VetLine

Toxoplasma IgM ELISA

(TOXVM0460)

Performance Characteristics

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1 Introduction

Toxoplasma gondii, the causative agent of toxoplasmosis, is an intracellular, ubiquitous tissue parasite. The final hosts of the pathogen are domestic cats and other felids. In felids as final hosts, the sexual development, and as in other intermediate hosts, the asexual development of Toxoplasma gondii takes place. Over 200 species of bird and mammals, including humans and farm animals such as pigs, cattle and sheep are known as intermediate hosts worldwide. In intermediate hosts only asexual reproduction of the pathogen by endodyogeny takes place.

Cats and other felids are usually infected with Toxoplasma gondii by ingesting bradyzoites in tissue cysts in the meat of infected prey or by ingesting oocysts. Oocysts are excreted with the feces of felids.

The excreted oocysts become infectious after a maturation period of about 2-5 days (sporulation). They are extremely resistant and can remain infectious in moist soil for several months to years.

In the course of infection with Toxoplasma gondii, tissue cysts form in the muscles, internal organs, retina and brain of the host. They represent an intracellular form of the parasites.

Symptoms in cats:

In cats, infection is usually asymptotic. During an approximately two to three weeks excretion phase of oocysts, there are occasional non-specific symptoms such as mild diarrhea, swelling of the lymph nodes and an increase in body temperature.

In immunosuppressed cats (e.g. diseases of FIP, FeLV and FIV) symptoms such as fever, difficulty breathing, pneumonia, gastrointestinal disease, hepatitis, eye disorders or central nervous system disorders occur more often.

Extremely severe courses are often seen in intrauterine infected kitten. These usually lead to the death of the animals very quickly.

Cats are relevant for zoonotic excretion of oocysts.

Proof of a Toxoplasma infection in cats is subject to reporting in Germany.

Toxoplasmosis is one of the most common zoonoses. Humans may become accidental infected by ingestion of oocysts excreted by the cat, e.g. infected while gardening or by eating raw or underheated meat from infected animals (e.g. raw sausage). An initial infection with Toxoplasma gondii during pregnancy is dangerous. If the pathogen is able to enter fetal tissue, it can cause severe damage to the fetus and cause a miscarriage. The reactivation of an existing infection in immunosuppressed people can cause serious disease or even death.

Evidence of the pathogen:

- Antibody detection using an immunofluorescence test or ELISA
- Microscopic detection of pathogens in tissue or fecal samples
- Detection by means of polymerase chain reaction in tissue samples or fecal samples

2 Intended Use

The NovaTec VetLine Toxoplasma IgM ELISA is intended for the qualitative determination of specific IgM antibodies against Toxoplasma gondii in serum samples from cats.

3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec Toxoplasma IgM 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	1,228	3,40
2	24	0,719	2,85
3	24	0,421	4,07

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	23,58	7,12
2	12	19,74	6,28
3	12	6,92	7,11

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 IgM ELISA, an internal study by NovaTec and an external study by an accredited laboratory in Germany were conducted with well defined serum samples from cats predetermined with Anti-Toxoplasma-gondii IIFT Katze (IgM), FI 2410-1010 MF from Euroimmun.

Materials

VetLine Toxoplasma IgM ELISA Lot: TOXVM- 131-1,TOXVM-132-1

27 positive samples

33 negative samples

Results

Total number of samples: 60

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Toxoplasma IgM ELISA	positive	27	2	**29**
	negative	0	31	**31**
	Σ	**27**	**33**	**60**

Diagnostic Sensitivity feline: 100,00 % (95 % confidence interval: 87,23 % - 100,0 %)

Diagnostic Specificity feline: 93,94 % (95 % confidence interval: 79,77 % - 99,26 %)

Agreement feline: 96,67 % (58/60)

Conclusion

The evaluation of the diagnostic performance of the VetLine Toxoplasma IgM ELISA was conducted internal by NovaTec and external at Laboklin (Germany).

The diagnostic sensitivity feline was 100,00 % and the diagnostic specificity feline was 93,94 % (agreement: 96,67 %).

Therefore, the acceptance criteria are met.