Toxocara species

Enzyme immunoassays for the diagnosis of toxocariasis

ELISA a **IMMUNOBLOT** kits are optimized and validated for detection of IgA and IgG antibodies in human serum and plasma

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Larval toxocariasis is a human parasitic disease caused by the larval stage of a dog roundworm – *Toxocara canis* and cat roundworm – *Toxocara cati*.

The infection is characterized by the presence of migrating larvae (larva migrans) in various organs. The Toxocara larvae hatching from infectious eggs in the human intestine penetrate the intestinal wall and migrate hematogenously into the liver, lungs, CNS, eyes, musculature and other organ systems. Larvae migration generates several clinical pathologies in the patient, such as visceral larva migrans, ocular toxocariasis and neurotoxocariasis.

Visceral larva migrans (VLM) – includes non-specific and variable clinical symptoms such as eosinophilia, leucocytosis, hepatomegaly, brief febrile episodes, mild gastrointestinal disorders, asthmatic attacks, pneumonic symptoms, lymphadenopathy and urticarial skin changes.

Ocular larva migrans (OLM) – the granulomatosus retina deficiencies that may result in the loss of sharp vision, strabismus, uveitis, chorioretinitis, retinaablation and the so-called "lightvision". In some cases full blindness of one or both eyes may also occur.

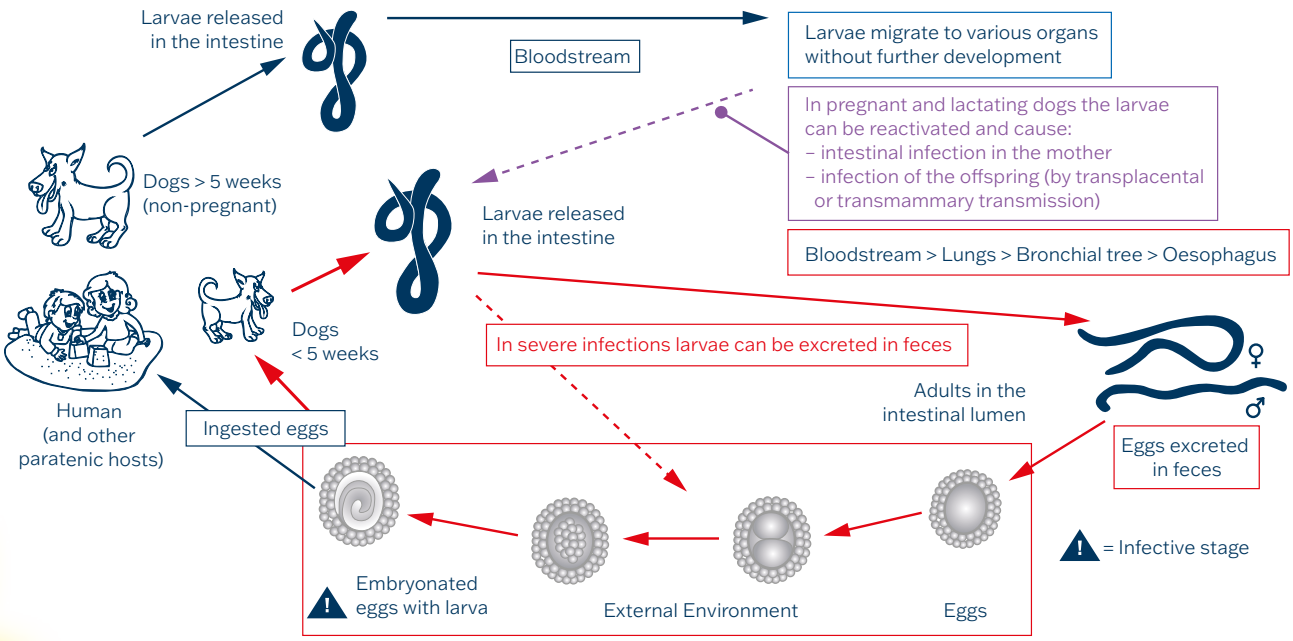
Neurotoxocariasis – Toxocara larvae in the peripheral or the central nervous system cause meningoencephalitis or other neurological manifestations such as eosinophilic meningomyelitis cerebral vasculitis, epilepsy, myelitis, radiculitis, affection of cranial nerves or skeletal muscle affection.

Diagnosis of Infection

Diagnosis of a disease is based on the evaluation of a data set: anamnesis, clinical manifestation and laboratory tests results.

Given the incomplete life cycle and non-specific symptoms serodiagnostic methods are reliable tools to detect Toxocara antibodies or antigens in humans. The most significant criterium is the presence of specific IgG antibodies against excretory-secretory antigens (TES).

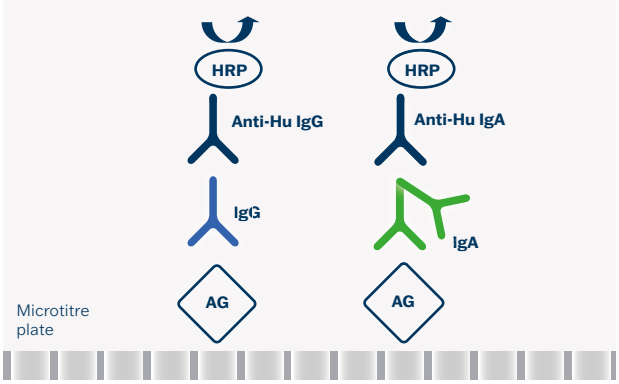
Determination of avidity for IgG antibodies enables to determine the phase of the disease, specific IgA antibodies complement the diagnostic mosaic.



ELISA

Test Principle

The assay is based on a sandwich type of ELISA method.



Summary Protocol

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 minutes at 37°C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 minutes at 37°C
	7.	Aspirate and wash the wells 4 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 minutes at 37°C
	10.	Add 100 µl Stopping solution 100 µl – Including blank
	11.	Read colour intensity at 450 nm

Antigens

Excretory-secretory ('ES') antigen isolated from cultured larvae of *Toxocara canis*

Clinical Application

- Laboratory screening test for detection of toxocariasis (larva migrans)
- Diagnostics and differentiation of toxocariasis infection stage (acute, chronic) by detection of IgG antibodies in human serum or plasma and determination of IgG avidity.
- Monitoring of antibody levels following therapy

User Comfort

- Ready-to-use components
- Colour-coded components
- Breakable colour-coded microplate strips
- CUT-OFF included
- Semiquantitative evaluation of results (Index of Positivity)
- Easy assay procedure

Advantages

- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Short total assay time
- Avidity test available (EIA Toxocara IgG)
- Ready for automation
- Customer support

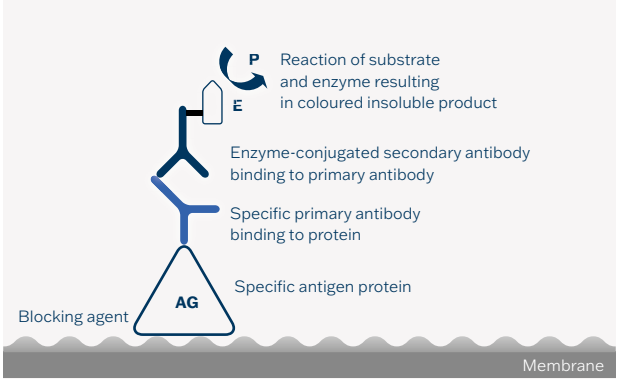
Test Characteristics

ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA Toxocara IgA	95.2%	95.8%
EIA Toxocara IgG	95.5%	95.5%

IMUNOBLOT

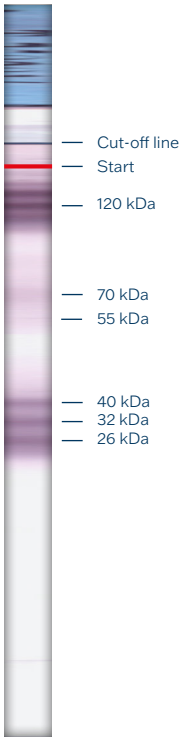
Test Principle

Electrophoretically separated Toxocara antigens are transferred to a nitrocellulose membrane.



Antigens

Excretory-secretory ('ES') antigen isolated from cultured larvae of Toxocara canis



TES 120 – set of secreted mucins Tc-muc1 – Tc-muc4
– surface coat antigens
– sensitive and specific for detection of toxocariasis
TES 70 – non-specific lectin
– associated with the nematode body cuticle
TES 40, 55 – non-specific glycoproteins
TES 32 – the most frequent
– associated with nematode body cuticle
– specificity for binding of saccharides (mannose and/or galactose)
– sensitive and specific for detection of toxocariasis
TES 26 – phosphatidylenolamine binding protein
– sensitive and specific for detection of toxocariasis

Summary Protocol

Step	Test steps
1.	Pipette Universal solution 2 ml
2.	Strips soaking 10 min. at room temperature – Shaker
3.	Aspirate
4.	Dilute samples – serum/plasma (8 µl + 1,5 ml)
5.	Pipette Controls and diluted samples 1.5 ml
6.	Incubate 60 min. at room temperature – Shaker
7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
8.	Pipette Conjugate 1.5 ml
9.	Incubate 60 min. at room temperature – Shaker
10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
12.	Inkubace 10 min. při laboratorní teplotě – Shaker
13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
14.	Sticking and evaluation of strips

Clinical Application

- Differentiation of specific and non-specific fractions of the ES antigen by immunoblot
- Confirmation of ELISA test results as well as confirmation of ambiguous results obtained due to cross-reactions with other helminthoses.

User Comfort

- Ready-to-use components
- Colour-coded strips
- Positive and Negative controls
- CUT-OFF control is present on the strip
- Interchangeable components
- Easy assay procedure

Advantages

- Possibility to detect Toxocara antibodies in vitreous humour
- Easy interpretation and reproducibility of results
- High diagnostic sensitivity and specificity
- Compatibility with all commercial immunoblot processing systems
- Customer support

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
BLOT Toxocara IgG	95.8%	99.0%

Clinical Data

High Specificity of Immunoblot

(Elimination of false positive result in EIA tests due to cross-reactivity with other helminth diseases)

Sample	Confirmation BLOT Toxocara	Reaction in EIA tests					
		Toxocara	Trichinella	Echinococcus	Cysticercus	Fasciola	Schistosoma
1	+	++	+	+	+/–	–	
2	–	+	+	+	+	+/–	
3	–	+++	++	+++	+	++	
4	–	+/–	+/–	+/–	+/–	+/–	+/–
5	–	+	+++	+/–	+/–	–	
6	–	+	–	+	–	–	
7	–	+	+	+/–	–	+/–	
8	–	+	+	+	+/–	+/–	
9	+	+++	+	+/–	–	–	+
10	–	+	+	+/–	–	–	
11	+	+++	+	+	–	+	

Results of Cross-Reacting Pathogens or Factors Category

EIA Toxocara IgA

Category	n	Positive Result
Borrelia spp.	20	0
RF	14	0
Schistosoma spp.	3	0
Echinococcus spp.	2	0
Ascaris lumbricoides	1	0
Toxoplasma gondii	9	0
Treponema pallidum	18	0
Total	**67**	**0**

EIA Toxocara IgG

Category	n	Positive Result
Borrelia spp.	20	0
RF	14	0
Schistosoma spp.	3	0
Echinococcus spp.	2	0
Ascaris lumbricoides	1	0
Toxoplasma gondii	9	0
Treponema pallidum	18	0
Total	**67**	**0**

Ordering information

ELISA

Cat. No.	Product	No. of Tests
TcA096	EIA Toxocara IgA	96
TcG096	EIA Toxocara IgG	96

*available also in Smart version

IMMUNOBLOT

Cat. No.	Product	No. of Tests
TcGB16	BLOT Toxocara IgG	16

TestLine®

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CERTIFIED
ISO 9001 · ISO 13485

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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