Canine Toxoplasma Antibody ELISA

An ELISA test to detect antibodies against Canine Toxoplasma gondii infections in serum and plasma samples of Dogs.

REF EVL-EIAD1013-AB01

Σ 96
Effective, januari 2011

Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aquí en el kit.

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1 INTRODUCTION
Toxoplasma gondii infections induce in dogs undiagnosed fever, encephalopathy, pulmonary disease, uveal disease, hepatic disease myopathy or lymphadenopathy.

Infected dogs will shed Oocytes (10-20 μm) in the faeces (in this way dogs can transmit the disease to humans, but in most cases this is not the case) for only 1 to 2 weeks. Sometimes dogs develop a necrologic form of the disease, increased CSF protein/leucocytes (mono and neutro) may occur. Most people are infected by eating contaminated meat instead of being infected by dog/dog faeces. Detection of early disease stage is very important especially in absence of clinical signs.

Following antibody signs is also very important in measuring efficiency of antitoxoplasmic therapy. Infections can be treated by pyri- and sulfa- preparations (followed by spiramycin).

2 INTENDED USE OF THE TESTKIT
The Canine Toxoplasma ELISA test is designed to detect antibodies against toxoplasma infections. After washing the strips are incubated with the dog sera to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labeled anti-species conjugate is added to detect bound dog antibodies to toxoplasma antibodies. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3 PRINCIPLE OF THE TEST KIT
The test is based on the reaction of toxoplasma proteins with polyclonal dog antibodies. To this end toxoplasma proteins have been coated to a microtiter plate. The diluted dog serum/plasma sample is added to the wells of the coated plate. After washing the bound dog antibodies are detected by a HRPO conjugated antispecies conjugate. The color reaction in the wells is directly related to the concentration of toxoplasma antibodies in serum/plasma samples.

4 CONTENTS
- 12 x 8-well microtiter strips coated with Toxoplasma proteins
- 1 x strip holder
- 1 x 12 ml anti species IgG HRPO conjugated (anti-species) antibody
- 1 x 0.5 ml inactivated positive control serum (Freeze dried)
- 1 x 1 ml inactivated negative control serum (Freeze dried)
- 1 x 20 ml wash solution, 200 x concentrated. Dilute in de-ionized water before use!
- 1 x 18 ml ELISA buffer
- 1 x 8 ml substrate A
- 1 x 8 ml substrate B
- 1 x 8 ml stop solution
- 1 x plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS.
The kit should be stored at +4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.
Avoid repeated freezing and thawing as this increases non-specific reactivity.
6 WASH PROTOCOL
In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing
1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times
4. Turn the plate upside down and empty the wells with a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment
When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL
1. Open the package of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal, store them at ± 4 ºC and use them within 10 days. Wash the microtiter strips with washing solution according to the washing protocol. The washing solution provided must be diluted 200 x in de-ionized water!
2. Make 3 step dilutions of each sample in ELISA buffer starting 1:30 (90, 270, 810) in a round bottomed microtiter plate. Reconstitute directly before use the positive control in 0,5 ml and the negative control in 1 ml de-ionized water, divide into aliquots, and store immediately at -20°C until use. Make also a 3 step dilution of the positive and negative control.
3. Transfer 100 µl of these dilutions to the coated micro titer strips. Seal and incubate for 60 min. at 37 º C.
4. Wash as pointed out in wash protocol.
5. Dispense 100 µl conjugated anti-species antibody to all wells.
6. Seal and incubate 60 min. at 37 º C.
7. Wash as pointed out in wash protocol.
8. Mix equal parts of buffer A and B with gently shaking. Prepare immediately before use! Dispense 100 µl substrate solution to each well. Incubate 10-15 min. at room temperature (21°C.).
9. Add 50 µl stop solution to each well; mix well.
10. Read the absorbency values immediately within 10 min. ! at λ 450 nm ref 620nm.

8 PRECAUTIONS
- Handle all biological material as though capable of transmitting toxoplasma infections (Human pathogene).
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.
9 VALIDATION OF THE TEST
To standardize this ELISA test, a positive and negative control has to be tested. In order to confirm appropriate test conditions, the positive control should give an extinction higher than 1.000 OD units measured at $\lambda$ 450 nm and an endpoint titer higher than 90. The negative control should give an OD $\leq$ 0.400 units measured at $\lambda$ 450 nm and an end point titer of $\leq$ 30.

10 INTERPRETATION OF TEST RESULTS
Test samples are considered Toxoplasma positive if the absorbency is above 2 times the absorbency of the negative control. When a sample is negative > sample $< 2 \times$ negative it is suspected and should be retested after 2-3 weeks.

Intermediate antibody titers (90;270) in diseased animals showing signs suggestive of toxoplasma are considered positive and the dog will be suspected of shedding Canine Toxoplasma infection. A rise in antibody titer in a non vaccinated dog with Canine Toxoplasma infection represents an exaggerated, effective immune response.

In summary: a titer

- $< 30$ = no antibodies found
- $90$ = antibodies found, retest within 3 months (probably shedding Canine Toxoplasma antigen)
- $> 270$ = higher titer, considered to be protective.

SYMBOLS USED WITH EVL ASSAYS

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<th>Symbol</th>
<th>English</th>
<th>Deutsch</th>
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