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Toxoplasma IgM vet ELISA

RUO

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CONTENTS

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 Toxoplasma IgM vet 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. MATERIALS

4.1. Reagents supplied

1. **SORB MT Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Toxoplasma gondii antigens; in resealable aluminium foil.
2. **SAM DIL Sample Dilution Buffer:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
3. **STOP SOLN Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
4. **WASH SOLN 20x Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
5. **ENZ CONJ Conjugate:** 1 bottle containing 20ml of peroxidase labelled antibody to IgM in phosphate buffer (10 mM); coloured red; ready to use; white cap.
6. **SUB TMB TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
7. **CAL C Positive Control:** 1 vial containing 2 mL; coloured yellow; ready to use; red cap; ≤ 0.02 % (v/v) MIT.
8. **CAL B Cut-off Control:** 1 vial containing 3 mL; coloured yellow; ready to use; green cap; ≤ 0.02 % (v/v) MIT.
9. **CAL A Negative Control:** 1 vial containing 2 mL; coloured yellow; ready to use; blue cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Toxoplasma gondii antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use serum samples from cats with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with Sample Dilution Buffer. Dispense 10 µl sample and 1 ml Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.
Perform all assay steps in the order given and without any delays.
A clean, disposable tip should be used for dispensing each standard/control and sample.
Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.
If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.
Bichromatic measurement using a reference wavelength of 620 nm is recommended.
Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.
Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.3. Interpretation of Results

Normal value ranges for this ELISA should be established by each laboratory based on its own sample populations in the geographical areas serviced.
The following values should be considered as a guideline:

Cut-off	10 U	-
Positive	> 11 U	Antibodies against the pathogen are present.
Equivocal	9 – 11 U	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 U	The sample contains no antibodies against the pathogen.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
The performance data have been established with serum samples from cats.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	1.228	3.40
#2	24	0.719	2.85
#3	24	0.421	4.07

Interassay	n	Mean (IU/ml)	CV (%)
#1	12	23.58	7.12
#2	12	19.74	6.28
#3	12	6.92	7.11

10.2. Relative Specificity

The relative specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte (relative to the results of other serological tests).
Relative Specificity cats: > 98 % (95 % confidence interval: 87.23 % - 100.0 %)

10.3. Relative Sensitivity

The relative sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte (relative to the results of other serological tests).
Relative Sensitivity cats: 93.94 % (95 % confidence interval: 79.77 % - 99.26 %)

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.5. Cross Reactivity

Cross reactions cannot be excluded.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- **Only for research use.**
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1 Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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ABBREVIATIONS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
Toxoplasma IgM vet ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µl	-	-	-
Cut-off Control	-	-	100 µl	-	-
Positive Control	-	-	-	100 µl	-
Sample (diluted 1+100)	-	-	-	-	100 µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37±1 °C** Wash each well three times with 300 µl of Washing Buffer					
Conjugate	-	100 µl	100 µl	100 µl	100 µl
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µl of Washing Buffer					
TMB Substrate Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta