DIAGNOSTIKA GMBH

Instructions for Use

ACHRAB® Ganglionic Assay RIA

^{125}I-Radio Immuno Assay
for the Quantitative Determination of
Ganglionic Acetylcholine Receptor Autoantibodies
in Human Serum

RUO

REF RA122/25

25

2 – 8 °C

DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
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Contents

Symbols

RUO	Research Use Only		
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue number		Consult Instructions for Use

Hazard Pictograms

 Radioactive

1. Introduction and Principle of the Test

The ACHRAB® Ganglionic Assay RIA kit is for the quantitative determination of Ganglionic Acetylcholine Receptor (gAChR) Autoantibodies (Abs) in human serum. The assay is intended for use by professional persons only and for research use only. Not for use in diagnostic procedures.

Serum autoantibodies reactive with gAChR are implicated in impaired synaptic transmission at autonomic ganglia, specifically associated with Autoimmune Autonomic Ganglionopathy (AAG) and gastro-intestinal dysmotility. Functional gAChRs are pentameric, consisting of α3 and β4 subunits and are expressed predominately in autonomic ganglia. gAChR Abs primilary bind to the α3-subunit. The kit is easy to use and provides a specific and sensitive assay for gAChR Ab.

The assay depends on the use of recombinant gAChR complexed with ^{125}I-labelled epibatidine. The ^{125}I-labelled gAChRs are the incubated with test sera and the resulting complexes immunoprecipitated with anti-human IgG. The higher the concentration of autoantibody, the greater the amount of radioactivity precipitated.

2. Precautions

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. Refer to 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. It must not be administered to humans or animals under any circumstances. Avoid all actions likely to lead to ingestion; do not eat, drink or smoke where radioactive materials are being handled. Do not pipet by mouth. Avoid contact with skin and clothing. Wear protective clothing, disposable gloves, 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 copius amounts of water.

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. **^{125}I-labelled gAChR** **TRACER**  2 vials

lyophilized, activity < 6 kBq/vial at manufacture

4.2. **Negative Control** **CON -** 1 vial

0.1 ml, ready for use

4.3. **Positive Controls 1 & 2** **CON + I** **CON + II** 2 vials

0.1 ml each, ready for use,
for concentration ranges see QC Certificate

4.4. **Anti-human IgG** **ANTI-HUMAN-IGG** 1 vial

2 ml, ready for use

4.5. **Reconsitution Buffer** **BUFFER** 1 bottle

2 ml, ready for use
for reconstitution of ^{125}I-labelled gAChR

4.6. **Wash Solution** **WASH** 1 bottle

60 ml, ready for use,
Keep at 2-8°C except when in use

Additional materials and equipment required but not provided:

- Pipettes for dispensing 10 µl, 50 µl, 0.75 ml and 1 ml
- 4.5 ml conical plastic tubes and suitable rack
- Centrifuge capable of 2-8 °C and 1500 x g
- Suitable device for aspirating or decanting the supernatant
- Vortex mixer
- Gamma counter

5. Specimen Collection and Storage

Sera can be stored up to one week at 2 – 8 °C or at -20 °C for longer periods, preferably in aliquots. 20 µl is sufficient for one assay (duplicate 10 µl determinations). Repeated freezing and thawing or increase in storage temperature must be avoided. Do not use lipaemic or haemolized serum samples.

6. Preparation of Samples and Reagents

6.1 Samples

One the day of the assay, thaw the sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to the assay (preferably for 5 min at 10.000 x g) in a microfuge to remove any particulate material. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

6.2 Reconstitution of the Tracer

Approximately 10 min before use, reconstitute the lyophilized Tracers [TRACER] each with 0.75 ml Reconstituion Buffer [BUFFER]. Dissolve by vortexing briefly (about 5 sec) on a vortex mixer and then keep the vial upright.

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

7. Test Procedure

Allow all reagents, except wash solution, to stand at room temperature (20-25°C) for at least 30 minutes before use.

7.1. Pipette 10 µl (in duplicate) of the Negative Control [CON -], Positive Controls [CON + I] & [CON + II] and the sera, into labelled assay tubes (conical tubes recommended).

7.2. Add 50 µl freshly reconstituted ^{125}I-labelled gAChR [TRACER] into each tube.

7.3. Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature (20-25 °C) for 2 hours.

At the beginning of the incubation determine the total radioactivity for 2 minutes in at least 2 tubes in a gamma counter.

7.4. Add 50 µl Anti-human IgG [ANTI-HUMAN-IGG] to each tube.

7.5. Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature (20-25 °C) for 2 hours.

7.6. Add 1 ml of cold (2-8 °C) Wash Solution [WASH] to each tube and mix gently on a vortex mixer.

7.7. Centrifuge tubes at 1.500 x g for 20 minutes at 2-8°C.

7.8. Decant or aspirate the supernatant, taking care not to disturb the pellet.

7.9. Add 1 ml of cold (2-8 °C) Wash Solution [WASH] to each tube and carefully resuspend the pellet using a vortex mixer.

7.10. Centrifuge tubes at 1.500 x g for 20 minutes at 2-8°C.

7.11. Decant or aspirate the supernatant, taking care not to disturb the pellet.

7.12. Determine the counts (cpm) in each tube for 2 minutes in a gamma counter.

8. Calculation of Results

The radioactivity in the pellet represents the amount of ^{125}I-labelled gAChR bound by the gAChR Abs. This is often expressed as picomoles of labelled toxin bound per litre of test serum and the relationship between this parameter and pellet radioactivity can be calculated from the knowledge of:

- The counts per minute cpm determined with the gamma counter
- The specific activity A [Ci/mmol] of the ^{125}I-labelled toxin at the time it was used to label gAChRs.
- Decay factor D for the decay of ^{125}I between the tracer manufacture date and the day of the assay.
- The volume of serum used in the assay C (i.e. 10 µl).
- Counter efficiency B of the gamma counter used (e.g. 70 %, then B = 0.7)
- The conversion factor of Curie in decays per minute (2.22×10^{12} dpm/Ci or cpm/Ci)

Values for A and D are on the QC-Certificate included in each kit.

The simplified formula (1) to be used, is as follows:

$$\text{pmol/l} = \frac{(\text{cpm}_{\text{Pos. Control OR Sample}} - \text{cpm}_{\text{Neg. Control}}) \times 1000 \times D}{C \times A \times B \times 2.22\ \text{dpm/Ci}}$$

Example:

$$\frac{(5750\ \text{cpm} - 809\ \text{cpm}) \times 1000 \times 1.32}{10\ \mu\text{l} \times 1420\ \text{Ci/mmol} \times 0.7 \times 2.22\ \text{dpm/Ci}} = 295.56\ \text{pmol/l}$$

Typical Results (example only, not for use in calculation of actual results):

	cpm	**pmol/l**
Negative Control	608	**0.0**
Positive Control 1	4844	**134.2**
Positive Control 2	1505	**28.4**

With the following formula (2) a factor F can be calculated, which is lot specific and indicated on the QC-Certificate.

$$F = \frac{1000}{C \times A \times B \times 2.22\ dpm/Ci}$$

Should the counter efficiency B of your gamma counter differ from the one stated on the QC-Certificate, then F has to be recalculated accordingly.

Therefore the above formula (1) becomes simplified to the following formula (3):

$$pmol/l = (cpm_{Pos.\ Control\ OR\ Sample} - cpm_{Neg.\ Control}) \times D \times F$$

For assay to be valid, values achieved for both positive controls must be in target range indicated on QC-Certificate included in each kit.

9. Assay Characteristics

This kit is for research use only, the values below are not for use in diagnostic procedures.

The following assay cut off was determined:

Negative	< 10 pmol/l
Indeterminate	between 10 and 15 pmol/l
Positive	≥ 15 pmol/l

However each labaoratory should establish its own normal and pathological reference ranges for gAChR Ab levels. Also it is recommended that each labaoratory includes its own panel of control samples in the assay.

Sera from 50 individual healthy blood donors were assayed in the ACHRAB® Ganglionic Assay RIA. 50 (100%) were identified as being negative for gAChR Ab.

Sera from 30 patients with autoimmune diseases other than those with suspected AAG and related neurological disorders were assayed in the ACHRAB® Ganglionic Assay RIA. 29 (96.7%) of GAD or TSH Receptor autonantibody-positive samples were tested negative for gAChR Ab in this study, with 1 further sample (3.3%) providing indeterminate.

10. Modifications of Instructions for Use

Version_2: Section 5 refined to “Sera can be stored up to one week at 2 – 8 °C or at -20 °C for longer periods, preferably in aliquots.” Section 8 complemented with sample calculation and factor F.

Version_1: Creation of instructions for use. No changes to declare.

11. Literature

- Vernino et al.
  Autoantibodies in ganglionic acetylcholine receptors in autoimmune autonomic neuropathies.
  N. Engl. J. Med. 2003; **343**: 847-855

- McKeon et al.
  The ganglionic acetylcholine receptor autoantibody: oncological, neurological and serological accompaniments
  Arch. Neurol. 2009 **66**: 735-741

Pipetting Scheme

Allow all reagents, except Wash Solution to stand at room temperature (20-25 °C) for at least 30 minutes before use

		Negative Control	Positive Controls	Samples
[CON -]	µl	10		
[CON + I] & [CON + II]	µl		10	
Samples	µl			10
[TRACER]	µl	50	50	50

Vortex gently, in 2 tubes determine total counts for 2 minutes
Cover and incubate for 2 hours at room temperature (20-25 °C)

[ANTI-HUMAN-IGG]	µl	50	50	50

Vortex gently, cover and incubate for 2 hours
at room temperature (20-25 °C)

[WASH] (cold)	ml	1	1	1

Centrifuge for 20 minutes at 1500 x g at 2-8 °C
Aspirate or decant supernatant carefully

[WASH] (cold)	ml	1	1	1

Vortex gently to resuspend pellet
Centrifuge for 20 minutes at 1500 x g at 2-8 °C
Aspirate or decant supernatant carefully
Determine counts in tubes for 2 minutes in a gamma counter