fingerprints or smudges.

13 - Remove the adhesive film and measure the absorbance (OD) **within 10 minutes** after adding the stop solution:

- If available, perform a first read using a **405 nm** filter (the 405 nm read must be achieved first)
- Perform a read at **450 nm**
- Optional: perform a read at a wavelength of between 610 and 650 nm (620 nm recommended)

**Note:** If a 405 nm filter is not available, only use the high sensitivity range (CAL0-CAL4) and samples with renin values higher than the calibrator 4 (~100 µU/mL) have to be further diluted or reported as higher than this value.

**Note:** the optional 405 nm read allows to extend the concentration range of the calibration curve of the assay from calibrator 4 (~100 µU/mL) to calibrator 6 (~250 µU/mL). The exact value of each calibrator is printed on the vial label.

---

**ASSAY FLOWCHART – TOTAL INCUBATION TIME = 2h50**

<table>
<thead>
<tr>
<th>Wells</th>
<th>INC BUF</th>
<th>Test</th>
<th>CONJ Green</th>
<th>SUBS TMB</th>
<th>STOP SOLN</th>
<th>Read OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrators</td>
<td>100 µL</td>
<td>100 µL</td>
<td>Incubate 1h 700 rpm 18-25°C</td>
<td>Incubate 1h 700 rpm 18-25°C</td>
<td>Incubate 20 min 700 rpm 18-25°C</td>
<td>100 µL</td>
</tr>
<tr>
<td>Controls</td>
<td>100 µL</td>
<td>100 µL</td>
<td>Wash 5 x with 300 µL Wash Buffer Solution and aspirate</td>
<td>Wash 5 x with 300 µL Wash Buffer Solution and aspirate</td>
<td></td>
<td>100 µL</td>
</tr>
<tr>
<td>Samples</td>
<td>100 µL</td>
<td>100 µL</td>
<td></td>
<td></td>
<td></td>
<td>100 µL</td>
</tr>
</tbody>
</table>

1) 405 nm  
2) 450 nm  
Option 620 nm
8. QUALITY CONTROL

Good Laboratory Practices (GLP) require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. CALCULATION OF RESULTS

1- Optional OD correction*: subtract readings at 620 nm (610 to 650 nm) from the readings at 405 and/or 450 nm.

2- For each duplicate, calculate the mean absorbance (OD) of calibrators, controls and samples.

3- Construct 1 or 2 calibration curve(s) depending on the use or not of the extended range mode from the 405 nm data:
   - **Calibration curve for HIGH SENSITIVITY RANGE (~0-100 µIU/mL)**
     Prepare a calibration curve by plotting the (corrected*) mean OD values at 450 nm of calibrators CAL0, CAL1, CAL2, CAL3 and CAL4 (y-axis) against their concentration (x-axis) indicated on the vial.
     - Concentration of samples with (corrected*) mean OD values at 450 nm ranging between CAL0 and CAL4 must be read directly from this calibration curve.
   
   - **Optional: calibration curve for EXTENDED RANGE MODE (~100-250 µIU/mL)**
     Prepare a calibration curve by plotting the (corrected*) mean OD values at 405 nm of calibrators CAL0, CAL3, CAL4, CAL5 and CAL6 (y-axis) against their concentration (x-axis) indicated on the vial.
     - Concentration of samples with (corrected*) mean OD values at 405 nm ranging between CAL4 and CAL6 must be read directly from this calibration curve.

   *Note: The extended range calibration curve should not be used to read samples with an OD at 450 nm < CAL4

4- The **third-order polynomial** mathematical fitting model is recommended for both calibration curves. Other data reduction functions may give slightly different results.

Read the values of the samples from the curve, correcting by the dilution factor if necessary.

Do not extrapolate data with OD values higher than the highest calibrator of the selected range.

**Example of assay data:** for illustration only and must under no circumstances be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>Concentration µIU/ml</th>
<th>High sensitivity range OD 450 nm</th>
<th>Extended range OD 405 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL0</td>
<td>0</td>
<td>0.023</td>
</tr>
<tr>
<td>CAL1</td>
<td>4.2</td>
<td>0.131</td>
</tr>
<tr>
<td>CAL2</td>
<td>25</td>
<td>0.68</td>
</tr>
<tr>
<td>CAL3</td>
<td>50</td>
<td>1.31</td>
</tr>
<tr>
<td>CAL4</td>
<td>91</td>
<td>2.38</td>
</tr>
<tr>
<td>CAL5</td>
<td>170</td>
<td>1.24</td>
</tr>
<tr>
<td>CAL6</td>
<td>225</td>
<td>1.49</td>
</tr>
<tr>
<td>CONTROL 1</td>
<td>37.4</td>
<td>0.99</td>
</tr>
<tr>
<td>CONTROL 2</td>
<td>129</td>
<td>1.02</td>
</tr>
</tbody>
</table>

10. LIMITATION OF THE PROCEDURE

- Samples presenting cloudiness, haemolysis, hyperlirpemia or containing fibrin may give inaccurate results.

- The significance of active renin measurements can be meaningfully interpreted only when the patient is studied under controlled conditions and with defined sodium balance.

- Since a number of physiological factors can influence the renin secretion, conditions under which samples are collected must be carefully controlled:
  a. the patient must not have taken any antihypertensive medication for 8 days.
  b. posture must be controlled: he must have been lying down for more than one hour or upright for more than one hour.
  c. sodium content in the diet must be known and eventually verified by the measurement of natriuria over a period of 24 hours (60 to 200 mEq/24 h).
It must be known that physiological factors affect renin secretion:

- a. both levels of inactive and active renin increase during pregnancy,
- b. menstrual cycle: increase of the level on the second phase of the cycle (sampling is to be done if possible during the first phase),
- c. active renin level decreases with age,
- d. Nycthemeral cycle affects also the concentration: sampling is to be done between 7 AM and 10 AM if possible.

It must be also noted that various medications could affect the renin secretion:

- a. Diuretics, inhibitors of the conversion enzyme (Captopril, Enalapril), vasodilators (Dihydralazine, Minoxidil, Prazozine,) could provide a stimulation of the renin-angiotensin system.
- b. Beta adrenergic-blocking agents (Labetalol, Clonidin, Methyl-dopa,) could provide inhibition on the renin-angiotensin system.
- c. Cathepsin B (0.1 U/mL) has been found to interfere with this test. **Do not use this test for patients being administered Cathepsin B.**

Do not extrapolate sample values beyond the last calibrator of the range used (CAL4 for 450 nm data or CAL6 for 405 nm data). Dilute the samples concerned and retest.

**Do not use the extended range mode from data at 405 nm to read sample values below CAL4, use the 450 nm data instead.**

**Do not use results if reading is performed more than 10 minutes after adding the stop solution.**

11. EXPECTED NORMAL VALUES

It is critically important to recognize that many factors (posture, age, sodium intake, menstrual cycle) can influence the active renin level. Thus, and with every diagnostic test, it is recommended that each laboratory establishes its normal range values according to its own population.

In order to determine the normal range of RENIN-ELISA, 124 samples from fasting subject males and females, without estrogen-progesterone treatment, apparently healthy subjects ages 4 to 89 years were analyzed using the RENIN-ELISA kit.

The results are shown in the Table below:

<table>
<thead>
<tr>
<th>POSITION</th>
<th>n</th>
<th>Mean (µIU/mL)</th>
<th>5th perc. (µIU/mL)</th>
<th>95th perc. (µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPINE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean men</td>
<td>22</td>
<td>19.0</td>
<td>2.2</td>
<td>42.4</td>
</tr>
<tr>
<td>Mean women</td>
<td>33</td>
<td>15.2</td>
<td>2.2</td>
<td>44.0</td>
</tr>
<tr>
<td>UPRIGHT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean men</td>
<td>25</td>
<td>19.0</td>
<td>2.7</td>
<td>60.3</td>
</tr>
<tr>
<td>Mean women</td>
<td>44</td>
<td>24.4</td>
<td>6.2</td>
<td>68.0</td>
</tr>
</tbody>
</table>

12. PERFORMANCE CHARACTERISTICS

12.1 Measurement Range of the assay

The samples must be measured in the range between the lower limit of detection and the highest concentration of the calibration range, i.e. between 1.5 µIU/mL and 100 µIU/mL (high sensitivity) or 250 µIU/mL (extended mode).

12.2 Traceability

The assigned renin values of the RENIN-ELISA kit are expressed in International Units and are traceable to the WHO reference materials (68/356).

12.3 Precision

12.3.1 Intra-Assay

The intra-assay (within-run) variation was determined by 27 to 30 measurement of 3 samples covering the whole measuring range of the calibration curve.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Mean value (µIU/mL)</td>
<td>31.7</td>
<td>99.4</td>
<td>191</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.8</td>
<td>5.1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

12.3.2 Inter-Assay

The Inter-assay (between-run) variation was determined using 5 tests measured in 15 to 45 runs in duplicate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>CONTROL 1</th>
<th>CONTROL 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>15</td>
<td>43</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>Mean value (µIU/mL)</td>
<td>33.4</td>
<td>63.2</td>
<td>197</td>
<td>39.0</td>
<td>133</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.6</td>
<td>5.0</td>
<td>5.3</td>
<td>5.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>
12.4 Detection limit
The Limit of Detection (LOD or analytical sensitivity) of the RENIN-ELISA kit is defined as being the lowest detectable concentration that differs from zero with a probability of 95% calculated by adding 2 standard deviations to the mean of 30 replicate analysis of the zero calibrator (CAL0).

### Analytical Sensitivity (Limit of Detection)

| LOD    | 0.5 µIU/mL |

The Limit of Quantitation (LOQ or functional sensitivity) of the RENIN-ELISA kit is defined as being the concentration measured by the imprecision profile at a CV of equal to 15%. It was calculated by testing 10 specimens in duplicate in 30 runs using 3 different kit lots. The mean, standard deviation, and %CV were then determined for each sample.

### Functional Sensitivity (Limit of Quantitation)

| LOQ    | 1.5 µIU/mL |

12.5 Recovery
Renin solutions from the 7 calibrators were mixed 1:1 to 3 plasma samples pools with various initial Renin concentrations. Each sample (non-spiked and spiked) was assayed in duplicates in one run. Renin concentrations were measured and the recovery percentage were calculated.

### Recovery

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pool A</th>
<th>Pool B</th>
<th>Pool C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µIU/mL)</td>
<td>49.0</td>
<td>105</td>
<td>119</td>
</tr>
<tr>
<td>Mean recovery (%)</td>
<td>96.9</td>
<td>94.4</td>
<td>97.8</td>
</tr>
<tr>
<td>Range of recovery (%)</td>
<td>92.5 - 99.2</td>
<td>87.8 - 98.0</td>
<td>89.3 - 109</td>
</tr>
</tbody>
</table>

12.6 Dilution - Linearity
Studies were performed to evaluate the linearity of the assay using 3 EDTA plasma samples of different concentrations. The samples were assayed as neat and serially diluted with DIL-CAL0 down to the LOQ (dilution factor down to 1:64 or 1:128).

### Dilution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pool 1</th>
<th>Pool 2</th>
<th>Pool 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µIU/mL)</td>
<td>96.9</td>
<td>257</td>
<td>223</td>
</tr>
<tr>
<td>Range of Dilution</td>
<td>1:2 to 1:64</td>
<td>1:2 to 1:128</td>
<td>1:2 to 1:128</td>
</tr>
<tr>
<td>Mean recovery (%)</td>
<td>108</td>
<td>104</td>
<td>99</td>
</tr>
<tr>
<td>Range of recovery (%)</td>
<td>102 - 115</td>
<td>98 - 109</td>
<td>93 - 107</td>
</tr>
</tbody>
</table>

### Linearity

| Intercept | 1.31 | 0.45 | -0.39 |
| Slope (Y=measured) | 1.01 | 1.00 | 0.99 |
| R² | 0.99 | 0.99 | 0.99 |

These results show the good linearity of the dilution test over the reported measuring range of this assay (LOQ to the highest calibrator).

12.7 Specificity
The specificity of the assay is guaranteed by the use of two complementary monoclonal antibodies. Human renin is recognized. A study was performed to evaluate the effect of Pro-Renin on the Renin assay using molar concentrations of both molecules to take into account the differences in molecular weights.

Cross Reaction with pro-renin is below 0.007% (mean value obtained when pro-renin is spiked at a concentration of 10 ng/ml in 3 different plasma EDTA samples with known renin values).

12.8 Hook effect
No hook effect was observed with this assay up to 30000 µIU/mL.
12.9 Interferences

An interference study was evaluated according to CLSI EP17-A2 guideline. No significant* interference was observed when plasma samples were spiked with any of the following substances at the concentration given below.

- Human haemoglobin (2 mg/mL)
- Triglycerides from hyper-lipidemic EDTA plasma (7.4 g/L)
- Triglycerides from a commercial Intralipid solution (5 g/L)
- Human albumin (spiked up to 60 mg/mL)
- Bilirubin (0.15 mg/mL)
- Cathepsin D (0.5 U/mL)
- Various treatments for hypertension: Captopril (5 µg/mL), Renitec (0.3 µg/mL), Loxen (60 µg/mL), or Lasilix (60 µg/mL)

* No significant interference corresponds to variation in Renin concentrations between spiked and unspiked samples (Controls) within +/- 10 %.

CAUTION 1 - Cathepsin B has been found to interfere with this test (tested at 0.1 U/mL). Do not use this test for patients being administered Cathepsin B.

CAUTION 2 - The immunoassay is protected against potential interferences with heterophilic antibodies such as HAMA and rheumatoid factors (RF) by adding a protection. Nevertheless, we cannot assure that there will never be a “false positive” result due to the presence of heterophilic antibodies in a patient sample.

13. METHOD COMPARISON

A study was performed to compare the results of the RENIN-ELISA kit to RENIN III GENERATION RIA assay (Cisbio Bioassays) using 299 EDTA plasma samples and 3 different kit lots.

The equivalence of the concentration reported by the RENIN III GENERATION RIA assay with respect to the international reference (WHO 68/356) was obtained by multiplying the results from the RIA kit (pg/mL) by a factor of 1.8 to obtain µIU/mL.

Passing Bablok regression analysis and difference plots were applied to these samples, yielding the following regression:

\[
\text{RENIN - ELISA} = 0.98 \times \text{RENIN III GENERATION RIA} - 1.44 \text{ µIU/mL}
\]

The 95% confidence interval for the slope was 0.95 to 1.01, and the 95% confidence interval for the intercept was -2.24 to -0.70 µIU/mL for the 299 patient samples having Renin concentrations from 2.4 to 234 µIU/mL.

14. Bibliography


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