



## Instructions for Use


# LEMS<sup>®</sup> Assay RIA


**125I-Radio Immuno Assay  
for the Quantitative Determination of Antibodies  
to the Voltage-gated P/Q-Calcium Channel (VGCC)  
in Serum**



REF RA117/25

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




 2 – 8 °C

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## Symbols

 IVD	In-Vitro-Diagnostic Device		EC Declaration of Conformity
 CONT	Contents		Expiry Date
 LOT	Lot Number		Store
	Manufactured by		Sufficient for
 REF	Catalogue Number		Consult Instructions

## Hazard Pictograms



radioactive

## 1. Introduction and Principle of the Test

The primary physiological disorder in the **Lambert-Eaton Myasthenic Syndrome (LEMS)** is the decreased release of the neurotransmitter acetylcholine from the nerve terminal into the synaptic gap.

This presynaptic defect is caused by antibodies against a membrane protein of the nerve cell, the voltage-gated calcium channel (VGCC).

As the typical clinical symptoms of LEMS are very similar to those of the generalized myasthenia gravis, the syndrome may initially be misdiagnosed as myasthenia gravis. Therefore, the determination of VGCC antibodies is of great help for the differential diagnosis of these syndromes.

About 60 percent of patients with a Lambert-Eaton syndrome have an associated small cell lung carcinoma. Usually the diagnosis of LEMS precedes the radiological appearance of the carcinoma by two years and sometimes by 5 years or more. Therefore, the diagnosis of LEMS is often the earliest clue to an underlying tumor.

The principle of the test for the determination of antibodies to VGCCs is similar to that of the ACHRAB<sup>®</sup>-Assays for the determination of antibodies against the postsynaptic acetylcholine receptor.

Calcium-channel proteins labelled with <sup>125</sup>Iodine are used for the LEMS-assay. For the radioactive labelling the fact is used that these membrane proteins have a strong affinity to conotoxin, a toxin produced by a cone snail which binds almost irreversibly to the VGCCs.

There are several subtypes of calcium-channel proteins according to their electrophysiological characteristics. Each of them binds to different conotoxines. Apparently, the VGCCs of the P/Q-subtype are responsible for neurotransmitter release at the neuromuscular junction and are probably the target antigen for the autoantibodies. This subtype binds to the  $\omega$ -Conotoxin MVIIIC from the cone snail *Conus magus*.

For the assay the  $\omega$ -Conotoxin MVIIIC is labelled with <sup>125</sup>I and then mixed with prepurified VGCCs. In this way the P/Q-calcium channels are labelled specifically and indirectly. The labelled protein is incubated with patient serum. During this incubation autoantibodies bind to the protein. Thereafter, the resulting complexes are precipitated with anti-human IgG. The concentration of autoantibody is proportional to the amount of radioactivity in the resulting pellet.

The concentration of the samples are calculated using the specific activity of the conotoxin and considering the individual nonspecific binding. The results are obtained as pmol/litre.

## **2. Precautions**

- For in vitro use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- This radioactive product may only be received, stored and used by persons so authorized and by laboratories covered by such authorization. It must not be administered to humans or animals under any circumstances.
- Do not eat, drink or smoke where radioactive materials are being handled.
- Do not pipet by mouth.
- Wear disposable gloves when handling radioactive materials.
- The kit components "Negative and Positive Controls" are made with human serum. All sera used are tested for HIV I/II antibodies, HCV and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.



## **3. Storage and Stability**

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels.

Do not mix various lots of any kit component within an individual assay.

#### 4. Contents of the Kit

4.1	<b>125I-Tracer (T)</b> for the measurement of the total binding lyophilized, activity < 6 kBq 125I- $\omega$ -Conotoxin MVIIC labelled VCGGs	<b>TRACER (T)</b>		2 vials
4.2	<b>125I-Tracer (NSB)</b> for the measurement of the nonspecific binding lyophilized, activity < 6 kBq 125I-labelled $\omega$ -Conotoxin MVIIC and VGCCs, saturated with unlabelled $\omega$ -Conotoxin MVIIC	<b>TRACER (NSB)</b>		2 vials
4.3	<b>Negative Control</b> 0,2 ml, ready for use, normal human serum	<b>CON -</b>		1 vial
4.4	<b>Positive Control</b> 0,25 ml, 1:10 prediluted, ready for use human serum containing antibodies against VGCC, for concentration ranges see qc certificate	<b>CON +</b>		1 vial
4.5	<b>Anti-human IgG</b> 4 ml, ready for use	<b>ANTI-HUMAN-IGG</b>		2 vials
4.6	<b>Wash Solution</b> 120 ml, ready for use, PBS with 0.01 % Triton X-100	<b>WASH</b>		1 bottle
4.7	<b>Dilution Buffer</b> for diluting the controls and patient sera 10 ml, ready for use, contains sodium azide (< 0,1 %)	<b>DIL</b>		1 vial

Additional materials and equipment required but not provided:

- Pipettes for 25, 50, 125  $\mu$ l and 1 ml
- Conical plastic tubes and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 1500 x g
- Dist. water
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter

## 5. Specimen Collection and Storage

Serum is to be used in the assay. Do not use lipemic or grossly haemolized serum. Do not use plasma. Repeated freezing and thawing should be avoided. Samples which appear turbid should be centrifuged before assay to remove any particulate material.

Samples can be stored up to one week at 2 - 8 °C or at - 20 °C for longer periods.

## 6. Preparation of Samples and Reagents

### 6.1 Patient samples

Patient samples should be stored at -20 °C. When required thaw at room temperature, mix gently, and centrifuge, if necessary, to remove any particulate material.

### 6.2 Dilution of Patient Samples and Negative Control

Dilute patient sera and Negative Control 1:10, i.e. 1+9, with the Dilution Buffer (for example 20 µl serum + 180 µl buffer). Do not dilute positive control as it is already diluted ready for use.

Centrifuge for 10 minutes at 3000 x g to remove any particulate material.

***Note: the Positive Control is already pre-diluted 1:10 !***

### 6.3 Reconstitution of the Tracer (T) and Tracer (NSB)

Approximately 10 min. before use reconstitute the lyophilized Tracers with each 0.7 ml distilled water. Dissolve by vortexing briefly (about 5 seconds) on a vortex mixer and then keep the vial approximately upright.

**Avoid continuous contact of the solution with the rubber stopper** as some of the tracer may stick to the stopper resulting in a significant reduction in total tracer cpm.

A slightly cloudy solution will be formed containing about 15,000 cpm per 50 µl.

The reconstituted Tracers are stable for only a few hours and should be used at once.

## 7. Test Procedure

- 7.1 The total binding as well as the nonspecific binding of each sample and the controls has to be measured in the assay.  
Therefore, one set of duplicate tubes for the total binding is requested as well as one set of duplicate tubes for the nonspecific binding.  
We recommend to use conical tubes.
- 7.2 Add 25  $\mu$ l of the Positive Control and 25  $\mu$ l of the 1:10 diluted Negative Control and 1:10 diluted samples, respectively, to the corresponding tubes of each set.
- 7.3 Add 50  $\mu$ l Tracer (T) into each tube of the set for the total binding.  
Add 50  $\mu$ l Tracer (NSB) into each tube of the set for the nonspecific binding.
- 7.4 Mix on a vortex mixer and incubate for 1 hour at room temperature.  
During this incubation count the total radioactivity of at least 2 tubes out of each set in a gamma counter.
- 7.5 Add 125  $\mu$ l Anti-human IgG to each tube and mix on a vortex mixer.  
Incubate 1 hour at room temperature.
- 7.6 Add 1ml of the Wash Solution to each tube and mix thoroughly.  
Centrifuge all tubes for 10 minutes at 4,000 x g or 20 minutes at 1,500 x g (preferably use a refrigerated centrifuge).
- 7.7 Decant or aspirate the tubes, taking care not to disturb the pellet.
- 7.8 Add 1 ml of the Wash Solution to each tube and carefully resuspend the pellets using a vortex mixer for a few seconds.
- 7.9 Centrifuge the tubes again for 10 minutes at 4,000 x g or 20 minutes at 1,500 x g (preferably use a refrigerated centrifuge).
- 7.10 Decant or aspirate the tubes carefully.
- 7.11 Count the tubes in a gamma counter for 2 minutes.

## 8. Calculation of Results

The following parameters are used for the calculation of the concentration of the antibodies:

- The difference of the cpm (total binding) minus the cpm (nonspecific binding)
- Faktor D for the decay of  $^{125}\text{I}$  between labelling date and assay date
- Dilution factor of the sample, usually = 10 (see Sample Preparation, paragraph 6.2).
- Sample volume, usually = 25  $\mu\text{l}$
- Specific activity of the toxin in dpm/fmol
- Counter efficiency Z ( % )

The relationship between the antibody-concentration and the radioactivity in the pellet can be calculated as follows:

$$\frac{(\text{cpm}_{\text{T-Sample}} - \text{cpm}_{\text{NSB-Sample}}) \times D \times \text{dilution factor}}{\text{volume of sample } (\mu\text{l}) \times \text{spec. activity of toxin} \times Z}$$

The usual values for the dilution factor (10), the volume of the sample (25  $\mu\text{l}$ ), the lot-depending activity of the toxin (dpm/fmol, indicated on the q.c. certificate) and the counter efficiency (70%, i.e. 0.7) can be summarized to the factor F.

The value of F is specific for each lot of tracer and is given in the q.c. certificate included in each kit.

Therefore the above formula becomes simplified to:

$$\text{Antibody concentration} = (\text{cpm}_{\text{T-Sample}} - \text{cpm}_{\text{NSB-Sample}}) \times D \times F$$

The result obtained is expressed in pmol/litre.



The decay factor D is the radioactivity at the time of manufacturing divided by the radioactivity at the time of the assay performance. This factor D can be taken from the following table. The date of manufacturing (labelling) is given in the qc certificate included in each kit.

Week of assay after labelling date	Factor D
1. - 2.	1.12
2. - 3.	1.22
3. - 4.	1.32
4. - 5.	1.43
5. - 6.	1.55
6. - 7.	1.68
7. - 8.	1.82

For example, if labelling was on the 01. November, then 1. - 2. week after labelling means the week of 08. - 15. November with a factor D of 1.12.

F is calculated assuming a counter efficiency of 70%. If the gamma counter used has a differing efficiency the value of F has to be adjusted accordingly. For example, if the counter efficiency is 74%, then F has to be corrected by  $0.7 / 0.74 = 0.95$ .

Finally, the calculated concentration of the Negative Control in pmol/l is subtracted from the calculated concentrations of all samples and controls in pmol/l.

**The difference thus obtained is reported as result in pmol/l.**

### Calculation Example:

F is given as 0.114 and D is 1.32 (assay performed 3. to 4. week after labelling date). For a dilution of samples of 1:10 and a counter efficiency of 70% the actual calculation factor to be used then becomes 0.150.

Sample	Mean cpm <sub>T</sub>	Mean cpm <sub>NSB</sub>	cpm <sub>T</sub> - cpm <sub>NSB</sub>	x F x D = pmol/l	Sample – Neg. Control pmol/l
Negative Control	544	429	115	17	—
Positive Control	3,167	326	2,841	426	<b>409</b>
Patient Serum	1,582	355	1,227	184	<b>167</b>

### 9. Reference Range

The recommended reference range – in accordance with results published by the University of Oxford, UK (see literature Motomura et al., 1995) is up to 40 pmol/l. Samples with concentrations above 40 pmol/l can be considered positive.

### 10. Assay Characteristics

#### Clinical Specificity

Samples from 160 individual healthy blood donors were measured. 160 (100%) were identified as being negative for VGCC Ab.

#### Clinical Sensitivity

Samples from 50 patients diagnosed with LEMS were. 50 (100%) were identified as being positive for VGCC Ab.

### **Clinical Accuracy**

There was no interference from autoantibodies to the acetylcholine receptor, 21-hydroxylase, glutamic acid decarboxylase, thyroid stimulating hormone receptor, thyroid peroxidase, thyroglobulin, dsDNA, or from Rheumatoid Factor.

### **Lower Detection Limit**

The negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at 2 standard deviations was 2.9 pmol/L

### **Interference**

No interference was observed when samples were spiked with the following materials; haemoglobin up to 500 mg/dL, bilirubin up to 20 mg/dL or intralipid up to 3000 mg/dL.

### **Precision**

#### Intra-Assay

sample	number	mean	cv (%)
1	25	145 pmol/l	6.9
2	25	62 pmol/l	15.5

#### Inter-Assay

sample	number	mean	cv (%)
1	20	142 pmol/l	14.6
2	20	61 pmol/l	14.3

## **10. Literature**

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