DIAGNOSTIKA GMBH

Instructions for Use

VGKC Antibody Assay RIA

^{125}I-Radio Immuno Assay for the Quantitative Determination of Antibodies to the Voltage-Gated Potassium Channel (VGKC)

CE

IVD

REF RA115/25

Σ 25

+2 +8 °C 2 – 8 °C

DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • D-22459 Hamburg • Germany
Tel +49-40-555 87 10 • Fax +49-40-555 87 111
Internet: http://www.dld-diagnostika.de • E-Mail: contact@dld-diagnostika.de

Contents

Symbols

I V D	In-Vitro-Diagnostic Device	CE	EC Declaration of Conformity
CONT	Contents		Expiry Date
LOT	Lot Number	+2 +8 °C	Store
	Manufactured by	Σ	Sufficient for
REF	Catalogue Number		Consult Instructions

Hazard Pictograms

radioactive

1. Introduction and Principle of the Test

The VGKC autoantibody RIA assay kit is intended for use by professional persons only, for the quantitative determination of VGKC autoantibodies (VGKC Ab) in human serum. Serum VGKC Ab have been detected in peripheral nervous system disease specifically associated with the clinical spectrum of acquired neuromyotonia (NMT) and cramp-fasciculation syndrome (CFS), and disorders of the central nervous system, including Morvan syndrome, epilepsy and limbic encephalitis (LE). Detection and measurement of VGKC Ab are useful in the diagnosis and management of autoimmune Voltage-Gated Potassium Channelopathies and related neurological disorders. The kit is easy to use and provides a specific and sensitive assay for VGKC Ab.

In the VGKC autoantibody radioimmunoassay (RIA), VGKC Ab in patient sera and controls are allowed to interact with detergent solubilised VGKCs extracted from rabbit brain tissue and complexed with ^{125}I-labelled α-dendrotoxin (known to react with Kv1.1, 1.2 and 1.6 subtypes of the VGKC). After incubation at 2 – 8 ^{o}C overnight, the resulting antigen-antibody complexes are immunoprecipitated by the addition of anti-human IgG. After a second incubation of 1½ hours, assay buffer is added and the samples centrifuged. Unbound ^{125}I-labelled alpha-dendrotoxin-VGKC complex is removed from the tubes by aspiration of the supernatant. The level of radioactivity remaining in the tube is proportional to the antibody

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 kit components at 2-8 °C before use!

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

Store kit components at 2-8 °C before use!

4.1 **^{125}I-labelled VGKC** **TRACER** 2 vials
2 x 0.75 ml; lyophilized, activity < 15 kBq per vial,
Reconstitute each vial by addition of 0.75 ml pure water and vortex gently to dissolve. **Use immediately**

4.2 **Negative Control** **CONTROL —** 1 vial
250 µl, ready for use,
normal human serum

4.3 **Positive Control** **CONTROL + I & II** 2 vials
2 x 250 µl, ready for use
human serum containing antibodies against VGKC,
for concentration ranges see qc certificate

4.4 **Anti-human IgG** **ANTI-HUMAN-IGG** 1 vial
2 ml, ready for use

4.5 **Assay Buffer** **ASSAY BUFFER** 1 bottle
60 ml, ready for use

Additional materials and equipment required but not provided:

- Pipettes for 50 µl, 0.75 µl and 1 ml
- 4.5 ml conical plastic tubes and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3,000 x g
- Dist. water
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter

5. Specimen Collection, Storage and Preparation

Serum samples, citrate, heparin and EDTA plasma samples can be run. Samples 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.

When required, thaw test sera at room temperature and mix gently to ensure homogeneity.
Dilute 1:10 using Assay Buffer (e.g. 15 µl serum plus 135 µl assay buffer). Centrifuge diluted serum prior to assay (preferably for 5 min at 10-15,000 x g in a microfuge) to remove any particulate matter.

6. Test Procedure

We recommend to use conical tubes.

6.1 Pipett 50 µl of the Negative Control and both Positive Controls and 50 µl of the 1:10 diluted samples (diluted in Assay Buffer), respectively, to the corresponding tubes of each set.

6.2 Add 50 µl of freshly reconstituted tracer into each tube.

6.3 Mix on a vortex mixer and incubate for 16-20 hours hours at 2-8 °C covered with a suitable cover.
During this incubation count the total radioactivity of 2 tubes out of each set in a gamma counter.

6.4 Add 50 µl Anti-human IgG to each tube and mix on a vortex mixer. Incubate 1 ½ hours at room temperature covered with a suitable cover.

6.5 Add 1ml of cold (2-8 °C) Assay Buffer to each tube and mix thoroughly.
Centrifuge all tubes for 20 minutes at 3,000 x g (preferably use a refrigerated centrifuge).

6.6 Decant or aspirate the tubes, taking care not to disturb the pellet.

6.7 Add 1 ml of cold (2-8 °C) Assay Buffer to each tube and carefully resuspend the pellets using a vortex mixer for a few seconds.

6.8 Centrifuge the tubes again for 20 minutes at 3,000 x g (preferably use a refrigerated centrifuge).

6.9 Decant or aspirate the tubes carefully.

6.10 Count the tubes in a gamma counter for at least 1 minute.

7. Calculation of Results

The radioactivity in the pellet represents the amount of receptor-toxin complex bound by VGKC antibodies. This is usually expressed as picomoles of toxin bound per litre of test serum. The relationship between this parameter and the radioactivity in the pellet can be calculated as follows:

$$\frac{(\text{cpm Sample} - \text{cpm Negative Control}) \times D}{\text{Volume Neat Sample (µl)} \times \text{spec. Activity Toxin (Ci/mmol)} \times Z \times U}$$

cpm = measured counts per minute
D = decay factor; decay of the ^{125}I between labelling date and day of the assay
Z = counter efficiency (for example 0.7)
U = conversion factor between counts per minute and Curie (2.22×10^{12} dpm/Ci)

The values in the denominator can be calculated separately and assigned to the factor F. Using this factor the results are obtained as pmol/l.
The value of F is specific for each lot of labelled receptor and is given in the qc certificate included in each kit.

Therefore the above formula becomes simplified to:

Concentration VGKC Ab = (cpm Sample - cpm Neg. Control) x D x F

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. The certificate includes a table showing the decay factor for each date during the shelf life of the assay.

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
8. - 9.	1.98
9. – 10.	2.14
10. – 11.	2.32

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.

Calculation Example

F is given as 0.044, D is 1.22 (assay performed 2. to 3. week after labelling date), then the calculation factor becomes 0.0537.

Sample	mean cpm	mean cpm – cpm Neg. Contr.	Concentration of VGKC in pmol/l
Negative Control	1,673	0	0
Control 1	8,875	7,202	387
Control 2	3,995	2,322	125

8. Reference Ranges

Studies on healthy blood donors suggest a preliminary cut-off of 85 pmol/l. Therefore, all values above 85 pmol/l can be considered positive.

9. Assay Characteristics

Clinical Specificity

Samples from 100 individual healthy blood donors were measured. 98 (98%) were identified as being negative for VGKC Ab.

Clinical Sensitivity

Serum samples from 30 patients with suspected Voltage-Gated Potassium Channelopathies and related neurological disorders were assayed in the VGKC Ab RIA. 27 (90%) were positive for VGKC Ab.

Clinical Accuracy

None of 138 patients with autoimmune diseases other than those with suspected Voltage-Gated Potassium Channelopathies and related neurological disorders were positive for VGKC Ab except for 1 (out of 17) patient with Type 1 Diabetes (IA-2 Ab positive) and 2 (out of 26) patients with Rheumatoid Arthritis. This study indicated no interference from autoantibodies to thyroglobulin, thyroid peroxidase, the TSH receptor, aquaporin-4, 21-hydroxylase, GAD and the acetylcholine receptor in the VGKC Ab RIA.

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 4.5 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 3,000 mg/dl.

Precision

Intra-Assay

sample	number	mean pmol/l	cv (%)
1	20	102	5.7
2	20	150	5.8
3	20	332	3.7

Inter-Assay

sample	number	mean pmol/l	cv (%)
1	12	89	6.6
2	12	138	6.4
3	12	320	5.4

10. Literature

Hart et al.
“Autoantibodies detected to expressed K+ channels are implicated in Neuromyotonia.”
Ann Neurol 41 (1997), 238 - 246

Vincent et al.
“Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis.”
Brain 127 (2004), 701 - 712

Tan et al.
“Clinical spectrum of voltage-gated potassium channel autoimmunity.”
Neurology 70 (2008), 1883 - 1890

Pipetting Scheme

	T	Negative Control	Positive Controls	Patients
Negative Control µl		50		
Pos. Controls I & II µl			50	
diluted Patient Sample µl				50
^{125}I - VGKC µl	50	50	50	50

Mix carefully (Vortex) and incubate for 16 - 20 hours at 2 - 8 °C

Anti-human-IgG µl		50	50	50

Mix carefully (Vortex) and incubate for 90 minutes at room temperature

Assay Buffer (2-8 °C) ml		1	1	1

Centrifuge for 20 minutes with 3,000 x g (preferably cool)

Aspirate or decant supernatant carefully (except T)

Assay Buffer (2-8 °C) ml		1	1	1

Mix carefully (Vortex)

Centrifuge for 20 minutes with 3,000 x g (preferably cool)

Aspirate or decant supernatant carefully (except T)

Count tubes in a gamma counter for at least 1 minute