ENG

CE

ACHR-AB

(May 2019 – Model 14)

1. NAME AND INTENDED USE

ACHR-AB is a radioreceptor assay kit (RRA) for the measurement of the acetylcholine receptor autoantibodies in human serum or plasma EDTA.

2. INTRODUCTION

Autoantibodies to the acetylcholine receptor (AChRAb) are responsible for failure of the neuromuscular junction in myasthenia gravis. Measurement of the antibodies can be of considerable value in disease diagnosis.

3. PRINCIPLE

Adult and foetal forms of the acetylcholine receptor differ by one of their subunits (the gamma subunit in foetal receptor is replaced by the epsilon subunit in adult receptor). Furthermore AChRAb in some sera recognise the foetal form of the receptor preferentially whereas AChRAb in other sera recognise the adult form of the receptor preferentially. Consequently, a carefully balanced mixture of detergent solubilised foetal and adult forms of the receptor is the optimum preparation for AChRAb assays. This mixture of receptors is labelled with ^{125}I-labelled alpha bungarotoxin. In the assay labelled receptors (^{125}I-AChR) are incubated with test sera and any resulting complex of labelled receptor and receptor antibody immunoprecipitated with anti human IgG. After centrifugation and a wash step, the precipitate is counted.

4. REAGENTS

Each kit contains enough reagents for 25 tubes. The expiry date is printed on the external label.

REAGENTS	QUANTITY	STORAGE
ANTISERUM: ready to use. Anti-human IgG, buffer and sodium azide.	1 1.5 mL vial	2-8°C until the expiry date.
^{125}I-RECEPTORS: freeze dried. Alpha bungaratoxin ^{125}I labelled acetylcholine receptor, buffer, animal proteins and red dye. ≤ 40 kBq (≤ 1.5 µCi).	1 vial qs 1.3 mL buffer	2-8°C until the expiry date. 2-8°C 2 weeks after reconstitution.
NEGATIVE CONTROL (N): ready to use. Human serum and sodium azide.	1 0.1 mL vial	2-8°C until the expiry date.
POSITIVE CONTROL (P) : ready to use. Human serum containing acetylcholine receptor antibodies and sodium azide.	1 0.1 mL vial	2-8°C until the expiry date.
DILUENT: ready to use. Normal human serum and sodium azide.	1 1 mL vial	2-8°C until the expiry date.
WASHING SOLUTION: ready to use. Buffer and sodium azide.	1 70 mL bottle	2-8°C until the expiry date.
BUFFER: ready to use. Buffer and sodium azide. For tracer reconstitution use only.	1 4 mL vial	2-8°C until the expiry date.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate security.
A summary of these is given below :
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

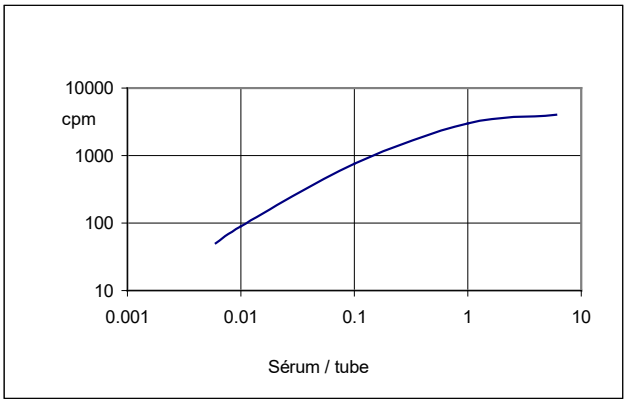
5.3. Handling precautions
Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid microbial contamination of the reagents.
Fully respect the incubation times and the temperature during the assay.

6. SPECIMEN COLLECTION AND PREPARATION
The assay is performed only on serum or on EDTA plasma. Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results. Sera to be analysed should be assayed soon after separation or stored in aliquots at or below - 20°C.
Incorrect storage can lead to loss of AChRAb activity.
Specimen should be thawed before using at room temperature then mixed (vortex).
Centrifuge the serum prior to assay to remove any particulate matter ; please do not omit this centrifugation step. Do not refreeze samples for later use.

7. ASSAY PROCEDURE
7.1. Material required
Precision micropipettes or similar with disposable tips, capable of dispensing 5 µL, 50 µL and 1 mL (or 1 mL multipipette type). Their calibration should be checked regularly.
Distilled water.
Disposable tubes.
Vortex-type mixer.
Refrigerated multitube centrifuge at 2-8°C (1500 g minimum).
Test tube rack with inversion possibility.
Gamma scintillation counter calibrated for 125 iodine measurement.

7.2. Reconstitution of the tracer
Reconstitute the tracer with 1.3 mL of Buffer up to 30 minutes before use. Recap the vial.
Mix gently by inversion to assure complete dissolution of the freeze-dried material. A clear solution will be formed. The labelled receptor preparation can be stored at 4°C for up to 2 weeks after reconstitution.
Do not freeze the reconstituted tracer preparation.

7.3. Assay of high concentrated sera
The relationship between acetylcholine receptor antibody concentration and cpm bound in the assay is not linear at high concentrations and the linear portion varies from sample to sample. In order to overcome this problem, one has to dilute antibody positive sera in normal human serum and assay several ^{125}I dilutions. The acetylcholine receptor concentration should then ^{125}I be determined for a dilution which falls in this linear range and the value obtained multiplied by the dilution factor to give the concentration in the neat sample.
Normal human serum (diluent) is provided in the kit so that this type of analysis can be made if required.



7.4. Protocol
The assay requires the following groups of tubes :
T group for the total activity determination,
Control group,
Sx group, for the samples to be assayed.
It is recommended to perform the assay in duplicate for the controls and samples.
Observe the order in which reagents are to be added :
Dispense 5 µL of Negative control, Positive control and serum samples into the correspondingly labelled tubes.
Add 50 µL of ^{125}I-acetylcholine receptor to each tube (including T group).
Mix each tube gently with a vortex-type mixer. **Cover** the tubes.
Incubate 2 hours at room temperature (18-25°C).
Add 50 µL of antiserum to each tube (except the T tubes).
Mix each tube gently with a vortex-type mixer. **Cover** the tubes.
Incubate 2 hours or one night at 2-8°C.
Add 1 mL of washing solution to each tube (except the T tubes).
Mix the contents of the tubes.
Centrifuge all the tubes (except the T tubes) at 3000 g for 20 mn at 2-8°C.
Eliminate the liquid by inversion (except the T tubes), taking care not to disturb the pellets.
Add 1 mL of washing solution to each tubes (except the T tubes).
Mix the content of the tubes.
Centrifuge all the tubes (except the T tubes) at 3000 g for 20 mn at 2-8°C.
Discard the supernatant by overturning the tubes on to adsorbing paper and leaving for one minute. Do not shake the overturned tubes on the paper.
Measure the remaining radioactivity bound to the tubes with a gamma scintillation. Count the tubes for 5 mn each.

8. QUALITY CONTROL

Good laboratory practices require that control samples be used in each series of assays to check the quality of the results obtained. These samples must be treated in exactly the same way as the samples to be assayed, and it is recommended that the results be analyzed with appropriate statistical methods.

9. RESULTS

9.1. Results using the inhibition of acetylcholine binding

The radioactivity in the pellet represents the amount of receptor-toxin complex bound by the receptor antibody. This is often expressed as nanomoles of toxin bound per litre of test serum and the relationship between this parameter and the pellet radioactivity can be calculated from a knowledge of :

The specific activity (K = Ci/mmol) of the ^{125}I-labelled toxin at the time it was used to label the receptor.

The decay of the ^{125}I-labelled toxin-receptor complex in the period between receptor labelling and the day of the assay (decay factor A).

The volume of serum used in the assay (C µL)

The counter efficiency (B).

The formula is as follows :

$$\text{nmol/L toxin bound} = \frac{(\text{cpm test sample} - \text{cpm negative control}) \times A}{C \times K \times B \times 2.22}$$

Decay factor (A) :

Assay date	Decay factor (A)
Up to one week	1,0
+1-2 weeks	1,1
+2-3 weeks	1,2
+3-4 weeks	1,3
+4-5 weeks	1,4
+5-6 weeks	1,5
+6-7 weeks	1,6
+7-8 weeks	1,75

9.2. Calculation example

If cpm test sample count = 2756 and cpm negative control count = 140 and the assay is carried out with 5 µL of test serum (C=5 µL) 2 weeks after the receptor labelling day (decay factor A = 1.2) using ^{125}I-toxin with a specific activity of 216 Ci/mmol to label and a counter efficiency (B) of 0.7, then :

$$\text{nmol/L toxin bound} = \frac{(2756 \text{ cpm} - 140 \text{ cpm}) \times 1.2}{5 \text{ µL} \times 216 \text{ Ci/mmol} \times 0.7 \times 2.22} = 1.9$$

9.3. Typical result (example only) : these data must not be substituted for results obtained in the laboratory. Test with 5 µL. The samples are diluted with the diluent.

Tubes	Content	Mean count in cpm	Toxine bound in nmol/L
T	Total activity	78000	
Negative control	Negative control	224	0
Positive control	Positive control	10043	4.9
Sample A Sample A/2	Sample A Sample A + diluent	3962 2120	1.9 0.95
Sample B Sample B/2	Sample B Sample B + diluent	1607 853	0.69 0.31
Sample C Sample C/2	Sample C Sample C + diluent	12249 6096	6.0 2,9
Sample D Sample D/10	Sample D Sample D + diluent	38513 13194	19 6.5

10. PROCEDURAL LIMITATIONS

To obtain reliable results with the ACHR-AB kit, respect strictly the procedure described.

Always store frozen serum samples carefully and do not allow increases in temperature above -20°C. Incorrect storage can lead to loss of ACHR-AB activity.

11. CLINICAL EVALUATION

Each laboratory must establish its own range of normal range. The values given below are only indicative.

Myasthenic patients	0 - 1500 nmol/L
Normal samples	0 - 0.2 nmol/L
Other auto-immune diseases	0 - 0.5 nmol/L

Values > 0.5 nmol/L may be considered positive for acetylcholine receptor antibodies.

11.1.Clinical sensitivity
Samples from 53 patients diagnosed with myasthenia gravis were assayed in the ACHR-AB assay and all 53 were identified as being positive. In a larger series, K. Otha et al (Autoimmunity 2003 ; 36 : 151-154) found 82% of 1740 patients with myasthenia gravis to be AChRAb positive using the ACHR-AB kit.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

Intra-essai			Inter-essai		
Sample	mol/l n=20	CV %	Sample	mol/l n=20	CV %
1	2.2	5	3	3.3	1.9
2	0.5	5.9	4	1.8	1.7

12.2. Specificity
The ACHR-AB assay is specific for acetylcholine receptor autoantibodies. There is no interference with the other autoantibodies and it gave negative results with anti-thyroglobulin autoantibodies, rheumatoid factors, anti-thyroperoxidase, anti-TSH receptor, anti-DNA and antimitochondrial autoantibodies.

12.3. Detection limit
The detection limit of the method defined as being the minimum detectable dose at 2 standard deviations of the value of the negative serum in cpm is 0.02 nmol/L.

12.4. Interference
No interference was observed when samples were spiked with the following materials; haemoglobin up to 5mg/mL, 20mg/dL bilirubin or intralipid up to 30mg/dL.

ASSAY FLOW-CHART

Groups of tubes	Controls Samples µL	^{125}I-receptor µL		Antiserum (anti-Humain IgG) µL		Washing solution µL		Washing solution µL	
T	-	50		-		-		-	
Negative control	5	50	Mix gently ---- Incubate 2 h at 18-25°C	50	Mix gently ---- Incubate 2 h at 2-8°C or 18-24h at 2-8°C	1000	Mix gently ---- Centrifuge 20 min at 2-8°C at 3000 g ---- Eliminate the liquid	1000	Mix gently ---- Centrifuge 20 min at 2-8°C at 3000 g ---- Eliminate the liquid ---- Count 5 min
Positive Control	5	50		50		1000		1000	
Samples	5	50		50		1000		1000	