BioVendor Group CLIA

Tick-Borne Infections

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

Diagnostic panel

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 ACA.

Disease stages

Early localized stage

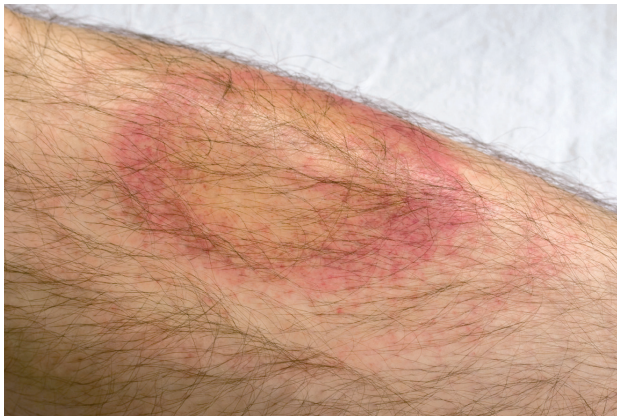
Lasts for days or weeks. It is characterized by erythema migrans (EM), which appears in only 50% of patients. Early symptoms of the disease may include “flu-like” symptoms, headache and lymphadenitis.

Early disseminated stage

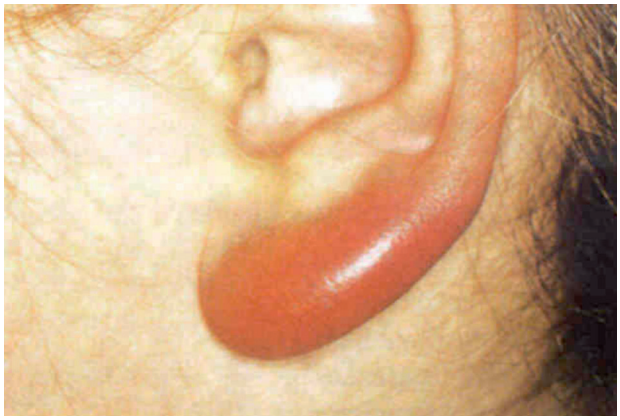
Lasts for weeks or months. Borrelia are disseminated by blood vessels and the lymphatic system (CNS, joints, heart, eye, skin – secondary EM). At this stage, the most frequently diagnosed symptoms are: neuroborreliosis, paresis neurofacialis, borrelial lymphocytoma (swollen earlobes, knucklebones, etc.)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

Late disseminated stage

Lasts for months or years. The most typically diagnosed immunopathological changes include acrodermatitis chronica atrophicans (chronic skin lesions – ACA), chronic neuroborreliosis, and borrelial arthritis



Erythema migrans



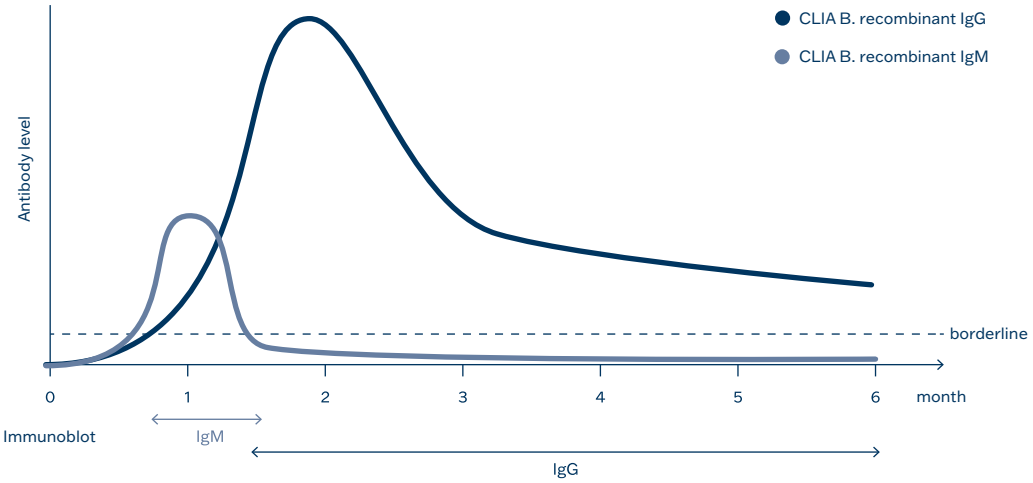
Borrelial lymphocytoma

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 ELISA or 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 kits are highly specific thanks to the use of a unique combination of recombinant antigens, leading to a high correlation with immunoblot results.

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

Antigens

CLIA Borrelia recombinant 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

The combination of recombinant antigens OspC (*B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. spielmanii*), VlsE (*B. garinii*), internal flagellin p41 (*B. afzelii*) and p39

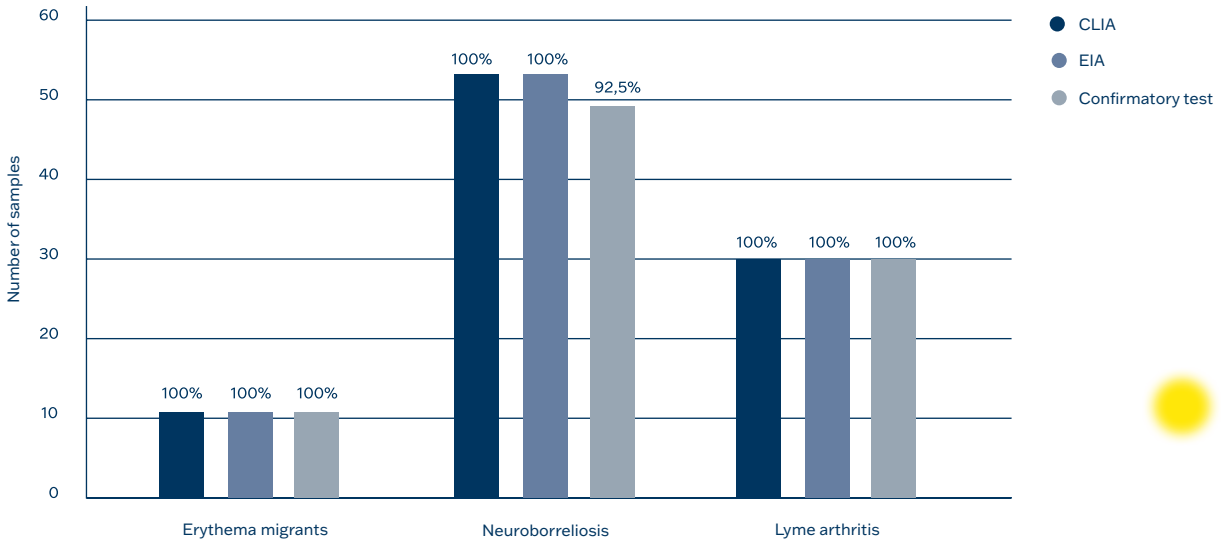
Test characteristics

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 %

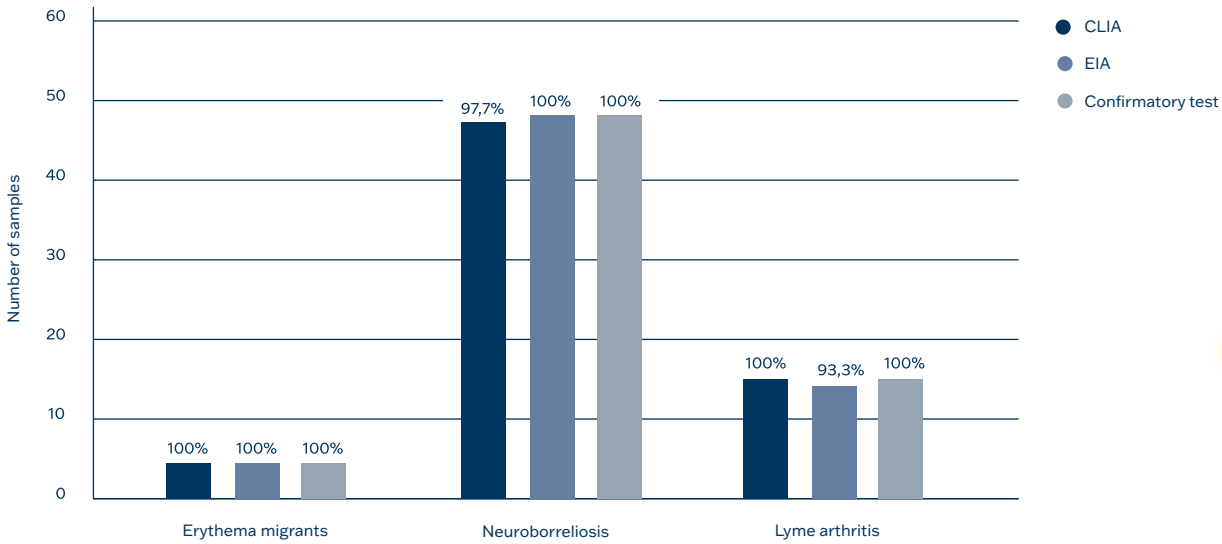
Correlation of methods

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



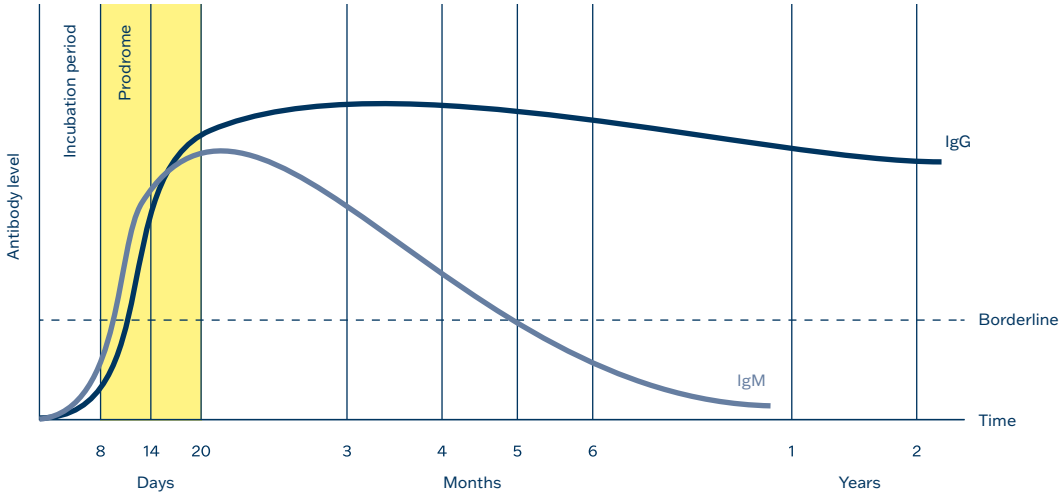
Tick-borne encephalitis

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



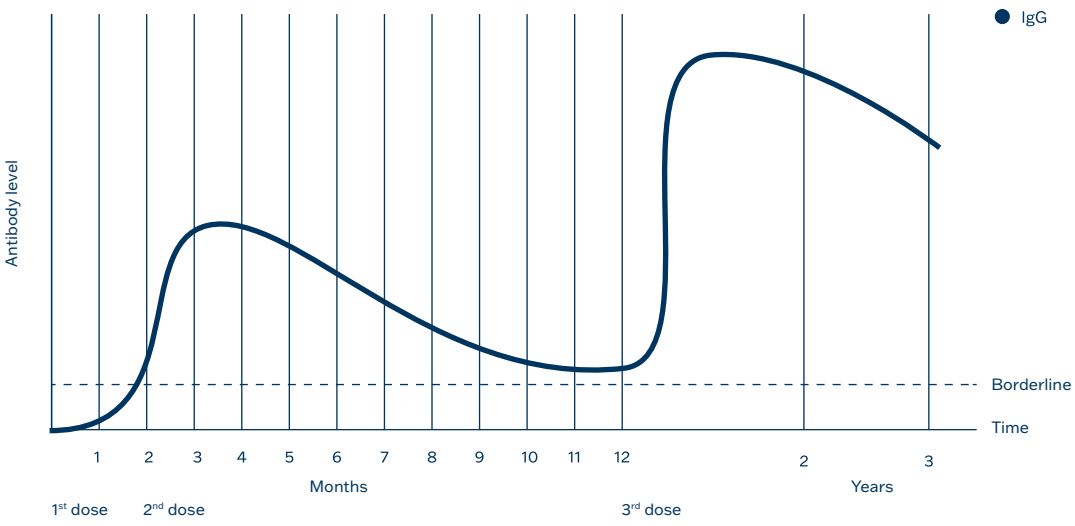
IgM antibodies can be detected at the beginning of the neural phase of the disease. The highest levels are obtained after 2–6 weeks from the onset of symptoms. They can last up to 10 months. The production of IgG antibodies usually takes more time, however they may sometimes be detected as soon as IgM.

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, IgM

A mixture of purified, inactivated, native TBE virus antigen and recombinant NS1 antigen

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 %

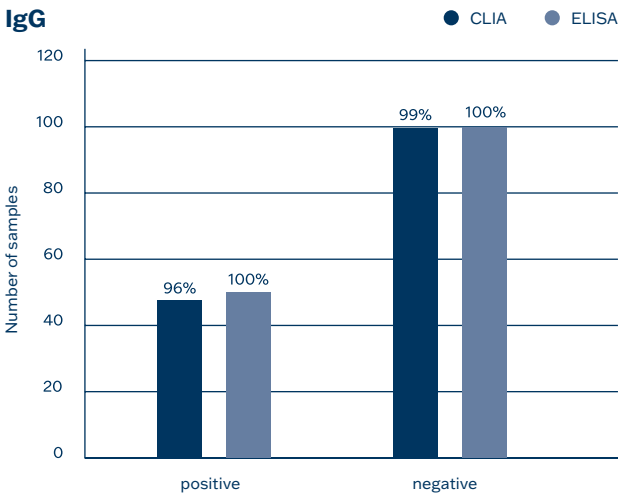
Correlation of methods

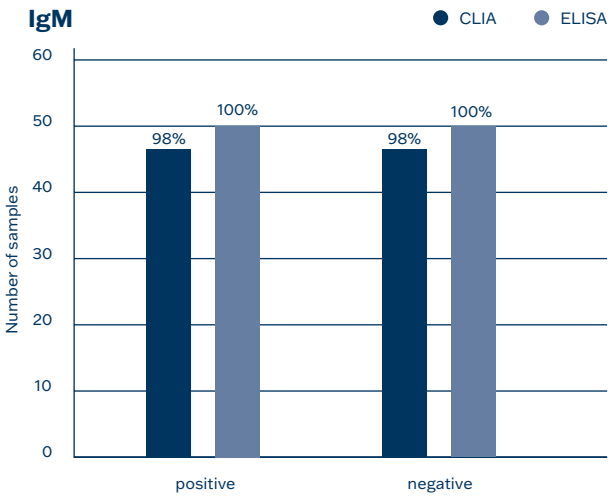
Correlation with VNT

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%	

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.

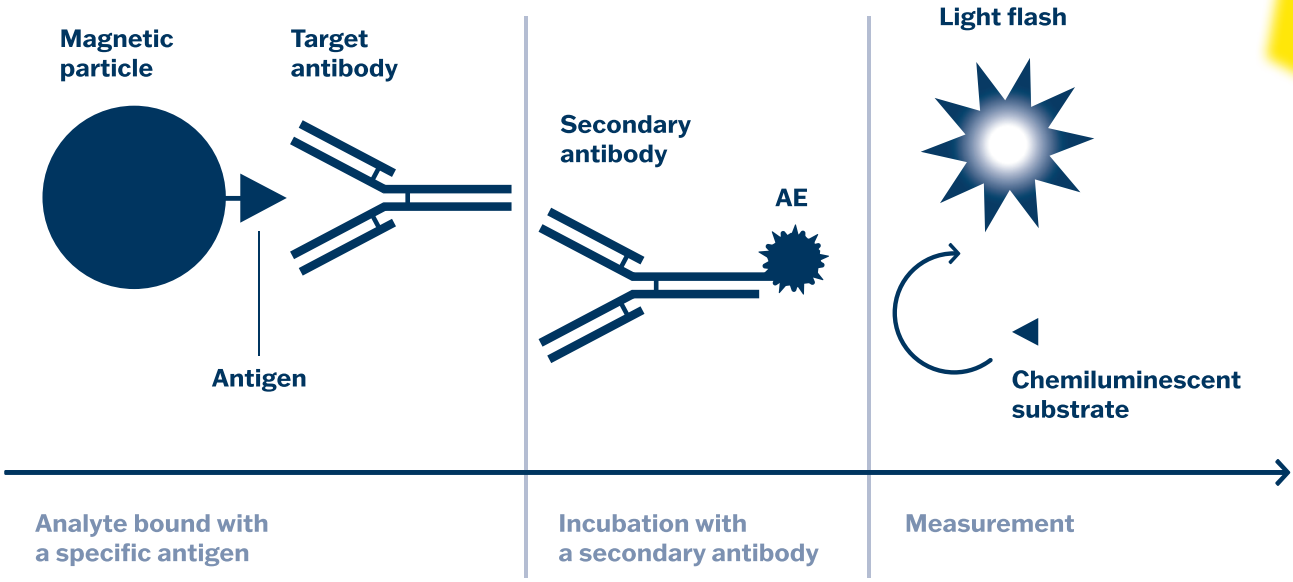
Correlation with ELISA





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 kits

Diagnostic CLIA kits are used to determine IgG and IgM antibodies against *Borrelia burgdorferi* s.l. or TBE virus in human serum or plasma on a KleeYa® analyzer. The results are reported in U/ml.

Control set CLIA

Control sera verify the the accuracy of results obtained by the CLIA kits.

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

Ordering information

CLIA kits

CLIA diagnostic kits are used to determine IgG and IgM antibodies against Borrelia burgdorferi s.l. or TBEV in patient serum or plasma on a KleeYa® analyzer.

Kit	Catalogue number	Number of tests
CLIA Borrelia recombinant IgG	CL-BRG100	100
CLIA Borrelia recombinant IgM	CL-BRM100	100
CLIA TBE Virus IgG	CL-TBG100	100
CLIA TBE Virus IgM	CL-TBM50	50

Control sets

Each set contains two vials of positive and two vials of negative control serum with the predetermined level of specific antibodies. They are designed to verify the accuracy of results obtained with CLIA kits.

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 TBE Virus IgG	CL-TBGCON	2 x 20
Control set CLIA TBE Virus IgM	CL-TBMCON	2 x 20

Contact us at

clia@biovendor.group

or visit our website

clia.biovendor.group

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