VetLine

Toxoplasma ELISA

(TOXVT0460)

Performance Characteristics

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1 Introduction

Toxoplasma gondii is a small intracellular parasite, whose live cycle has a sexual and an asexual phase. Sexual development is restricted to the intestinal cells of (probably exclusively) cats; the oocysts formed are excreted and due to their resistant cell walls they may be infectious under advantageous circumstances for at least 1 year. Animals and man are intermediate hosts for the asexual proliferation of T. gondii: the ingested parasites will proliferate explosively within the host cells lysing them eventually. They disseminate throughout the body via circulation and lymphatic system and though may infect any cell type. In muscle and brain cells cysts are formed which are spheroidal and about 5-100 µm in diameter. Cysts are virtually immortal in the intermediate host. Toxoplasma gondii infections induce in cats undiagnosed fever, encephalopathy, pulmonary disease, hepatic disease myopathy or lymphadenopathy. Infected cats will shed Oocytes (10-20 µm) in the faeces (in this way cats can transmit the disease to humans, but in most cases this is not the case) for only 1 to 2 weeks. Sometimes cats 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 catfaeces. 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 anti-toxoplasmic therapy.

Toxoplasma gondii is the most common parasite in humans, but its abundance (7-80 %) is highly dependent on the geographic area, the socio-economic status and the nutritional customs. Infection only rarely causes toxoplasmosis and usually clinical symptoms are absent, but may produce severe problems in immunosuppressed persons and fetus. Because only a primary infection during pregnancy may be dangerous and even fatal for the unborn (the probability of congenital infection is about 50 %), the recent onset of an infection must be excluded. In pregnant women in over 98 % of cases, the absence of IgM excludes the possibility of recent infection. In newborns the very presence of anti-toxoplasma IgM is sufficient to confirm a congenital toxoplasmosis, since maternal IgM, unlike IgG, does not cross the placental barrier. But a significant number of infected infants do not develop detectable IgM levels and thus are false negative. In immunosuppressed patients toxoplasmosis causes severe complications mostly by reactivation of an earlier latent infection.

Species	Disease	Symptoms	Mechanism of infection
Toxoplasma gondii	Toxoplasmosis	Acquired Toxoplasmosis: lymphadenopathy, retinochorioditis Congenital Toxoplasmosis: hydrocephalus and microcephaly, intracranial calcifications, chronical chorioretinitis	Direct: oocysts (cats): ingestion by food including water, which is contaminated by feces of cats or contaminated soil. Indirect: Ingestion of cysts by eating raw or insufficiently cooked meat, esp. pork Congenital infection of the fetus

Infection may be identified by

- PCR
- Indirect immunofluorescence (IIF)
- Serology: Detection of antibody production by ELISA

2 Intended Use

The NovaTec VetLine Toxoplasma ELISA is intended for the quantitative determination of antibodies against Toxoplasma in veterinary serum.

3 Principle of the Assay

The quantitative immunoenzymatic determination of antibodies against Toxoplasma is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Toxoplasma antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Protein A/G conjugate is added. This conjugate binds to the captured Toxoplasma specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) Substrate Solution which gives a blue reaction product. The intensity of this product is proportional to the amount of Toxoplasma specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec Toxoplasma ELISA kit was determined by comparing 12-24 replicates of at least 2 different samples in one assay (within-run) and by comparing at least 2 different samples assayed in 12 different runs (between-run).

In accordance with the procedures prescribed in the instruction, the mean ($\bar{x}$) and the standard deviation (s) of the absorbance values (E) were determined.

The CV value [%] was then calculated using the following formula:

$$CV = s/\bar{x} \times 100\ \%$$

Acceptance Criterion: CV < 15 %

Results

Table 1: Within-Run Precision

Sample	n	Mean (E)	CV [%]
1	24	0,575	2,23
2	24	1,218	2,18
3	24	1,056	3,23

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	43,61	9,97
2	12	8,54	11,47
3	12	118,07	5,54

Conclusion

The acceptance criterion was met for all samples.

4.2 Analytical Specificity

4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides

Introduction

Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters were performed.

Material and Test Condition

Different members of the NovaLisa® and VetLine ELISA line were used including assays for the detection of different antibody isotypes (IgA, IgM, IgM, IgM + IgM, total antibody with protein A/G) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH, Dirofilaria and FeLV.

Defined positive resp. negative or equivocal samples were used.

A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides). The results obtained with

the sample with added “interfering substance” should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

Conclusion

The internal specifications of 60-140 % were always fulfilled.
Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides.
These results are also in agreement with literature data.

This conclusion is valid for the NovaLisa® as well as for the VetLine version of the assays.

Dimeski, G. (2008) Interference Testing, Clin Biochem Rev 29, S43 – S48
Tate, J. and Ward, G. (2004) Interferences in Immunoassay, Clin Biochem Rev 25, 105 – 120

4.2.2 Cross-Reactivity

Cross-reactions with closely related pathogens cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

To evaluate the diagnostic performance of the NovaTec Toxoplasma ELISA, internal studies were conducted by NovaTec in comparison to pred-defined samples. Samples are of feline origin.

Materials

VetLine Toxoplasma ELISA Lot: TOXVT- 054-1

Production date: 2017-02 Expiry date: 2018-02-28

27 positive samples feline

17 negative samples feline

31 positive samples canine

34 negative smples canine

Results

Total number of samples feline: 44

Table 3: Diagnostic Sensitivity and Specificity feline

		Demand		
		positive	negative	**Σ**
VetLine Toxoplasma ELISA	positive	27	0	**27**
	negative	0	17	**17**
	Σ	**27**	**17**	**44**

(Equivocal results were not included in the calculations)

Diagnostic Sensitivity feline: 100 % (95 % confidence interval: 87,23 % - 100,0 %)

Diagnostic Specificity feline: 100 % (95 % confidence interval: 80,49 % - 100,0 %)

Agreement feline: 100 % (44/44)

Total number of samples canine: 65

Table 4: Diagnostic Sensitivity and Specificity canine

		Demand		
		positive	negative	**Σ**
VetLine Toxoplasma ELISA	positive	28	3	**31**
	negative	3	31	**34**
	Σ	**31**	**34**	**65**

(Equivocal results were not included in the calculations)

Diagnostic Sensitivity canine: 90,32 % (95 % confidence interval: 74,25 % - 100,0 %)

Diagnostic Specificity canine: 91,18 % (95 % confidence interval: 76,32 % - 100,0 %)

Agreement canine: 90,76 % (59/65)

Conclusion

For **feline samples** the diagnostic sensitivity was > 98 % and the diagnostic specificity was > 98 % (agreement: > 98 %).

For **canine samples** the diagnostic sensitivity was 90,3 % and the diagnostic specificity was 91,2 % (agreement: 90,8 %).