DIAGNOSTIKA GMBH

Instructions for Use

Canine ACHRAB RIA

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

For Veterinary Diagnostic

REF RA119/25

25

2 – 8 °C

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Contents

Symbols

CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for <n> determinations
REF	Catalogue number		Consult Instructions for Use

Hazard Pictograms

	Radioactive		Warning

1. Introduction and Principle of the Test

In myasthenia gravis, autoantibodies directed against the the acetylcholine receptor of the motor end plate cause a disturbance of the neuromuscular transmission. Clinically, there is weakness and abnormal fatigability of skeletal muscles.

The Canine ACHRAB RIA assay utilizes recombinant canine specific acetylcholine receptors as antigen. These receptors are labelled with ^{125}I-alpha-bungarotoxin, a snake venom that binds highly specifically and almost irreversibly to the receptor. The canine acetylcholine receptor autoantibodies (cACHRAB) in the sample bind to the ^{125}I-labelled receptors. The antibody-receptor complexes are then immunoprecipitated with anti-IgG antibodies. The radioactivity measured in the precipitate reveals the acetylcholine receptor-autoantibody concentration in the serum.

2. Precautions

This kit is intended for in vitro use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified shelf life for reconstituted reagents. 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. 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. Material of human origin used in the preparation of the kit has 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 after handling. Monitor hands and clothing before leaving the designated area. 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 copious 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 Canine AChR**  2 vials

lyophilized, activity ~ 20 kBq/vial at manufacture

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

0.1 ml, ready for use

4.3. **Positive Control** **CONTROL +** 1 vial

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

4.4. **Anti-IgG Ab** 1 vial

1.5 ml, ready for use

4.5. **Reconsitution Buffer for ^{125}I-labelled Canine AChR** 1 bottle

4 ml, ready for use
for reconstitution of ^{125}I-labelled Canine AChR

4.6. **Wash Solution** 1 bottle

60 ml, ready for use

4.7. **Precipitation Enhancer** 1 vial

1 ml, ready for use
Mix thoroughly immediately before use  Warning

4.8. **Normal Serum** 1 vial

1 ml, ready for use

Additional materials and equipment required but not provided:

- Pipettes for dispensing 5 µl, 25 µl, 50 µl, 0.75 ml and 1 ml
- 3.5 ml assay tubes (round bottomed tubes are recommended when using precipitation enhancer) and suitable rack
- Refrigerated 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 to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 10 µL is sufficient for one assay (duplicate 5 µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples.

6. Preparation of Samples and Reagents

6.1 Samples

On the day of assay, thaw the sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at about 10,000 rpm i.e. about 10,000 g in a microfuge) to remove any particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

6.2 Reconstitution of ^{125}I-labelled Canine AChR

Immediately before use, reconstitute the lyophilized ^{125}I-labelled Canine AChR each with 0.75 ml Reconstitution Buffer for ^{125}I-labelled Canine AChR. Dissolve by mixing gently and keep the vial upright.

7. Test Procedure

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

1. Pipette 5 µl (in duplicate) of the Negative Control CONTROL -, Positive Control CONTROL + and the sera (undiluted), into labelled assay tubes.
2. Add 50 µl freshly reconstituted ^{125}I-labelled cAChR (see 6.2) into each tube.
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 and document the total radioactivity for 2 minutes in at least 2 tubes in a gamma counter.
4. Add 50 µl Anti-IgG Ab into each tube.
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.
6. Pipette 25 µL of Precipitation Enhancer into each tube.
7. Add 1 ml of **cold** (2 - 8°C) Wash Solution into each tube and mix gently on a vortex mixer.
8. Centrifuge tubes at 1.500 x g for 20 minutes at 2 - 8°C.
9. Decant or aspirate the supernatant, taking care not to disturb the pellet.
10. Add 1 ml of **cold** (2 - 8°C) Wash Solution into each tube and carefully resuspend the pellet using a vortex mixer.
11. Centrifuge tubes at 1.500 x g for 20 minutes at 2 - 8°C.
12. Decant or aspirate the supernatant, taking care not to disturb the pellet.
13. Determine the counts (cpm) in each tube for 2 minutes using a gamma counter.

8. Calculation of Results

The radioactivity in the pellet represents the amount of ^{125}I-labelled cAChR bound by the cAChR antibodies. This can be expressed as nanomoles of labelled cAChR bound per litre of test serum using the following equation with:

- The counts per minute [cpm] determined with the gamma counter
- The specific activity A [Ci/mmol] of the ^{125}I-labelled bungarotoxin at the time it was used to label the cAChRs.
- 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. 5 µl).
- Counter efficiency Z of the gamma counter used (e.g. 70 % → Z = 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:

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

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

$$F = \frac{1}{C \times A \times Z \times 2.22\ \text{cpm/Ci}}$$

Note: Should the counter efficiency Z of your gamma counter differ from the one stated on the QC-Certificate, then F must be recalculated accordingly.

Therefore the above formula (1) can be further simplified to the following formula (3):

$$\text{nmol/l} = (\text{cpm}_{\text{Pos. Control OR Sample}} - \text{cpm}_{\text{Neg. Control}}) \times D \times F$$

Example with C = 5µl, A = 213.7 Ci/mmol, Z = 70% = 0.7, cpm_{Sample} = 3233 cpm, $cpm_{Neg.\ Conrol}$ = 332 cpm and D = 1.22:

$$F = \frac{1}{5\ \mu l \times 213.7\ Ci/mmol \times 0.7 \times 2.22\ cpm/Ci} = 0.60*10^{-3}\ nmol/l*cpm$$

nmol/l cAChR bound =

(3233 cpm – 332 cpm) x 1.22 x $0.60*10^{-3}$ nmol/l*cpm = 2.13 nmol/l

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

	cpm	**nmol/l**
Negative Control	1004	**0.0**
Positive Control	7688	**4.1**

For assay to be valid, value achieved for the Positive Control must be in target range indicated on QC-Certificate included in each kit.

9. Assay Characteristics

9.1 Cut off

The following assay cut off was determined:

Negative	< 1.0 nmol/l
Positive	≥ 1.0 nmol/l

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

A published study showed: "At a cut-off of 1.3 nmol/l, the Canine ACHRAB RIA showed a sensitivity of 100%, a specificity of 98.20%, a PPV of 80.00% and a NPV of 100%. When only dogs with neuromuscular disease and megaesophagus were considered, the results were as follows: Sensitivity 100%, Specificity 97.50%, PPV 94.12%, NPV 100%." Translated orig. citation from: Tierarztl Prax Ausg K Kleintiere Heimtiere 2023; 51: 55–62, DOI 10.1055/s-0043-1760812

9.2 Assay Linearity

The relationship between canine 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 Serum 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.

9.3 Clinical Specificity

Sera from 24 individual healthy dogs were assayed in the Canine ACHRAB RIA. 23 (96%) were identified as being negative for cAChR Ab.

9.4 Clinical Sensitivity

Sera from 4 dogs diagnosed with myasthenia gravis were assayed in the Canine ACHRAB RIA. All 4 were identified as being positive for cAChR Ab.

10. Modifications of Instructions for Use

Version _3: Names of components as used by original manufacturer were adopted throughout IFU.

Version _2: Addition to section 9 is highlighted in gray.

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

11.Literature

- H. Bauer, G. Buhmann, J. van Renen, T. Steinberg, K. Putschbach, A. Fischer **Klinische Validierung eines neuen Radioimmunoassays für die Diagnose von Myasthenia gravis bei Hunden**
  Tierarztl Prax Ausg K Kleintiere Heimtiere 2023; 51: 55–62

- C.W Dewey et al
  Clinical Forms of Acquired Myasthenia Gravis in Dogs: 25 Cases (1988-1995).
  J. of Veterinary Internal Medicine (1997) 11: 50 – 57

- G.D Shelton et al
  Acquired Myasthenia Gravis. Selective Involvement of Esophageal, Pharyngeal and Facial Muscles.
  J. of Veterinary Internal Medicine (1990) 4: 281 – 284

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 Control	Samples
CONTROL -	µl	5		
CONTROL +	µl		5	
Samples	µl			5
^{125}I-labelled Canine AChR (fresh)	µl	50	50	50

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

Anti-IgG Ab	µl	50	50	50

Vortex gently
Cover and incubate for 2 hours at room temperature (20 – 25°C)

Precipiation Enhancer	µl	25	25	25
Wash Solution (cold)	ml	1	1	1

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

Wash Solution (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