RiaRSR™ VGCC Ab

P-Type Voltage-Gated Calcium Channel (VGCC) Autoantibody RIA Kit - Instructions for use

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INTENDED USE

The RSR Voltage-Gated Calcium Channel autoantibody radioimmunoassay (VGCC Ab RIA) kit is intended for use by professional persons only, for the quantitative determination of P-type VGCC autoantibodies (VGCC Ab) in human serum. Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease, often associated with small cell lung cancer, in which autoantibodies are directed against voltage-gated calcium channels (VGCCs). The VGCCs can be classified by their electrophysiological characteristics into at least 4 subtypes (T, L, N and P). In the case of LEMS, autoantibodies to P-type VGCCs are most important and the RSR's kit provides a specific and sensitive assay for VGCC Ab.

REFERENCES

M. Motomura et al
An improved diagnostic assay for Lambert-Eaton myasthenic syndrome.
J. Neurol. Neurosurg. Psychiatry (1995) 58:85-87

V. A. Lennon et al
Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes.
N. Engl. J. Med. (1995) 332:1467-1474.

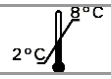
ASSAY PRINCIPLE

In RSR's VGCC Ab RIA, VGCC Ab in patient sera and controls are allowed to interact with detergent solubilised P-type VGCCs extracted from rabbit brain tissue and complexed with ^{125}I-labelled w-conotoxin MVIIC. After a 1 hour incubation, the resulting antigen-antibody complexes are immunoprecipitated by the addition of anti-human IgG. After a second incubation of 1 hour, assay buffer is added and the samples centrifuged. Unbound ^{125}I-labelled w-conotoxin MVIIC is removed from the tubes by aspiration of the supernatant. The level of radioactivity remaining in the tube is proportional to the antibody level in the test sample. Non-specific binding in the assay is determined using a preparation of VGCCs supplied in the kit which have been labelled with ^{125}I-conotoxin MVIIC in the presence of an excess of unlabelled conotoxin.

STORAGE AND PREPARATION OF TEST SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at 2 - 8°C for up to 2 weeks, or at −20°C or below for longer periods. 15 µL is sufficient for one assay. Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Do not use plasma. When required, bring test sera to room temperature and mix gently to ensure homogeneity. Dilute 1:10 using dilution buffer (e.g. 15 µL serum plus 135 µL dilution buffer). Centrifuge diluted serum prior to assay (preferably for 5 min at about 10,000 rpm i.e. about 10,000g in a microfuge) to remove any particulate matter.

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured by
	Sufficient for
	Expiry Date
	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

4.5 mL conical plastic tubes (Glass should not be used)
Pipettes capable of dispensing 25 µL, 50 µL 125 µL and 1 mL
Means of measuring various volumes to reconstitute or dilute reagents supplied.
Pure water.
Vortex mixer.
Refrigerated centrifuge capable of 1500 x g.
Gamma counter.

PREPARATION OF REAGENTS SUPPLIED

Store unopened kit and components at 2-8 °C.

A1	**^{125}I-Labelled VGCC T Tracer** **6kBq/vial** (at manufacture) 2 vials Lyophilised
	Reconstitute each vial by addition of 0.7 mL pure water and vortex gently to dissolve. Use immediately.
A2	**^{125}I-Labelled VGCC NSB Tracer** **6kBq/vial** (at manufacture) 2 vials Lyophilised
	Reconstitute each vial by addition of 0.7 mL pure water and vortex gently to dissolve. Use immediately.
B	**Dilution Buffer** 10 mL Ready for use
C	**Anti-Human IgG** 4 mL Ready for use
D	**Wash Solution** 120 mL Ready for use
E	**Negative Control** 0.2 mL
	Dilute 1:10 in dilution buffer (B) before use. For example 15 µL negative control (E) + 135 µL dilution buffer (B) on the day of use.
F	**Positive Control** 0.25 mL Ready for use.

ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20-25°C) for at least 30 minutes before use. An Eppendorf type repeating pipette is recommended for steps 2, 4, and 7.

1.	Dilute patient sera and kit negative control (E) 1:10 using dilution buffer [e.g. 15 µL serum plus 135 µL dilution buffer (B)]. Do not dilute positive control as it is already diluted ready for use.
2.	Pipette 25 µL (in quadruplicate) of diluted patient sera and diluted negative control (E) [all diluted 1:10 in dilution buffer (B)] and positive control (F) into labelled assay tubes. (The positive control is supplied ready diluted.)
3.	Pipette 50 µL of T ^{125}I VGCC (A1) into each of one set of duplicate tubes and 50 µL of NSB ^{125}I VGCC (A2) into the other set of duplicate tubes.
4.	Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature for 1 hour.
5.	Pipette 125 µL of anti-human IgG (C) into each tube.
6.	Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature for 1 hour.
7.	During this incubation, count 2 tubes of the T series and 2 of the NSB series for 60 seconds for total count checks.
8.	Pipette 1 mL of wash solution (D) into each tube and mix gently on a vortex mixer.
9.	Centrifuge each tube at 1500 x g for 20 minutes at 4°C.
10.	Aspirate or decant the supernatant.
11.	Pipette 1 mL of wash solution (D) into each tube and mix gently on a vortex mixer to resuspend the pellet.
12.	Repeat steps 8 and 9.
13.	Count each tube on a gamma counter for 120 seconds.

RESULT ANALYSIS

After subtracting the background count for each tube, the radioactivity in the pellet for the T tracer (T cpm) represents the amount of ^{125}I VGCC bound by the VGCC Ab plus non-specific binding, and the radioactivity in the pellet for the NSB tracer (NSB cpm) represents the amount of ^{125}I VGCC that is bound non-specifically (expressed as fmoles of labelled toxin bound). These values can then be converted to pmoles of labelled toxin bound per litre of test serum (pmol/L) by calculating the specific binding for each pellet and multiplying by 400 to adjust for the sample volume and dilution, and the change of unit (fmoles to pmoles). The following information that is required can be found on the QC record sheet:

(1). The conversion factor for dpm to fmole (***A***).
(2). The counter efficiency (***B%***).
(3). The decay factor for the decay of ^{125}I VGCC in the period between the QC assay (date on the QC record sheet) and the day of the assay (***C***).

The calculations are as follows:

$$\text{fmol/pellet for T tracer } (D) = \frac{100 \times \text{T cpm}}{A \times B \times C}$$

$$\text{fmol/pellet for NSB tracer } (E) = \frac{100 \times \text{NSB cpm}}{A \times B \times C}$$

For the negative/positive controls and test samples:

$$\text{pmol/L of VGCC Ab bound specifically} = (D - E) \times 400$$

If the pmol/L of VGCC Ab bound specifically to the positive control and each test samples is ***F*** and the pmol/L of VGCC Ab bound specifically to the negative control is ***G***.

The final pmol/L of VGCC Ab bound specifically to the positive control and test samples (H)

$= F - G$ pmol/L toxin bound

TYPICAL RESULTS (example only; not for use in calculation of actual results)

	T cpm	NSB cpm	(H) Final pmol/L bound specifically
Negative Control	566	369	-
Positive Control	2395	388	160

ASSAY CUT OFF

Negative	≤30 pmol/L
Positive	>30 pmol/L

This cut off has been validated at RSR. However each laboratory should establish its own normal and pathological reference ranges for VGCC Ab levels. Also it is recommended that each laboratory include its own panel of control samples in the assay.

CLINICAL EVALUATION

Clinical Specificity

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

Clinical Sensitivity

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

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 2.86 pmol/L

Inter Assay Precision

Sample	pmol/L (n=20)	CV (%)
1	157.6	13.0
2	71.7	15.5

Intra Assay Precision

Sample	pmol/L (n=25)	CV (%)
1	144.6	6.9
2	62.1	15.5

Clinical Accuracy

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

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.

Assay Drift

Little assay drift was observed in the VGCC Ab RIA. It is important that the incubation times and all other conditions specified in the instructions are adhered to for optimum assay performance.

SAFETY CONSIDERATIONS

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified shelf life for reconstituted reagents. Refer to Material Safety Data Sheet for more detailed safety information. The kit contains radioactive material. Users should make themselves aware of, and observe, any national and local legislation and codes of practice governing the use, storage, transportation and disposal of radioactive materials. Avoid all actions likely to lead to ingestion. Avoid contact with skin and clothing. Wear protective clothing and, where appropriate, personal dosimeters. Radioactive materials should only be used by authorised personnel and in designated areas. Wash hands thoroughly after handling. Monitor hands and clothing before leaving the designated area. Materials of human origin used in the preparation of the kit have been tested and found non-reactive for HIV1 and 2 and HCV antibodies and HBsAg but should, none-the-less, be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens, before disposal. Materials of animal origin used in the preparation of the kit have been obtained from animals certified as healthy but these materials should be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection or contact with skin, eyes or clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents to stand at room temperature (20–25°C) for at least 30 minutes before use.	
Dilute:	Patient sera and negative control
Pipette:	**25 µL** diluted patient sera and diluted negative control (diluted 1:10 in dilution buffer) and positive control (ready to use; do not dilute)
Pipette:	**50 µL** of T ^{125}I VGCC into each of one set of duplicate tubes and **50 µL** of NSB ^{125}I VGCC into the other set of duplicate tubes for each test sample or control
Tubes:	Mix gently on vortex mixer and cover
Incubate:	1 hour at room temperature
Pipette:	**125 µL** of anti-human IgG into all tubes
Tubes:	Mix gently on vortex mixer and cover
Incubate:	1 hour at room temperature
Check:	Count 2 tubes of the T series and 2 of the NSB series for 60 seconds for total counts
Pipette:	**1 mL** wash solution into all tubes
Tubes:	Mix gently on vortex mixer
Tubes:	Centrifuge at 1500 x g for 20 minutes at 4°C
Tubes:	Aspirate or decant supernatants
Pipette:	**1 mL** wash solution into all tubes
Tubes:	Mix on vortex mixer to resuspend pellet
Tubes:	Centrifuge at 1500 x g for 20 minutes at 4°C
Tubes:	Aspirate or decant supernatants
Count tubes for 120 seconds using gamma counter	