DIAGNOSTIKA GMBH

Instructions for Use

ACHRAB®Assay RIA

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

CE IVD

REF	RA001/25	RA105/100
Σ	25	100
+2 +8 °C	2 – 8 °C	

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Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number	+2 +8 °C	Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

Hazard Pictograms

radioactive

1 Introduction and Principle of the Test

Autoantibodies to the acetylcholine receptor are responsible for failure of the neuromuscular junction in myasthenia gravis and receptor antibody measurement can be of considerable value in disease diagnosis and follow-up.

In the receptor antibody assay, detergent solubilized acetylcholine receptors from a human cell line are labelled with ^{125}I-labelled alpha-bungarotoxin (a snake venom). The labelled receptors are then incubated with sera containing autoantibodies to the acetylcholine receptor, and the resulting complex of labelled receptor and receptor antibody immunoprecipitated with anti-human IgG. The higher the concentration of autoantibody the greater the amount of labelled receptor immunoprecipitated.

2 Precautions

- For in vitro use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- This radioactive product assay only be received, stored as 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 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

^{125}I-Receptor **TRACER** 1 (4) vial(s)

Activity < 75 kBq per vial lyophilized
^{125}I-alpha-Bungarotoxin labelled human receptor

radioactive

Negative Control **CONTROL -** 1 vial

0.1 ml, ready for use,
normal human serum

Positive Controls **CONTROL A** & **CONTROL B** 2 vials

each 0.1 ml, ready for use,
human serum containing antibodies against acetylcholine receptor, for concentration ranges see qc certificate

Anti-human IgG **ANTI - IGG** 1 vial

1.5 ml (5.5 ml), ready for use

Wash Solution **WASH BUFFER** 1 (2) bottle (s)

60 ml (120 ml), ready for use, PBS

Normal Serum **SERUM** 1 vial

1 ml (4 ml), ready for use,
normal human serum for diluting patient sera

Buffer **BUFFER** 1 (4) vial(s)

4 ml, ready for use,
for reconstituting labelled receptors

Additional materials and equipment required but not provided:

- Pipettes for 5, 50, 100 µl and 1 ml.
- 5 ml syringe and needle.
- Round-bottom plastic tubes and suitable rack.
- Centrifuge (preferable refrigerated) capable of 3,000 x g.
- Dist. water.
- Suitable device for aspirating the tubes.
- Vortex mixer.
- Gamma Counter.

5 Specimen Collection and Storage

Serum or plasma may be used in the assay. Do not use lipemic or grossly haemolized specimen. 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 Sample Preparation

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

First testing of an unknown sample should be done with 5 µl neat serum. Serum with high antibody levels should only be diluted with the Normal Serum supplied. The use of other diluent media can lead to invalid results. Only results in the linear range of the assay should be used (see linearity of dilution).

6.3 Reconstitution of Labelled Receptors

Approximately 30 min. before use reconstitute the labelled Receptors [TRACER] each with **2.7 ml** per vial of Buffer [BUFFER] supplied and mix the contents gently to dissolve. A slightly cloudy solution will be formed containing about 100,000 cpm per 100 µl. After reconstitution the receptor can be stored at 2 - 8 °C for at least 2 weeks.

7 Test Procedure

1. Add 5 µl each of negative control [CONTROL -] and positive controls [CONTROL A & B] and 5 µl neat patient serum or 5 µl patient serum diluted in Normal Serum in duplicate to round-bottom plastic tubes. Always include Negative and Positive Controls in each assay.
2. Add 100 µl of ^{125}I-Receptor [TRACER] to each tube and mix on a vortex mixer. Incubate for 2 hours at room temperature. Count the total radioactivity in three tubes during the incubation (rather than using separate tubes in order to save reagents).
3. Add 50 µl of Anti-human IgG [ANTI-IGG] to each tube and mix on a vortex mixer. Incubate for 30 minutes at room temperature or overnight (18 to 20 hours) at 2 - 8 °C.
4. Add 1 ml of Wash Solution [WASH BUFFER] to each tube.
5. Centrifuge all tubes for 20 minutes at 3,000 x g. The use of a refrigerated centrifuge is recommended.
6. Decant or aspirate the tubes, taking care not to disturb the pellet.
7. Add 1 ml of Wash Solution [WASH BUFFER] to each tube and re-suspend the pellets using a vortex mixer for at least 10 seconds.
8. Centrifuge all tubes for 20 minutes at 3,000 x g.
9. Decant or aspirate the tubes carefully.
10. Count the tubes in a gamma-counter.
11. Recommended counting time: 1 min.

8 Calculation of Results

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

$$\frac{(cpm_{Sample} - cpm_{Negative\ Control}) \times D}{\text{Volume of Sample (µl)} \times \text{spec. Activity of Toxin} \times Z \times U}$$

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 nmol/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 ACHRAB = (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.

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

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 0.33×10^{-3}, D is 1.22 (assay performed 2. to 3. week after labelling date), then the calculation factor for undiluted samples becomes 0.40×10^{-3}.

Sample	mean cpm	Mean cpm $- cpm_{Neg. Contr.}$	Concentration of ACHRAB in nmol/l
Negative Control	454	0	0
Patient Serum 1 (undiluted)	3,291	2,837	1.1
Patient Serum 2 (diluted 1 : 21)	2,439	1,985	16.7

9 Expected Values

Normal healthy blood donors show values up to approximately 0.15 nmol/l.

If patients with other autoimmune diseases and other neuromuscular diseases are included the upper normal limit at 95% specificity is 0.25 nmol/l. Until now no false positive values above 0.4 nmol/l have been observed.

Therefore, it is recommended to report a normal range up to 0.25 nmol/l and to consider values between 0.25 and 0.4 nmol/l as equivocal.

An example for the distribution of ACHRAB concentrations in different patient groups is shown in a histogram on the following page.

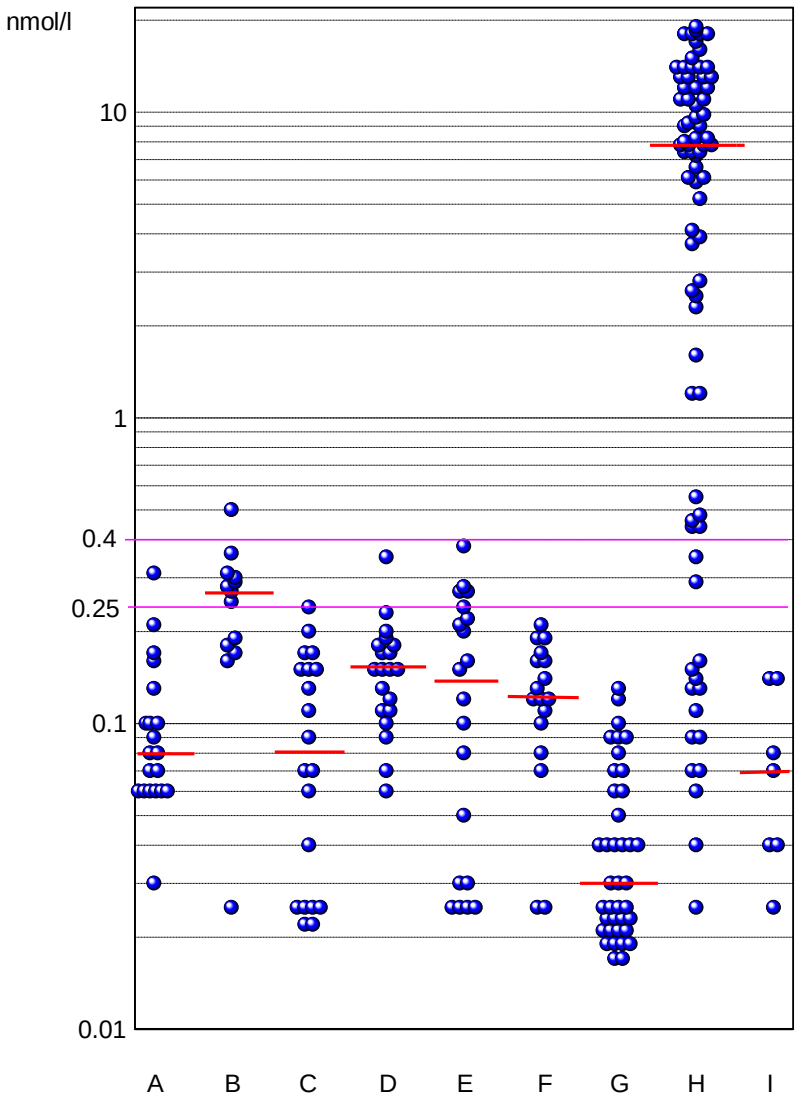
However, each laboratory should establish its own normal range and take the above mentioned figures only as a guideline.

10 Sensitivity and Specificity

The ACHRAB® Assay is specific for autoantibodies to the human acetylcholine receptor. Cross reactions of other autoantibodies have not been observed. For detailed results see graph on following page.

Lower detection limit: 0.02 nmol/l

Histogram of different patient groups



A	Graves' Disease	n = 13
B	Primary Biliary Cirrhosis	n = 13
C	Hashimoto, Tg-Antibody, positive	n = 20
D	Hashimoto, TPO-Antibody, positive	n = 20
E	SLE	n = 20
F	Rheumatoid Arthritis	n = 17
G	Normal Sera	n = 40
H	Myasthenia Gravis (generalized and ocular)	n = 76
I	Other Neuromuscular Diseases	n = 7

11 Range of Linearity

The relationship between antibody concentration and cpm bound in the assay is only linear over a limited range. Above this range, increases in antibody concentration do not lead to a proportionate increase in cpm bound and eventually a plateau effect is reached. Consequently, measurements above the linear range are not reliable, giving falsely low values.

The linear range as well as the plateau-range differ for each individual serum sample and therefore, it is necessary to test the linearity of dilution in each case. During the follow-up of a patient the knowledge of the preceding values gives a good estimate of the appropriate dilution factor.

Until now all sera tested with this kit were linear below 1.5 nmol/l.

On the following page dilution curves are shown as examples of the range of linearity.

Sera 1 and 2 when measured undiluted gave values of appr. 12 nmol/l; after dilution into the linear range the correct value was found to be
66 and 74 nmol/l, respectively.

Sera 3 and 4 when measured undiluted gave values of 8 and 9 nmol/l; after dilution into the linear range the correct value was found to be
25 and 29 nmol/l, respectively.

12 Reproducibility

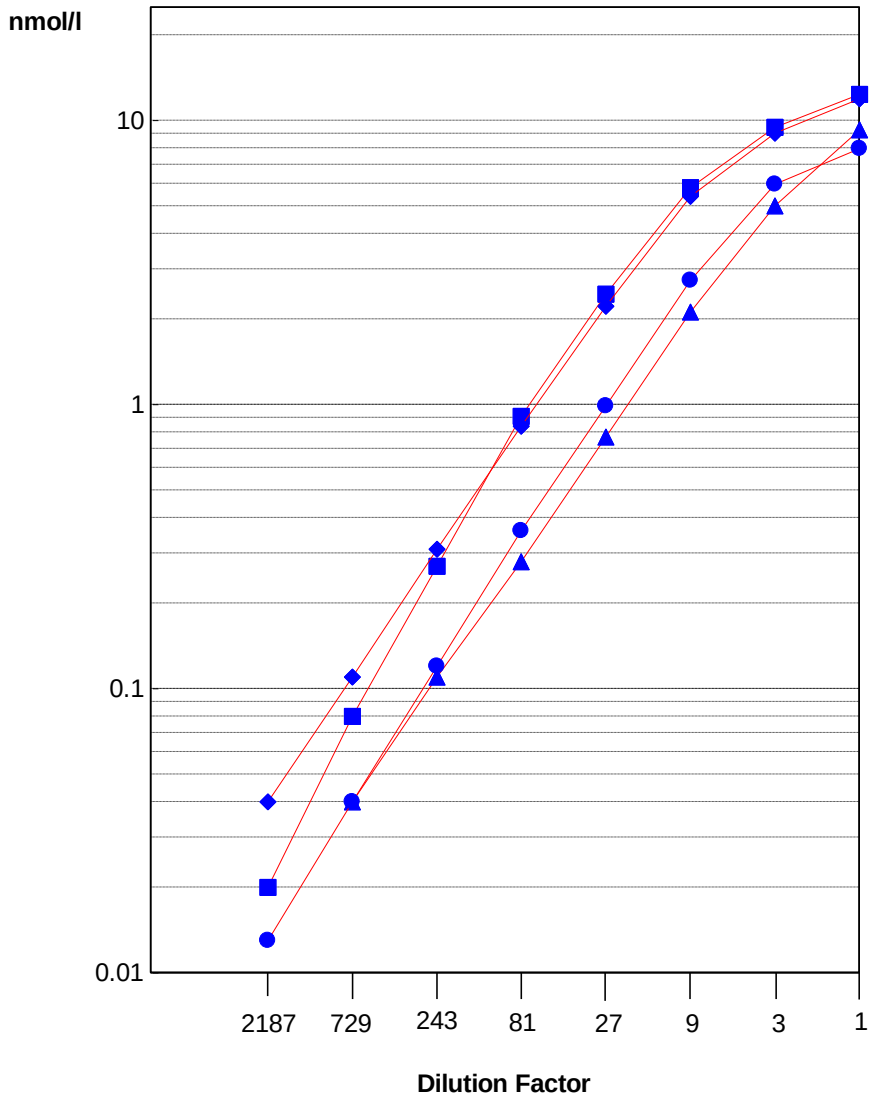
The intra and inter assay coefficients of variation (cv) were measured using two samples at different concentration.

intra assay variation (n=10)			inter assay variation (n=12)		
sample	mean (nmol/l)	cv (%)	sample	mean (nmol/l)	cv (%)
1	3.3	1.9	1	2.2	5.0
2	1.8	1.7	2	0.5	5.9

13 Dilution Curves

The patient sera shown in the figure were measured in the ACHRAB® Assay undiluted (dilution factor = 1) and after dilution with normal human serum. The concentrations calculated from measurements within the linear range are:

- ■ Serum 1 : 66 nmol/l
- ◆ Serum 2 : 74 nmol/l
- ● Serum 3 : 29 nmol/l
- ♦ Serum 4 : 25 nmol/l



14 References

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15 Changes to declare

IFU has been re-formatted.

In sections 6, 7 and pipetting scheme the name of components as indicated on labels has been included to provide more clarity.

No changes have been made to kit components or execution of protocols.

Pipetting Scheme

		T	Negative Control	Positive Controls	Sample
[CONTROL -]	µl		5		
[CONTROL A & B]	µl			5	
Patient Sample	µl				5
[TRACER]	µl	100	100	100	100

Mix carefully (Vortex) and incubate for 2 hours at room temperature

[ANTI-IGG]	µl		50	50	50

Mix carefully (Vortex) and incubate for 30 minutes at room temperature
(alternative: incubate for 18 – 20 hours at 2 – 4 °C)

[WASH BUFFER]	ml		1	1	1

Centrifuge for 20 minutes with 3,000 x g (preferably cool)
Aspirate or decant supernatant carefully (except T)

[WASH BUFFER]	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 1 minute