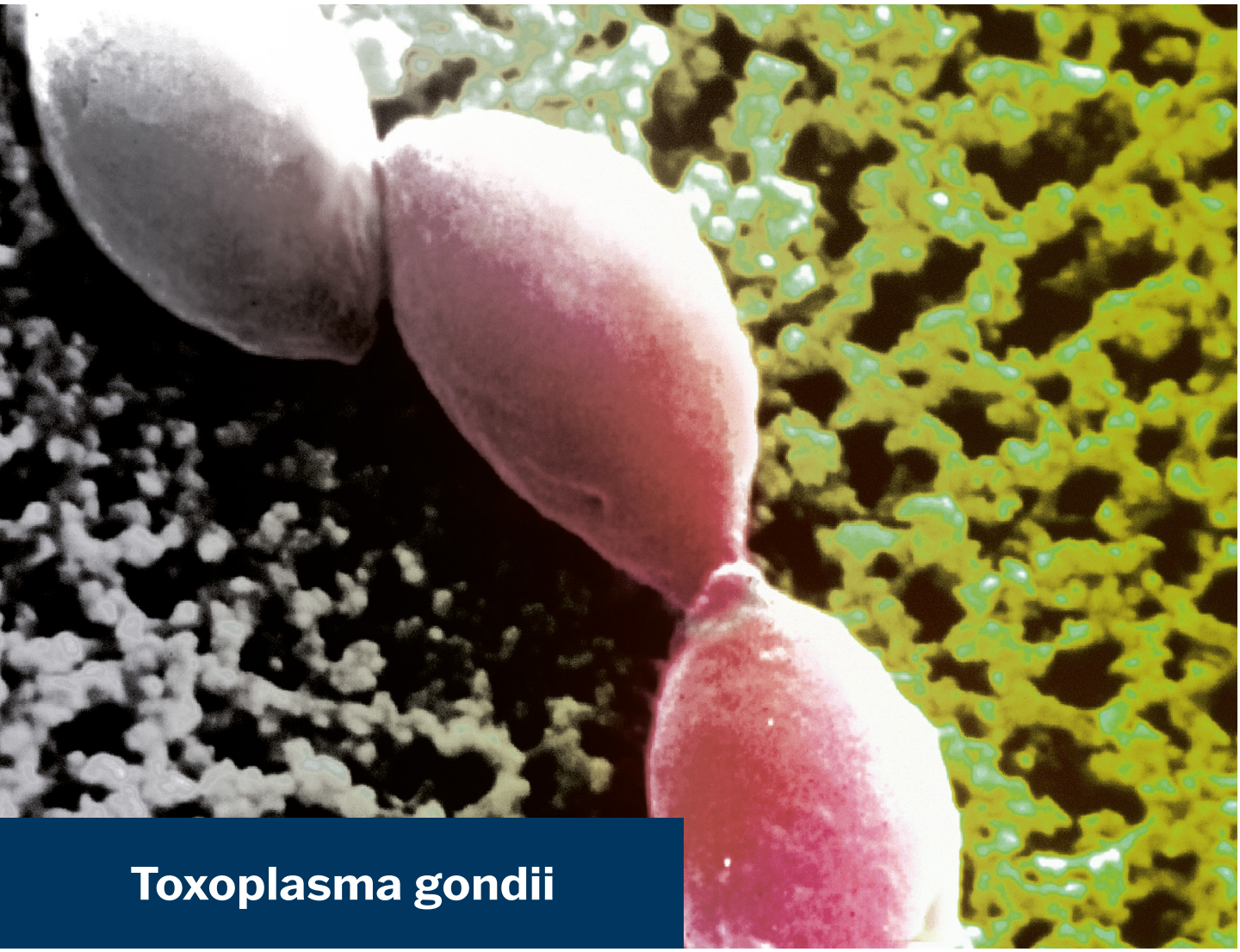

Toxoplasma gondii

Enzyme immunoassays for the diagnostics of toxoplasmosis

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

IVD CE 2265

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

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Introduction

Toxoplasmosis is a widespread parasitic disease caused by protozoan Toxoplasma gondii – a parasite with a complicated life cycle consisting of several morphologically different stadia. Primary hosts are members of the feline family. Humans and most warm-blooded animals can be infected by either primarily infected food (insufficiently heat-treated meat) or by ingestion of oocysts (secondary contaminated food or contaminated fingers, objects, etc.).

Acquired toxoplasmosis in immunocompetent individuals is usually asymptomatic or can manifest itself with flu-like symptoms (subfebrility, fatigue, lymphadenopathy, muscle aches) and has no lasting ill effects. Severe life-threatening infections (encephalitis, hepatitis, chorioretinitis, myocarditis, generalized form of the disease) may develop in immunocompromised patients usually because of a reactivation of a latent infection.

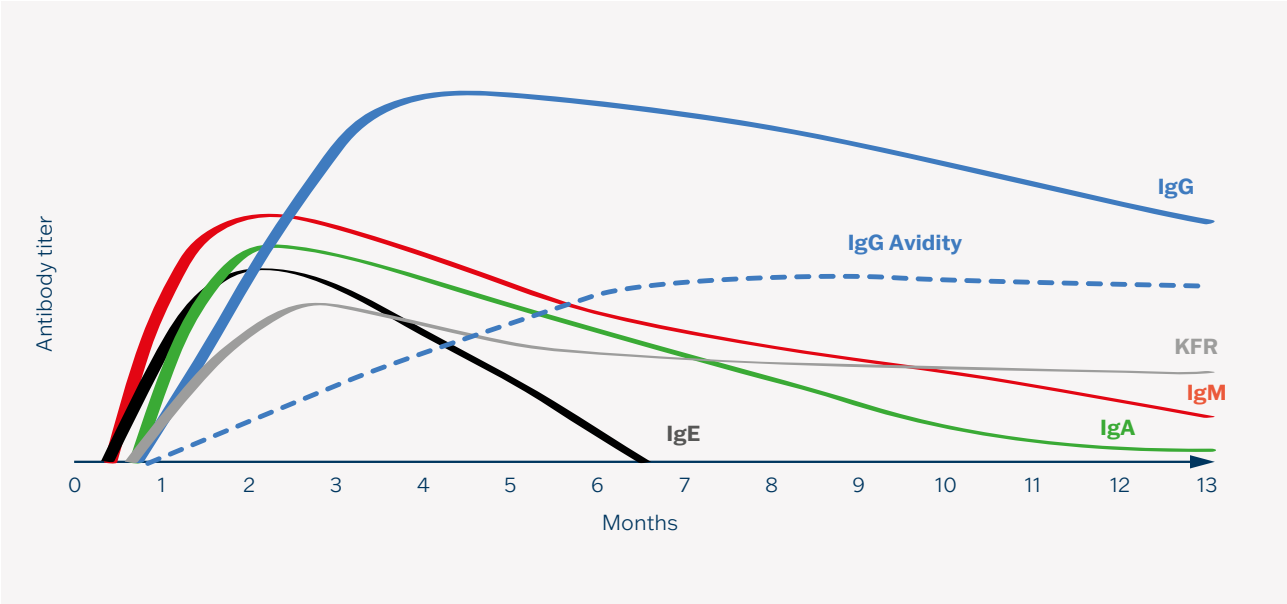
Congenital toxoplasmosis is caused by transmission of infection from mother to foetus and it might result in severe damages of the foetus (brain calcification, hydrocephalus, vision disorders, mental affections), still birth or abortion.

Diagnosis of infection

Diagnosis of the disease is based on epidemiological anamnesis, clinical manifestation and laboratory tests. Direct detection of the parasite is not available for routine diagnostics. Serology is the most important tool for laboratory diagnostics of toxoplasmosis.

- Screening – determination of total antibodies by complement fixation test (CFT)
- Determination of specific IgA, IgE, IgM, IgG antibodies and IgG avidity by ELISA and confirmation of results by Immunoblot

Antibody response



ELISA

IgM antibodies:

- Highly sensitive marker of acute infection
- Disadvantage: long-time persistence after the beginning of infection (more than 1 year)

IgA antibodies:

- Sensitive and specific marker of acute infection
- Persistence for 6-9 months from the beginning of infection

IgE antibodies:

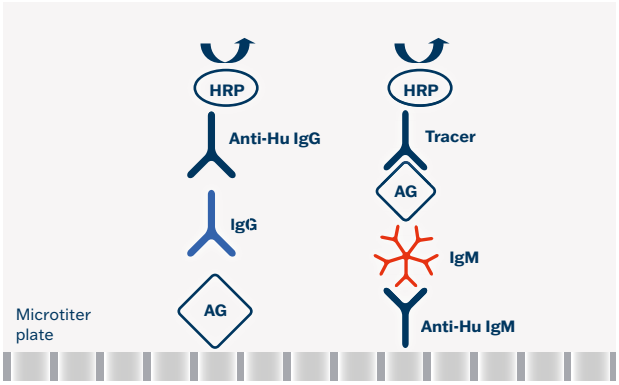
- Highly specific marker of acute infection
- Persistence up to 6 months from the beginning of infection

IgG antibodies:

- Anamnestic antibodies
- Persist for years and ensure protection against new infection
- IgG avidity reflects stage of infection

Test Principle

Sandwich ELISA | Capture ELISA



Antigens

Purified and inactivated native *Toxoplasma gondii* antigen (RH strain).

Clinical application

- Diagnostics and differentiation of toxoplasmosis stage by detection of IgA, IgE, IgG a IgM specific antibodies in human serum or plasma and determination of IgG avidity

Summary protocol

Step	Test steps
1.	Dilute samples – serum/plasma 1:101 (10 µl + 1 ml)
2.	Pipette Controls and diluted samples 100 µl – Blank = empty well
3.	Incubate 60 min. at 37 °C
4.	Aspirate and wash the wells 5 times
5.	Add 100 µl Conjugate or Tracer – Blank = empty well
6.	Incubate 60 min. at 37 °C
7.	Aspirate and wash the wells 5 times
8.	Add 100 µl Substrate (TMB-Complete) – Including blank
9.	Incubate 30 min. at 37 °C
10.	Add 100 µl Stopping solution – Including blank
11.	Read colour intensity at 450 nm

User comfort

- Ready-to-use components (except Tracer)
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF included
- Semiquantitative evaluation of results (Index of Positivity)
- Calibrators (EIA Toxoplasma IgG)
- Quantitative evaluation of IgG antibodies (IU/ml)

Advantages

- Identical assay procedure
- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Avidity test available (EIA Toxoplasma IgG)
- Ready for automation
- Customer support
- Independent verification (CKS)
- Short total assay time 1.5 hour

Test characteristics

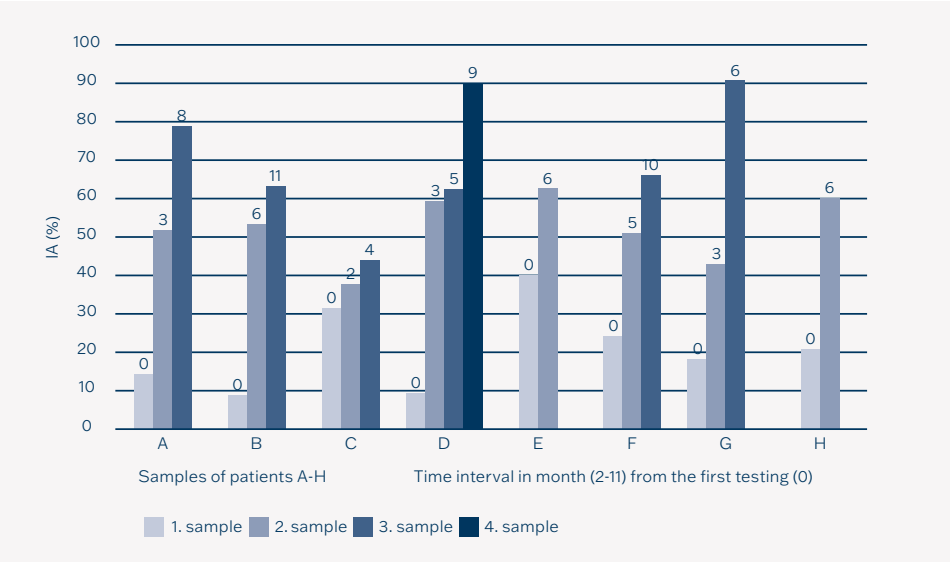
ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA Toxoplasma IgA	96.9%	99.0%
EIA Toxoplasma IgE	96.9%	99.0%
EIA Toxoplasma IgG	98.9%	99.2%
EIA Toxoplasma IgM	96.4%	97.9%

Avidity

Determination of avidity of IgG antibodies

Antibody avidity reflects binding strength of the binding between antigen and antibody. Antibodies with low avidity are created firstly in the process of primary infection . During the progress of infection the immune response matures and the avidity increases. Highly avid antibodies are being observed in the latent phase of infection. During secondary infection or reactivation the memory B-Cells immediately produces IgG antibody with high avidity.

Determination of avidity of IgG antibody allows more accurate identification of the infection stage.

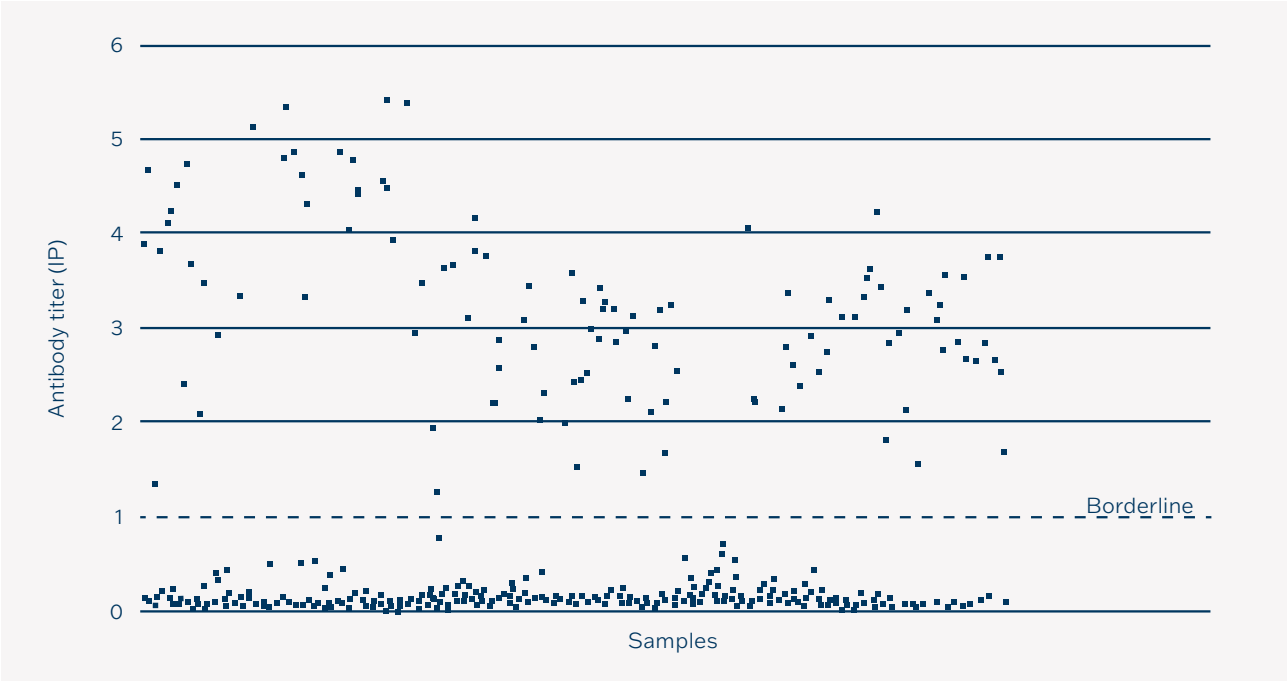


Clinical data

Seroprevalence and dynamics of IgG antibody classes at blood donors (CZ)

Blood donors (n = 483)

Positive (n = 119) (seroprevalence)	Negative (n = 364)
25%	75%



Types of kits

SmartEIA kits are designed for automated processing using the Agility® analyser.

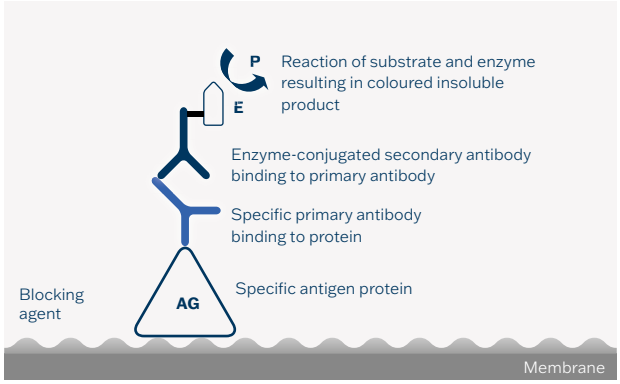
EIA

SmartEIA

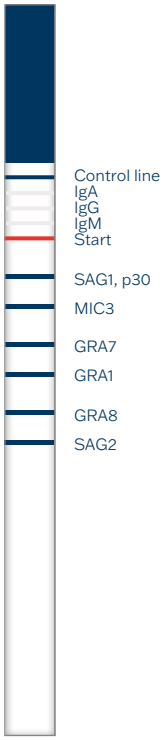
IMMUNOBLOT

Test principle

Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



Antigens



SAG1 p30; a highly immunogenic and antigenic surface antigen involved in the activation of strong immune response of tachyzoites during the acute phase of toxoplasmosis. A good serological marker for antibodies against *T. gondii* in the acute and chronic phases of infection; high titres of IgG, IgM and IgA.

MIC3 p90; is strong adhesin and one of the major vaccine candidates. It is a dimeric 90 kDa micronemal cysteine-rich protein. It is expressed in tachyzoite, bradyzoite and sporozoite and has excellent immune properties.

GRA1 p24; a highly immunogenic protein whose reactivity correlates with the chronic phase of the disease to a large extent.

GRA7 p29; expressed in all infectious forms of toxoplasma. It elicits a strong antibody response in the acute phase of infection. It is considered an important diagnostic tool, suitable for the chronic phase of infection.

GRA8 p35; highly immunogenic protein, more suitable for the diagnosis of acute toxoplasmosis than chronic infections

SAG2 p22; a major surface protein known as a binding ligand, characterised by good antigenicity and immunogenicity. Effective for the detection of IgG antibodies in patients with acute toxoplasmosis.

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 1:51 (30 µl + 1,5 ml)
5.	Pipette Controls and diluted samples 1.5 ml
6.	Incubate 30 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 30 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.	Incubate 15 min. at room temperature – 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

- Detailed determination for the presence of anti-Toxoplasma gondii specific antibodies
- Diagnostic of Toxoplasma gondii infection, confirmative test for ELISA
- Useful method to trace immune profile of mothers' and new-borns' sera – determination of congenital toxoplasmosis

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

Test characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Toxoplasma IgA	95.2%	99.1%
BLOT-LINE Toxoplasma IgG	95.6%	98.0%
BLOT-LINE Toxoplasma IgM	95.6%	98.0%

Advantages

- Easy interpretation and reproducibility of results
- High diagnostic specificity and sensitivity
- Compatibility with all commercial immunoblot processing systems
- Customer support

Interpretation results

IgE	IgA	IgM	IgG	Interpretation
–	–	+	–	Acute infection (occurs rarely)
+	+	+	low +	Acute infection
+	+	+	+	Acute infection
–	+	+	+	Acute or post-acute infection
–	low +	+	+	Post-acute infection
–	–	+	+	Post-acute or latent infection
–	–	–	+	Latent infection
–	–	–	–	Specific antibodies were not proven

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Ordering information

ELISA

Cat. No.	Product	Units
TgA096	EIA Toxoplasma IgA	96 wells
TgE096	EIA Toxoplasma IgE	96 wells
TgG096	EIA Toxoplasma IgG	96 wells
TgM096	EIA Toxoplasma IgM	96 wells
SK-TgA096	SmartEIA Toxoplasma IgA	96 wells
SK-TgE096	SmartEIA Toxoplasma IgE	96 wells
SK-TgG096	SmartEIA Toxoplasma IgG	96 wells
SK-TgM096	SmartEIA Toxoplasma IgM	96 wells
xxxTLN	CKS negative	3.5 ml
xxxTLP	CKS positive	3.5 ml

SmartEIA kits are designed for automated processing using the Agility® analyser

IMMUNOBLOT

Cat. No.	Product	No. of Tests
TgAL20	BLOT-LINE Toxoplasma IgA	20
TgGL20	BLOT-LINE Toxoplasma IgG	20
TgML20	BLOT-LINE Toxoplasma IgM	20

Related products

KFR

Cat. No.	Product
TgKF02	Toxo – CF – Ag lyophil. (0,2 ml)
TgKF05	Toxo – CF – Ag lyophil. (0,5 ml)
Tg-KF1	Toxo – CF – Ag lyophil. (1 ml)
KFAs00	CF – AMBOCEPTORset (0,5 ml)
KFAs01	CF – AMBOCEPTORset (1 ml)
KFC001	CF – COMPLEMENT (1 ml)
TgKFN2	Toxo – CF – Negative Control lyophil.
TgKFP2	Toxo – CF – Positive Control lyophil.

TestLine Clinical Diagnostics Ltd.
Krizikova 68, 612 00 Brno, Czech Republic
+420 549 121 203
sales@testlinecd.com
www.testlinecd.com

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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