


**Acetylcholine Receptor
Autoantibody (AChRab) RIA kit from
RSR – Instructions for use**



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INTENDED USE

The RSR Acetylcholine Receptor autoantibody (AChRab) kit is intended for use by professional persons only for the quantitative determination of acetylcholine receptor (AChR) autoantibodies in human serum. Autoantibodies to the AChR are responsible for failure of the neuromuscular junction in myasthenia gravis. Measurement of the antibodies can be of considerable value in disease diagnosis.

REFERENCES

K Ohta et al

Frequency of Anti AChR ϵ subunit-specific antibodies in MG.

Autoimmunity 2003 36: 151-154

I. Matthews et al

Muscle-specific receptor tyrosine kinase autoantibodies – a new immunoprecipitation assay.

Clinica Chimica Acta 2004 348: 95-99

D. Beeson et al

A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis.

Neurology 1996 47: 1552-1555











ASSAY 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, labelled with ¹²⁵I-labelled alpha bungarotoxin provides the basis for RSR's AChRab assay kit. In the assay labelled receptors (¹²⁵I-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.

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. Duplicate 5µL determinations are sufficient for one assay. Repeated freeze thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of antibody activity. Do not use lipaemic or haemolysed serum samples. Plasma may be used if EDTA has been used as the anticoagulant. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 g in a microfuge) to remove any particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

IFU SYMBOLS

Symbol	Meaning
	EC Declaration of Conformity
	In Vitro Diagnostic Device
	Catalogue Number
	Lot Number
	Consult Instructions
	Manufactured By
	Expiry Date
	Store
	Positive Control
	Negative Control

MATERIALS SUPPLIED IN 25, 50 AND 100 TUBE KITS

MATERIAL	25 Tube	50 Tube	100 Tube
¹²⁵ I AChR	1 x 1.3mL	2 x 1.3mL	4 x 1.3mL
Negative control	1 x 100µL	1 x 100µL	1 x 100µL
Positive control	1 x 100µL	1 x 100µL	1 x 100µL
Anti Human IgG	1 x 1.5mL	2 x 1.5mL	1 x 5.5mL
Normal human serum	1 x 1mL	2 x 1mL	1 x 4mL
Washing solution	1 x 70mL	2 x 70mL	2 x 120mL
Reconstitution buffer	1 x 4mL	2 x 4mL	4 x 4mL
Precipitation Enhancer	1 x 1mL	2 x 1mL	4 x 1mL

MATERIALS REQUIRED AND NOT SUPPLIED

3mL assay tubes (round bottomed tubes are recommended when using precipitation enhancer)
 Suitable rack for assay tubes
 Pipettes capable of dispensing 5µL, 50µL and 1mL
 Refrigerated centrifuge capable of 1500 x g
 Vortex mixer
 Suitable apparatus for aspirating assay tubes
 Gamma counter

PREPARATION OF REAGENTS SUPPLIED

A	125I labelled AChR Lyophilised	40kBq/vial (at manufacture)
	Reconstitute each vial with 1.3mL of reconstitution buffer (G) and mix gently to dissolve. Once reconstituted, store at 2 – 8°C for up to 2 weeks.	
B	Negative control	Ready for use
C	Positive control (see label for concentration range)	Ready for use
D	Anti human IgG	Ready for use
E	Normal human serum	Ready for use
F	Wash solution	Ready for use
G	Reconstitution Buffer	Ready for use
H	Precipitation enhancer	Ready for use

ASSAY PROCEDURE

Allow all reagents excluding assay buffer, to stand at room temperature (20-25°C) for at least 30 minutes prior to start of assay. A repeating Eppendorf type pipette is recommended for steps 2, 4, 6, 7 and 10.

- Pipette 5µL of undiluted patient sera and kit controls (B, C) into respectively labelled assay tubes in duplicate.
- Pipette 50µL of ¹²⁵I AChR (A).
- Cover the tubes with a suitable cover and mix on a vortex mixer and incubate at room temperature for 2 hours.
- Pipette 50 µL of anti human IgG (D) into each tube.
- Cover the tubes with a suitable cover, mix on a vortex mixer and incubate at 2 – 8°C for 2 hours (an overnight [17 – 21 hours at 2 – 8°C] incubation period can be used if necessary).
- Pipette 25µL of precipitation enhancer (H) into each tube.
- Pipette 1mL washing solution (F) into each tube and mix on a vortex mixer.
- Centrifuge each tube for 20 minutes at 2 – 8°C at 1500g.
- Aspirate or decant the supernatant.

- Pipette 1mL washing solution (F) into each tube, resuspend the pellet using a vortex mixer.
- Repeat centrifugation step 8.
- Aspirate or decant the supernatant and count each tube for ¹²⁵I for 2 minutes on a gamma counter.

RESULT ANALYSIS

The radioactivity in the final pellet is proportional to the amount of labelled AChR bound by AChRAb. This can be expressed as nano moles of labelled AChR bound per litre of test serum using the following equation;

$$\text{nmoles/litre AChR bound} = \frac{(\text{cpm test sample} - \text{cpm negative control}) \times A}{C \times K \times B \times 2.22}$$

where;

A is the decay factor for ¹²⁵I between the receptor labelling and the day of assay; **B** is the counter efficiency; **C** is the volume of serum used in the assay (µL) and **K** is the specific activity (Ci/mmoles) of the ¹²⁵I toxin at the time it was used to label the AChR. Values for A, C and K are provided with each kit lot on a separate sheet.

TYPICAL RESULTS (Example only, not for calculation of actual results)

Total counts 77,120	Mean counts per min in pellet	Conc. nmol/L
Sample		
Kit negative control (B)	608	
Kit positive control (C)	10680	4.4
Patient serum 1	6502	2.6
Patient serum 2	2202	0.7
Patient serum 3	15555	6.6

Example Calculation

If a test sample counts are 2756 cpm, negative control counts are 608 cpm and the assay was carried out with 5µL of test serum, 2 weeks after the labelling date using toxin with a specific activity of 216 Ci/mmoles and with a counter efficiency of 0.7, then A = 1.2, B = 0.7, C = 5 and K = 216.

$$\text{AChRAb Conc.} = \frac{(2756 - 608) \times 1.2}{5 \times 216 \times 0.7 \times 2.22} = 1.5 \text{ nmol / L}$$

Assay Calibrators

As an alternative to using the calculation described above a set of calibrators (0.25, 1, 4 and 8 nmol/L; available from RSR) can be run in each assay and AChRAb concentration read off a calibration curve.

Assay Linearity

The relationship between acetylcholine receptor antibody concentration and cpm bound in the assay is only linear over a limited range. To overcome this problem, antibody positive sera can be diluted several times in the normal human serum (E) provided and assayed.

Antibody concentrations can then be calculated using binding data from within the linear range. The linear range for different patient sera is often different but 0.5 – 5 nmol/L is a useful guide. For example, we can consider a test serum sample which gives a value of 9 nmol/L undiluted. Three fold, 9 fold and 27 fold dilutions give values of 12, 13 and 13 nmol/L respectively (after correction for the dilution factor), all 3 dilution values being clearly in the linear range as they agree well. The result for this sample is then expressed as the mean of these 3 values i.e. 12.7 nmol/L.

ASSAY CUT OFF

Cut off	nmol/L
Negative	< 0.5 nmol/L
Positive	≥ 0.5 nmol/L

CLINICAL EVALUATION

Clinical Specificity

113 samples from healthy blood donors were assayed in the AChRAb RIA assay. 100% were identified as being negative for AChRAb.

Clinical Sensitivity

Samples from 53 patients diagnosed with myasthenia gravis were assayed in the AChRAb RIA assay and all 53 were identified as being positive for AChRAb. In a larger series, K. Ohta et al (Autoimmunity 2003 36 151-154) found 82% of 1740 patients with myasthenia gravis to be AChRAb positive using the RSR AChRAb kit.

Lower Detection Limit

The AChRAb RIA kit negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.02 nmol/L.

Inter Assay Precision

Sample	nmol/L (n=20)	CV (%)
1	2.2	5
2	0.5	5.9

Intra Assay Precision

Sample	nmol/L (n=20)	CV (%)
1	3.3	1.9
2	1.8	1.7.

Clinical Accuracy

No interference from autoantibodies to thyroid peroxidase, to GAD, to 21-OH, to double stranded DNA or rheumatoid factor was detected in the AChRAb RIA assay.

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 30 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for AChRAb levels.

SAFETY CONSIDERATIONS

Follow the instructions carefully. Observe expiry dates stated on the labels and the specified stability 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. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should none the less be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection and contact with skin, eyes and 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 (excluding assay buffer) and samples to reach room temperature before use.	
Pipette:	5μL patients serum and kit controls (B, C)
Pipette:	50μL ¹²⁵ I AChR (A)
Tubes:	Cover and mix on vortex mixer.
Incubate:	2 hours at room temperature.
Pipette:	50 μL of anti human IgG (D) into all tubes.
Tubes:	Cover and mix on vortex mixer.
Incubate:	2 hours at 2 – 8°C.
Pipette:	25μL precipitation enhancer (H).
Pipette:	1mL wash solution (F)
Tubes:	Cover and mix on vortex mixer.
Tubes:	Centrifuge each tube for 20 minutes at 2 – 8°C at 1500g
Tubes:	Aspirate/Decant supernatant
Pipette:	1mL wash solution (F)
Tubes:	Cover and mix on vortex mixer to resuspend pellet.
Tubes:	Centrifuge each tube for 20 minutes at 2 – 8°C at 1500g
Tubes:	Aspirate/Decant supernatant
Count tubes for ¹²⁵ I for 2 minutes using gamma counter	