BioVendor Group CLIA

Tick-borne infections

Lyme borreliosis (*Borrelia burgdorferi* s.l.)
Tick-borne encephalitis (TBE virus)

Diagnostic panel

CE IVD

The kits are CE-IVD certified and intended for professional use.

CLIA kits are optimized and validated for the determination of antibodies in human serum and plasma

Designed for the platform

Kleeya®

Tick-borne infections

Many infectious diseases need a vector, which transmits the disease. Ticks are the major transmission vectors for many infectious diseases. Ticks occur worldwide and their life cycle usually lasts 2 years. The larvae hatch from the female-laid eggs, which immediately seek out a host on which to attach and feed. Fed larvae moult into unfed nymphs that remain on the host. After engorging on the host's blood, the nymphs moult into sexually mature adults that remain on the host to feed and mate. All stages of the lifecycle of the tick are relevant in the transmission of disease. Host animals can be both small terrestrial mammals and larger mammals and birds.

Ticks can carry a wide range of dangerous pathogens such as bacteria, spirochetes, rickettsiae, protozoa and viruses. The number of reported cases of tick-borne diseases in Europe and the US has increased significantly in recent decades.

Lyme disease (LB) and Tick-borne encephalitis are the most common tick-borne disease in the northern hemisphere. These diseases are debilitating and if not treated in time, they may have long-term health effects or become life-threatening. To start the immediate treatment and prevent serious health issues, rapid diagnosis of viral TBE and bacterial LB is absolutely essential. Serological tests detecting antibodies against Borrelia or TBE virus can help diagnose and determine the presence and stage of the disease.

Lyme borreliosis

Lyme borreliosis is a multisystem infectious disease caused by the spirochaete *Borrelia Burgdorferi* sensu lato. The infection is transmitted by ticks of the genus Ixodes.

There are three stages of Lyme borreliosis. Early localized, early disseminated and late disseminated infections.

The results of many studies show that all genospecies are involved not only in the development of EM, but also in the full range of clinical manifestations. However, *B. burgdorferi* sensu stricto is mainly related to joint disorders, *B. garinii* is associated with neurological symptoms and *B. afzelii* with chronic skin manifestations, especially acrodermatitis chronica atrophicans (ACA).

Disease stages

Early localized stage

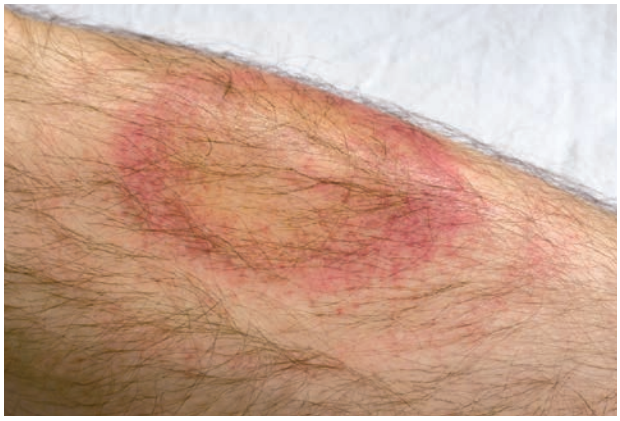
Lasts for days or weeks. It is characterized by erythema migrans (EM), which appears in approximately 50% of patients. Early symptoms of the disease may include “flu-like” symptoms, headache and lymphadenitis.

Early disseminated stage

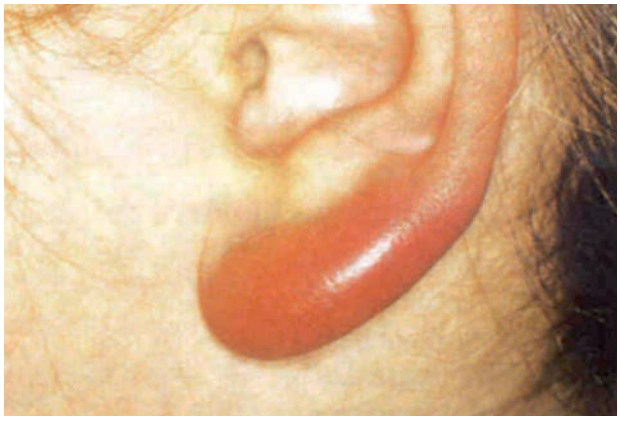
Lasts for weeks or months. Borrelia are disseminated by blood vessels and the lymphatic system (CNS, joints, heart, eye, skin – secondary EM). At this stage, the most frequently diagnosed symptoms are: neuroborreliosis, paresis neurofacialis, borrelial lymphocytoma (swollen earlobes, knucklebones, etc.) and Bannwarth syndrome.

Late disseminated stage

Lasts for months or years. The most typically diagnosed immunopathological changes include ACA, chronic neuroborreliosis, and borrelial arthritis



Erythema migrans



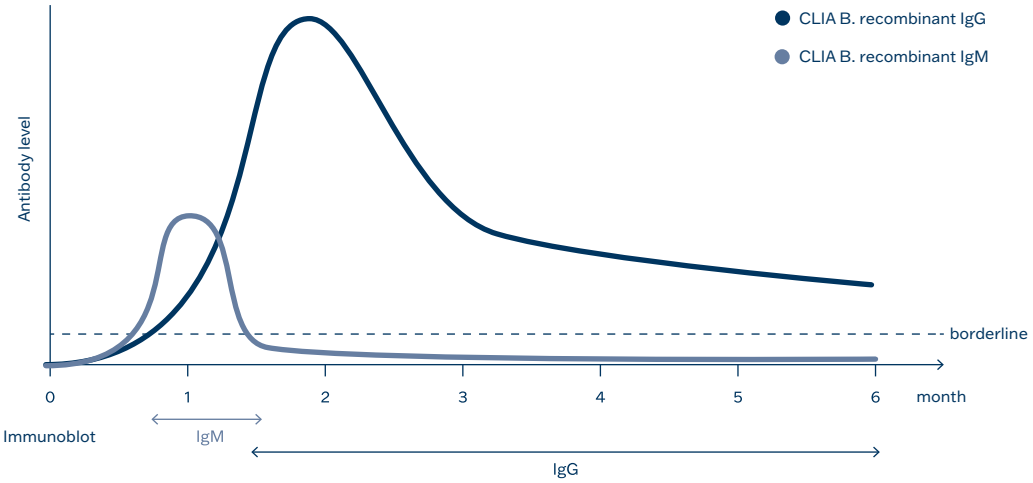
Borrelial lymphocytoma

Disease diagnosis

The diagnosis of the disease is based on patient history, clinical picture, and the results of laboratory tests. At present, the diagnostic methods of choice are screening of specific IgG and IgM class antibodies by means of CLIA and subsequent confirmation of the antibodies to specific antigens by means of immunoblot. Direct culture and electron microscopy are not methods suitable for routine testing.

Serological diagnosis of borreliosis is difficult due to the great genetic diversity of *Borrelia burgdorferi* s.l. species, possible cross-reactivity with unrelated antigens of other microorganisms and the significant heat shock response by *Borrelia*, producing a number of heat shock proteins (Hsp). Large differences in the serological reactivities of different individuals also complicate diagnosis. Early stage antibody production can be extremely slow, however, both IgG and IgM antibodies can persist for ten or more years.

Antibody response



Two-stage confimation of serology findings

IgG and IgM antibodies are determined in two steps. First, the CLIA method divides the samples according to positive or negative results; positive and borderline results are recommended to be confirmed by immunoblotting. If the test result is negative and the symptoms of the infection persist, a follow-up (control) sample is collected and measured in 2–3 weeks. The serological finding should be interpreted in the context of the results of other laboratory tests and the patient‘s clinical picture.

CLIA Borrelia recombinant and CLIA Borrelia CSF kits are highly specific thanks to the use of a unique combination of recombinant antigens, leading to a high correlation with immunoblot results.

1st level: Entry test for IgM and IgG antibody class using CLIA method

Positive or borderline test result

Negative test result

2nd level: Confirmation using immunoblot
in IgM and IgG antibody classes

Positive test result

Borderline test result

Negative test result

– Antibodies are not detected in the tested sample.

– If the symptoms persist, repeat sample collection 2–3 weeks later.

The clinical diagnosis of the disease status is based on a **comprehensive clinical picture** of the patient, not only on the serological result of the tested sample.

Two-level antibody detection (adapted from MiQ 12 2000 Lyme borreliosis, B. Wilske et al.)

Sensitivity for Various Stages of Lyme Borreliosis

Lyme Borreliosis Form	Diagnosis	Sensitivity by MiQ
Localized early	Erythema migrans	20–50%
Disseminated early	Borrelial lymphocytoma Erythema migrans multiple Neuroborreliosis Lyme arthritis and carditis	70–90%
Disseminated late	Acrodermatitis chronica atrophicans Late neuroborreliosis	90–100%

Routine evaluation model for borrelia serology

IgM		IgG		Evaluation
CLIA	BLOT	CLIA	BLOT	
–	–	–	–	No antibodies present.
+	+	–	–	Early stage of the disease.
+	+	+	+	High probability of acute infection.
–	–	+	+	Usually late stage of the disease.
+	–	–	–	Probably an unspecific CLIA reaction, the test result should be considered negative. If the symptoms last, it is recommended to perform a new test in 2–3 weeks.
–	–	+	–	
+	–	+	–	
+	+	+	–	Early stage of the disease, with more frequent positivity in Immunoblot or CLIA.
+	+	–	+	
+	–	+	+	Persisting or residual antibodies detected by CLIA or Immunoblot in IgM. The sample is already positive for IgG, meaning later stage of the infection.
–	+	+	+	
–	–	–	+	Disappearing residual antibodies after the treatment.
–	+	+	–	Extraordinary situation, the transition between IgM and IgG seropositivity.
–	+	–	–	Early stage of the disease, heat-shock protein activation or long-lasting post-treatment IgM antibodies.

Clinical application

- *Borrelia* spp antibody screening
- Lyme borreliosis detection
- Disease stage diagnosis
- Detection of intrathecal synthesis of specific antibodies

Antigens

CLIA Borrelia recombinant IgG, CLIA Borrelia CSF IgG

A combination of recombinant antigens VlsE (*B. afzelii, B. garinii, B. burgdorferi* sensu stricto), p83, p58, internal flagellin p41 (*B. afzelii*), OspA (*B. afzelii*), OspB, OspC (*B. afzelii*), p17, and NapA species *Borrelia burgdorferi* sensu lato

CLIA Borrelia recombinant IgM, CLIA Borrelia CSF IgM

Combination of recombinant antigens OspC (*B. afzelii*, *B. garinii, B. burgdorferi* sensu stricto, *B. spielmanii*), VlsE (*B. garinii*), internal flagelin p41 (*B. afzelii*), p39 of *Borrelia burgdorferi* sensu lato species

Specific Borrelia antigens

Antigens	Description
VlsE Ba VlsE Bg VlsE Bs	Variable major protein-like sequence, expressed | Species specific antigen | Main antigen of early and late antibody response to LB | Significantly increases test sensitivity (approx. 90% of samples of positive sera and CSF react in this antigen band)
p83	Main extracellular protein (product of p100 degradation) | Late antibody response antigen |Highly immunoreactive antigen, typical of neuroborreliosis
p58	OppA-2 (Oligopeptide permease 2) - membrane transporter | Considered as a marker of disseminated stage of Lyme disease
p41 Ba	Inner part of flagellin | Highly specific antigen of early antibody response
p39	BmpA (glycosaminopeptide receptor) | Antigen of late antibody response | Significant antigen for advanced disseminated form of LB, often associated with Lyme arthritis
OspB	Outer surface protein B | Antigen of late antibody response
OspA Ba	Outer surface protein A | Antigen of late antibody response, typical for neuroborreliosis
OspC Ba OspC Bg OspC Bs OspC Bsp	Outer surface protein C | Antigen of early antibody response | Immunodominant marker of IgM antibody response
NapA	Neutrophil activating protein A | Strong immunogen, main marker of Lyme arthritis pathogenesis
p17	DbpA (Decorin-Binding protein A) | Antigen of early and late antibody response, typical of neuroborreliosis

Ba – B. afzelii, Bg – B. garinii, Bs – B. burgdorferi sensu stricto, Bsp – B. spielmanii

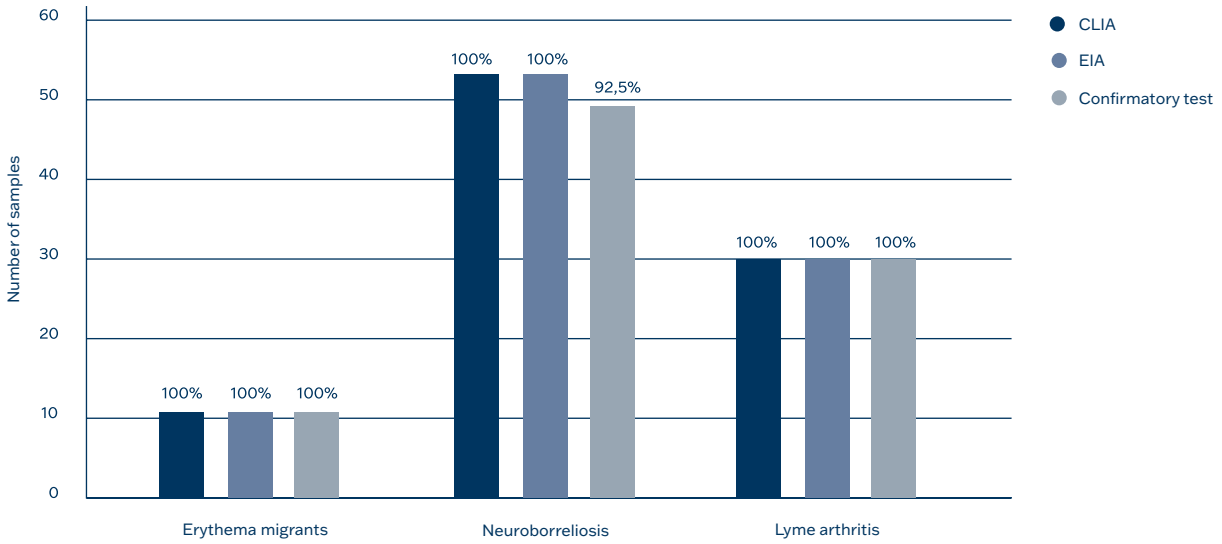
Test characteristics

Kit	Calibration range	Diagnostic sensitivity	Diagnostic specificity
CLIA Borrelia recombinant IgG	5–700 U/ml	98.99%	98.92%
CLIA Borrelia recombinant IgM	5–100 U/ml	98.59%	98.95%
CLIA Borrelia CSF IgG – serum, plasma	0–320 AU/ml	95.06%	99.15%
CLIA Borrelia CSF IgG – cerebrospinal fluid	0–320 AU/ml	96.88%	99.24%
CLIA Borrelia CSF IgM – serum, plasma	0–250 AU/ml	94.12%	99.88%
CLIA Borrelia CSF IgM – cerebrospinal fluid	0–250 AU/ml	97.78%	99.99%

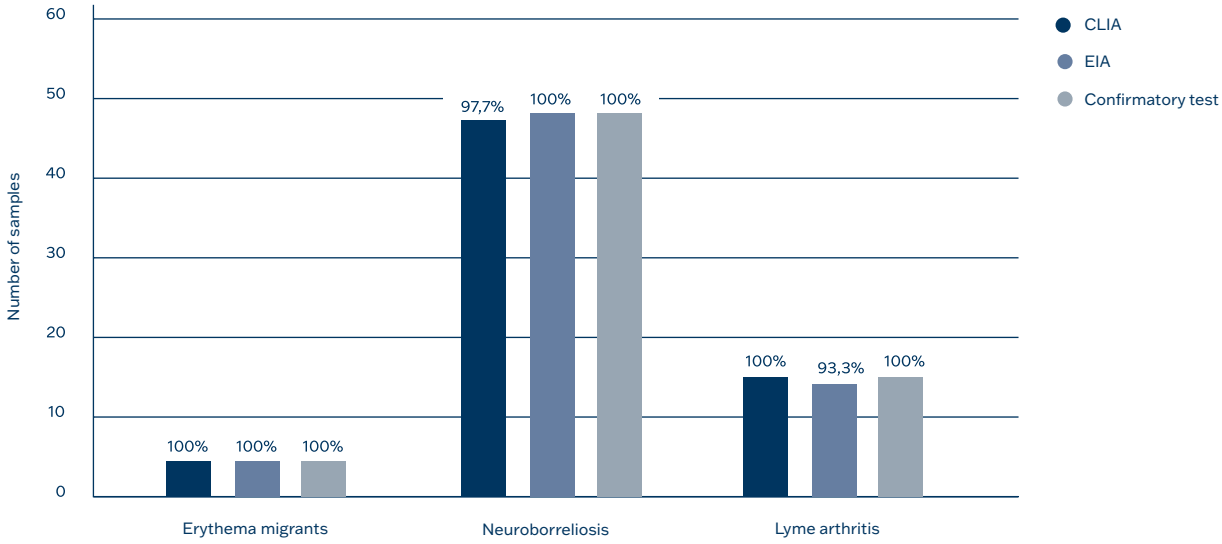
Correlation of methods

The reactivity of clinical specimens with the diagnosis of Lyme disease and typical clinical manifestations was compared by determining specific IgG and IgM antibodies using CLIA Borrelia recombinant IgG, resp. IgM. Established enzymatic immunoassays and confirmatory immunoblots were used for comparison.

IgG



IgM



Neuroborreliosis and intrathecal synthesis of specific antibodies

CLIA Borrelia CSF are designed for specific antibodies detection in serum, plasma and cerebrospinal fluid.

For rapid, routine determination of intrathecal antibody synthesis, use a combination of CLIA Borrelia CSF results and Antibody Index Software.

Antibody Index Software enables the evaluation of the antibody index (AI), i.e. the ratio of specific antibodies in the cerebrospinal fluid and serum in relation to the state of the blood cerebrospinal fluid barrier and the concentration of total immunoglobulins in CSF and serum.

According to the international recommendation of the European Union Concerted Action on Lyme Borreliosis (EUCALB), evidence of intrathecal antibody production is necessary for diagnosis of early and late neuroborreliosis (i.e. specific antibodies to Borrelia sp. produced in the cerebrospinal fluid (CSF) must be detected).

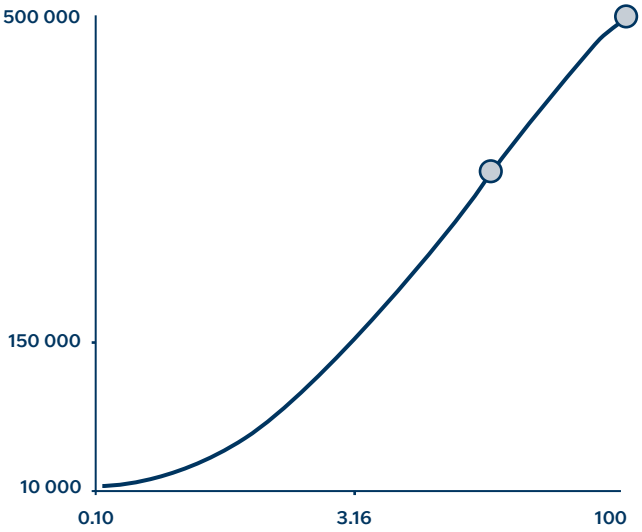
The antibody level in the CSF depends on the following parameters:

- Antibodies present in blood serum
- Permeability of blood-CSF barrier
- Intrathecal production of antibodies

The presence of specific antibodies as such (in the serum and/or CSF) cannot be deemed sufficient evidence.

Advantages

- Small amount of CSF sample needed to determine AI (32-92 µl)
- Possibility of Antibody Index determination within routine test
- Quick and easy evaluation with Antibody Index Software



The calibration curve is part of the SW and is generated automatically from the calibrators provided in the CLIA Borrelia CSF IgG, IgM kits.

Testing of antibodies in cerebrospinal fluid and detection of intrathecal synthesis

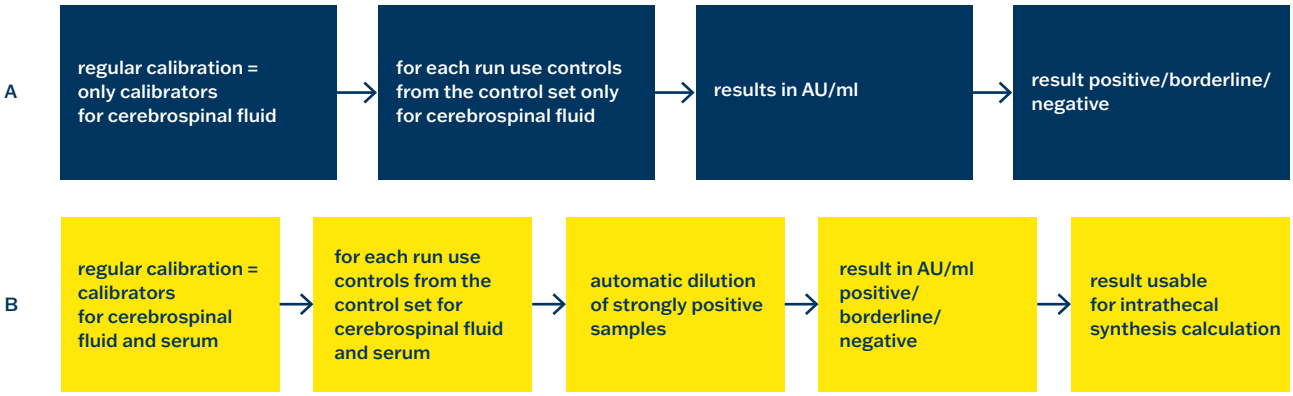
Difference between CLIA Borrelia recombinant and CLIA Borrelia CSF kits

	Generation of immunoassays	Determination in serum, plasma	Determination in cerebrospinal fluid	No. of tests in kits	Units	Suitable for determination of intrathecal synthesis	Control set
CLIA Borrelia recombinant	3rd generation (recombinant antigens used)	YES	NO	100	U/ml	NO	for serum
CLIA Borrelia CSF	3rd generation (recombinant antigens used)	YES	YES	50	AU/ml *	YES	for serum and cerebrospinal fluid

*AU/ml units serves for more precise intrathecal synthesis calculation, therefore they are not equal to U/ml.

Difference in workflow between cerebrospinal fluid examination and intrathecal synthesis determination

A	Determination of antibodies in cerebrospinal fluid	independent without detection in serum or paired sample determination without intrathecal synthesis calculation	there is no need to use serum calibrators for separate cerebrospinal fluid determination	there is no need to use serum controls from the control set for the separate determination in likvor	
B	Determination of intrathecal synthesis	paired sample determination of serum and cerebrospinal fluid	calibrators for both serum and cerebrospinal fluid must be used for analysis	for intrathecal synthesis determination, controls from the control set for both serum and cerebrospinal fluid must be used	a strongly positive sample is automatically further diluted for more precise intrathecal synthesis calculation



Serology of CSF and serum related to intrathecal antibody synthesis and Antibody Index determination

Serum	CSF	Intrathecal antibody synthesis	AI determination according to Reiber
–	+	Positive	YES – positivity confirmed (EUCALB recommendation)
+	+	Usually positive, but a passive transfer of antibodies via a disturbed blood-CSF barrier is possible	YES – necessary for detection of intrathecal synthesis
+	–	Possibly positive (provided that the measured absorbance values in the CSF and serum are close to absorbance ofthe CUT-OFF control)	YES – necessary for detection of intrathecal synthesis
–	–		

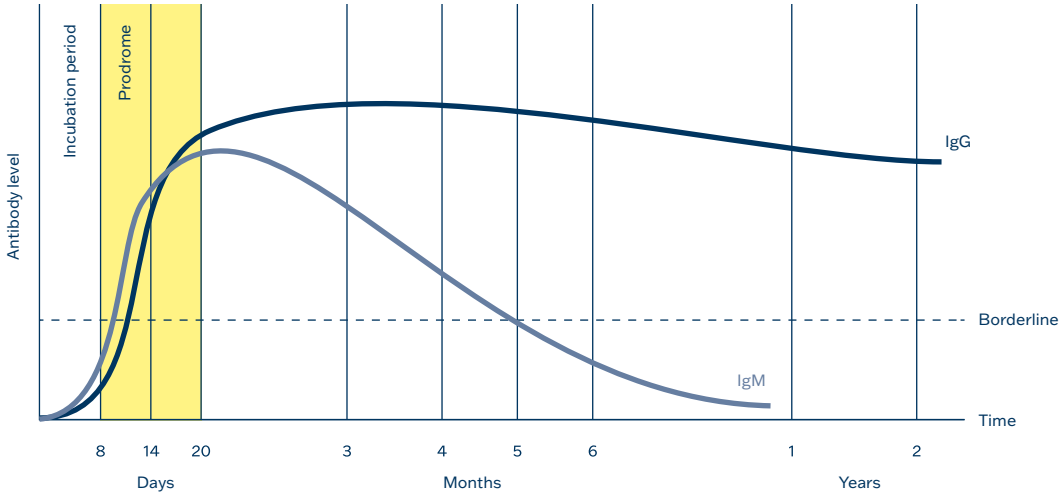
Tick-borne encephalitis

Tick-borne encephalitis is an infectious viral disease caused by arboviruses in the Flaviviridae family. It is a natural focal infection. The reservoir of the virus is small and large forest animals (e.g. small rodents). The vector of transmission is the various developmental stages of ticks. A human is most often infected through a tick bite, exceptionally by ingesting unheated infected milk. TMost cases of TBE occur during periods of peak tick activity (summer to autumn). Up to 70% of TBE infections are clinically inapparent. The manifestation of the disease is often biphasic. After the incubation period (3–14 days), non-specific flu-like symptoms (fever, headache and muscle aches, torpidity) begin. This is followed by several days of remission and then the development of the second (neural) phase of the disease (aggravated headaches, ophthalmoplegia, vomiting, malaise, meningeal symptoms, cranial nerve paralysis, and limb paresis). The acute phase of tick-borne encephalitis lasts 1–3 weeks. A more severe course, often with lasting consequences, can be observed in elderly patients.

Disease diagnosis

The diagnosis of tick-borne encephalitis is based on the patient history, clinical picture and results of laboratory tests. Laboratory methods include biochemical and cytological examination of cerebrospinal fluid (CSF) serological detection of specific IgM and IgG antibodies in the serum, plasma, and CSF. IgM antibodies are a serological marker of acute infection and the production can last up to 10 months. IgG antibodies protect the body against a new infection and can be detected over a long period (several years) after an infection or vaccination. Borderline results should be verified by a virus neutralisation test (VNT).

Antibody response



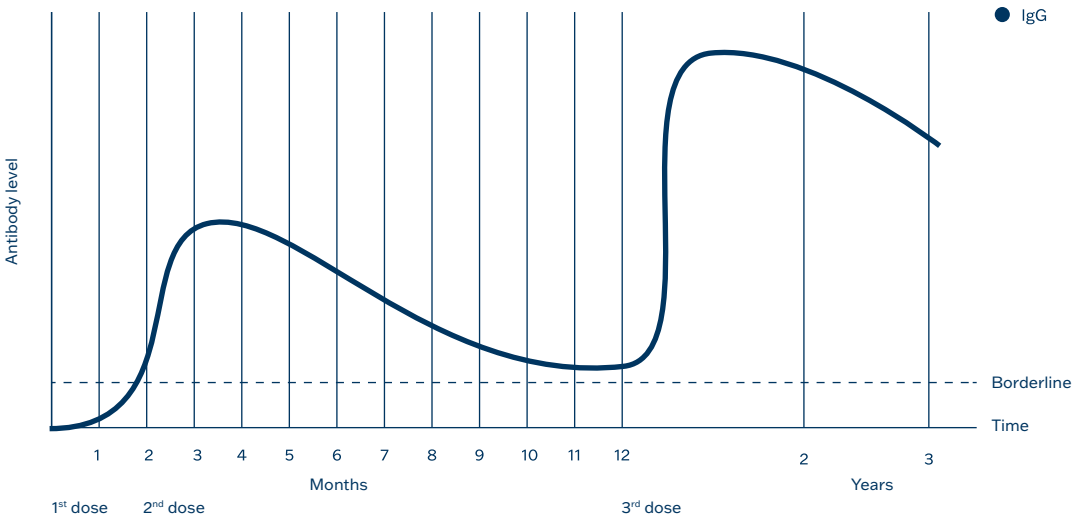
IgM antibodies can be detected at the beginning of the neural phase of the disease. The highest levels are obtained after 2–6 weeks from the onset of symptoms. They can last up to 10 months. The production of IgG antibodies usually takes more time, however they may sometimes be detected as soon as IgM.

Interpretation of serology results

IgM	IgG	Interpretation	Note
–	–	– negative anti-TBEV antibodies	– if acute infection is suspected, the test should be repeated after 2 weeks
–	+	– past infection – protective level of antibodies after the vaccination	– if acute infection is suspected, the test should be repeated after 2 weeks
+	–	– early acute phase of the infection	acute infection – IgG seroconversion will follow
+	+	– acute infection – recent vaccination	IgM antibodies can last up to 10 months after the infection

The serological finding can only be interpreted in the context of the results of other laboratory tests and the patient's clinical picture.

Post-vaccination antibody response



Interpretation of results – after vaccination

Result	Interpretation	Note
IgG – U < 18 U/ml	negative anti-TBEV antibodies	**not sufficient baseline immunity after vaccination** – it is recommended to carry on with the vaccination scheme (if there is no seroconversion within the first 4 weeks after the vaccination, a booster dose should be considered)
IgG +/- U = 18–22 U/ml	borderline anti-TBEV antibodies	**successful imunization** – the result should be verified by VNT or a booster dose should be administrated, the antibody level must be checked after 2–4 weeks
IgG + U > 22 U/ml	positive anti-TBEV antibodies	**seroconversion** – proceed in accordance to the vaccination scheme

Clinical application

– Disease diagnosis
– CLIA TBE Virus IgG: Evaluation of vaccination effectivness
– CLIA TBE Virus IgM: Identification of acute infection

Antigens

CLIA TBE Virus IgG	CLIA TBE Virus IgM
Mixture of purified and inactivated native tick-borne encephalitis virus antigen and recombinant NS1 antigen	Mixture of recombinant tick-borne encephalitis virus antigens: Envelope and NS1 protein

Test characteristics

Kit	Calibration range	Diagnostic sensitivity	Diagnostic specificity
CLIA TBE Virus IgG	3–600 U/ml	96.00%	99.00%
CLIA TBE Virus IgM	3–380 U/ml	98.00%	97.87%

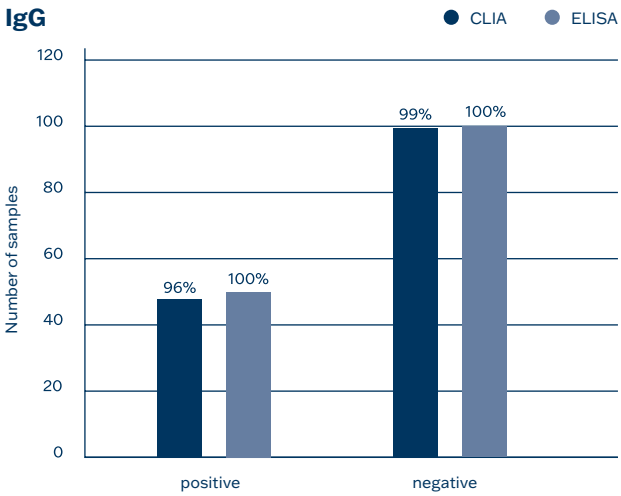
Correlation of methods

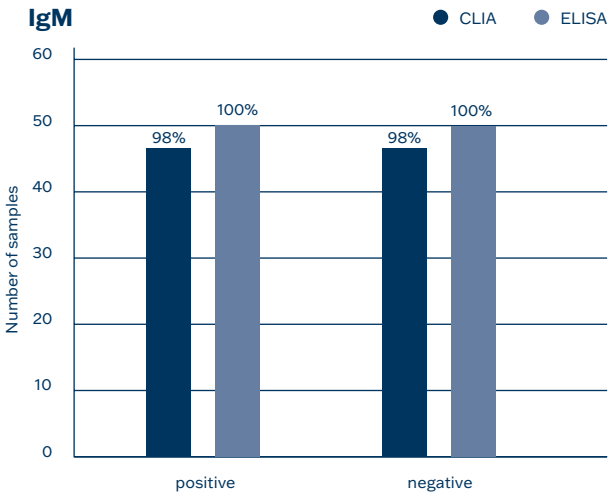
Correlation with VNT

Diagnostic kits CLIA TBE Virus IgG and EIA TBE Virus IgG were compared with the gold standard VNT method. A significant correlation was found for both products.

Method		CLIA TBEV IgG		EIA TBEV IgG	
		pos	neg	pos	neg
VNT	pos	18	0	18	0
	neg	1	1	1	1
Agreement		95.0%		95.0%	

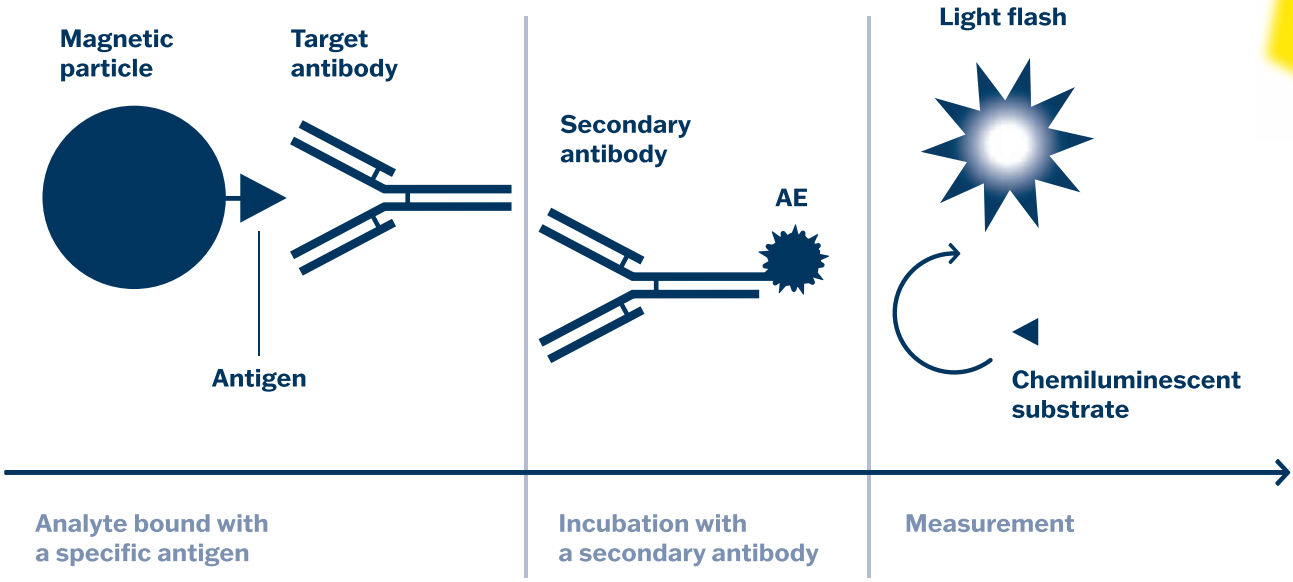
Correlation with ELISA





How does CLIA method work?

CLIA is a fully automated, fast, specific and sensitive method. It combines magnetic particle-mediated antigen / antibody immunocomplex separation and flash chemiluminescence to achieve sensitive detection. The use of magnetic particle suspension facilitates automation, significantly shortens reaction times and improves the specificity of the determination. Flash chemiluminescence of acridinium ester provides an intense light signal even at very low concentrations and its intensity is measured in relative units of light (RLU). CLIA kits are designed for use on the KleeYa® automated platform.



CLIA kity

Diagnostic CLIA kits are used for the detection of IgG and IgM antibodies against *Borrelia burgdorferi* s.l. or TBE virus in human serum or plasma on the KleeYa® analyzer. Results are reported in U/ml. CLIA Borrelia CSF kits allow detection of antibodies in cerebrospinal fluid and determination of intrathecal synthesis. The results are reported in AU/mL.

Control sets

Control sera are intended to verify the accuracy of results obtained using CLIA kits. CLIA Borrelia CSF serves also for examination of antibodies in cerebrospinal fluid and determination of intrathecal synthesis.

Ease of use

- Fully automated method
- Kits include all necessary reagents, incl. calibrators
- Ready-to-use reagents in the reaction cartridges
- Control sera available as independant sets
- Quantitative determination (U/ml)

Advantages

- High diagnostic sensitivity and specificity
- Low sample (10 µl) and reagent consumption
- Short test time (30 min)
- Wide measuring range
- Full traceability of reagent consumption and number of tests available using RFID tags
- LIS connectivity available
- Superior customer service

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Ordering information

CLIA kits

Diagnostic CLIA kits are used for the detection of IgG and IgM antibodies against *Borrelia burgdorferi* s.l. or TBEV in patient serum or plasma on the KleeYa® analyzer, CLIA Borrelia CSF also in cerebrospinal fluid.

Kit	Catalogue number	Number of tests	
CLIA Borrelia recombinant IgG	CL-BRG100	100	
CLIA Borrelia recombinant IgM	CL-BRM100	100	
CLIA Borrelia CSF IgG	CL-BCSFG50	50	IVD CE 2265
CLIA Borrelia CSF IgM	CL-BCSFM50	50	IVD CE 2265
CLIA TBE Virus IgG	CL-TBG100	100	
CLIA TBE Virus IgM	CL-TBM050	50	

Control sets

Each kit contains positive and negative controls with a declared range of the respective antibodies. They are intended to verify the accuracy of results obtained using CLIA kits. CLIA Borrelia CSF serves also for examination of antibodies in cerebrospinal fluid and determination of intrathecal synthesis.

Kit	Catalogue number	Number of tests
Control set CLIA Borrelia recombinant IgG	CL-BRGCON	2 x 20
Control set CLIA Borrelia recombinant IgM	CL-BRMCON	2 x 20
Control set CLIA Borrelia CSF IgG	CL-BCSFGCON	4 x 10
Control set CLIA Borrelia CSF IgM	CL-BCSFMCON	4 x 10
Control set CLIA TBE Virus IgG	CL-TBGCON	2 x 20
Control set CLIA TBE Virus IgM	CL-TBMCON	2 x 20

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