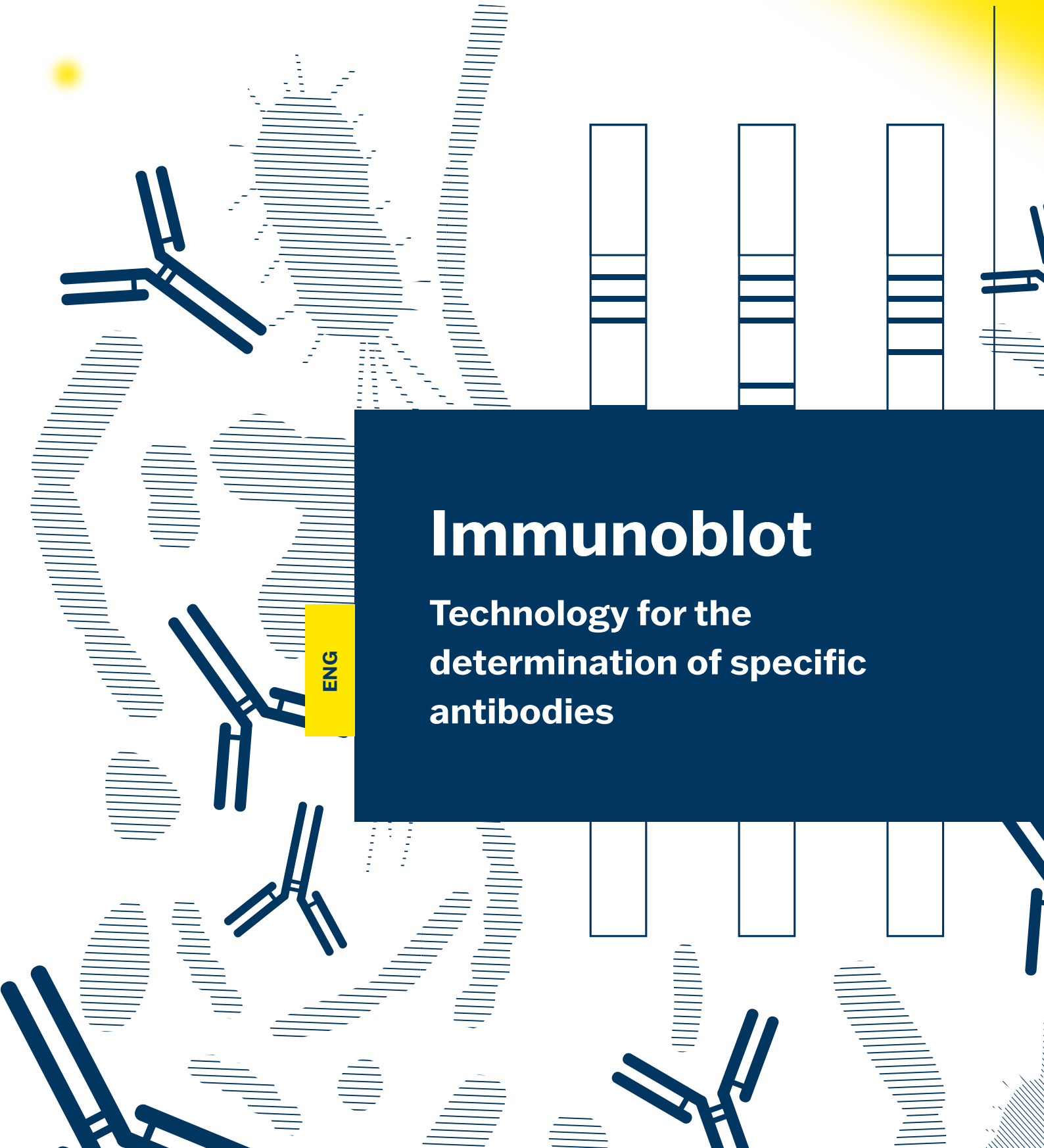
BioVendor Group®



Immunoblot

Technology for the determination of specific antibodies

ENG

Reliable and robust immunoblot kits

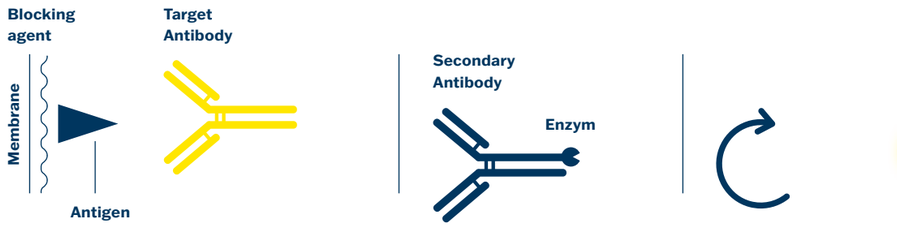
Immunoblot kits enable the simultaneous detection of multiple markers in serum, plasma, cerebrospinal, and synovial fluid. They are an important tool for the differential diagnosis of diseases with similar symptoms. They are used for the confirmation of borderline or positive ELISA.

Processing can be done automatically or manually. More precise evaluation can be achieved with easy-to-use software which can be downloaded for free on our website.

An innovative antigen composition and in-house production ensure the tests are of the upmost quality with a high sensitivity and specificity.

BLOT-LINE Test principle

A method using **highly specific recombinant antigens** that are transferred on nitrocellulose membrane.



Specific primary antibody binding to protein

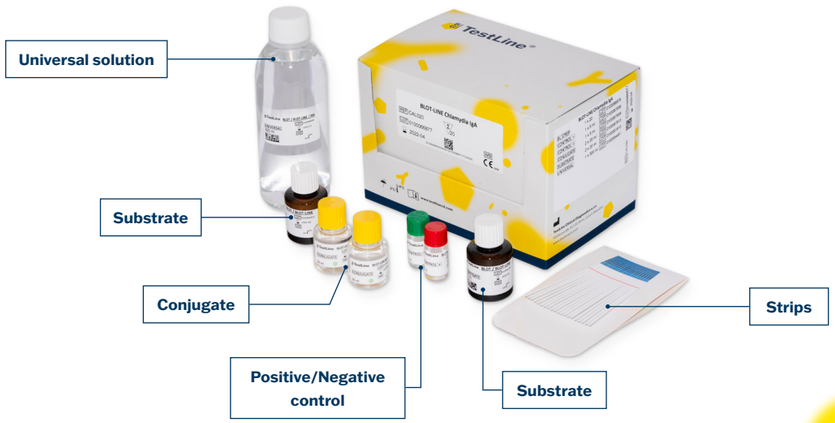
Enzyme–conjugated secondary antibody binding to primary antibody

Reaction of substrate and enzyme resulting in coloured insoluble product

(Western) BLOT Test principle

A method using electrophoretically separated **whole-cell antigens** that are transferred to a nitrocellulose membrane.

BLOT-LINE kit



Clinical application

- Confirmatory method for screening of serological methods (CLIA, ELISA, etc.)
- Differential diagnosis
- **Infectious serology:** Anaplasma phagocytophilum, Bordetella sp., Borrelia sp., CMV, Chlamydia sp., EBV, Helicobacter pylori, Mycoplasma pneumoniae, Toxocara sp., Toxoplasma gondii, Treponema pallidum, Yersinia sp.
- **Autoimmunity:** ANA, ANCA-3

User comfort

- Ready-to-use components
- Colour-coded strips
- Positive and Negative controls
- Control line on the strip
- Interchangeable components
- Easy assay procedure
- Possibility of software evaluation

Advantages

- Easy interpretation and reproducibility of results
- High diagnostic sensitivity and specificity of tests
- Compatibility with all commercial immunoblot processing systems
- Customer support
- Identical assay procedure for all of the tests

Summary protocol

Step		Test steps
	1.	Pipette Universal solution 2.5 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1.5 ml) – cerebrospinal fluid 1:2 (0.75 ml + 0.75 ml) – synovial fluid 1:17.5 (90 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.	Dry and evaluate strips

Workflow

1. Strip processing – 2 hours
2. Strip drying and sticking
3. Protocol scanning
4. Evaluation and data output

For software evaluation use office scanner

Evaluation

Software evaluation of the Immunoblot results

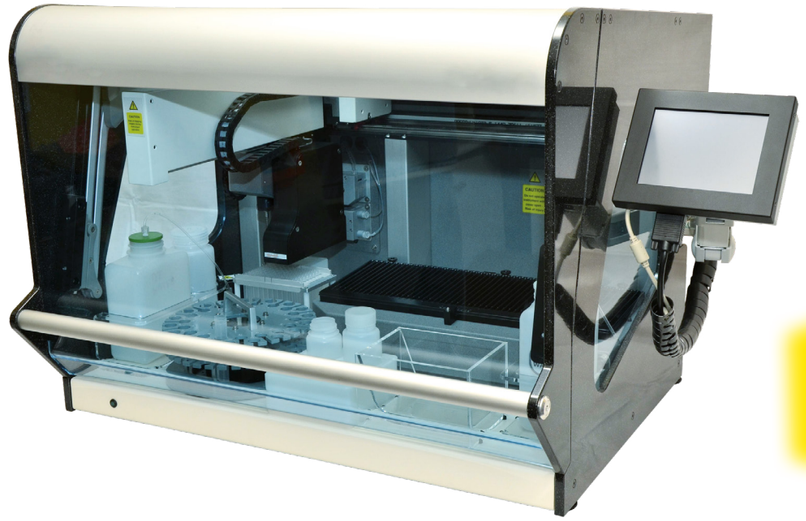
Software evaluation is an easy and fast way to get standardized results of laboratory tests. Result processing is user-friendly and straightforward, and the software solution significantly reduces workload and increases efficiency.

Automation

Possibility of automated processing using any open Immunoblot instrument

The complex solution of Immunoblot TestLine kits and RoboBlot

- Fully automated processing of the kits
- Minimization of manual handling and operating time
- Control of the entire process using Immunoblot software and touch screen analyzer
- Connectivity to LIS
- Full compatibility of RoboBlotTM, BLOT-LINE kits and Immunoblot Software (TestLine)



Safety

- Pipetting with disposable plastic tips
- Level detection using a pressure sensor
- Bar code reader for samples

Control and evaluation

- Touch screen device control, LAN communication with LIS
- Integrated camera for scanning strips at the end of the run (200-300 dpi)
- Connection with the evaluation program Immunoblot Software (TestLine)

Capacity

- High throughput – 50 samples
- Flexibility – 5 different methods simultaneously

Certification

CE marked; 98/79EC (IVDD); EN 61326-1:2006; EN 61326-2-6:2006; EMC Directive 2004/108/EEC

IMMUNOBLOT kits for the diagnostics of **Anaplasmosis**

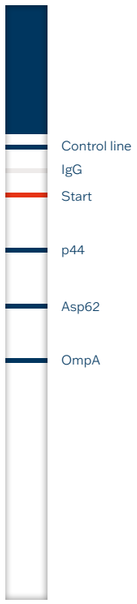
Introduction

Human granulocytic anaplasmosis (HGA) is a disease caused by Anaplasma phagocytophila bacteria. Clinically, the disease can manifest itself in various ways – from entirely asymptomatic to rather serious forms. Characteristic manifestations may include: fever, headache, muscle and joint aches, skin alterations (erythema migrans similar to Lyme borreliosis, mostly accompanied by maculopapular rash and hemorrhage), hepatosplenomegaly and lymphadenopathy. Antibodies are detectable approximately two weeks after the outbreak of the infection, which is why acute infections (30-60% positivity) are not always diagnosed correctly. During the convalescence time we usually find positivity in 70-90% of samples.

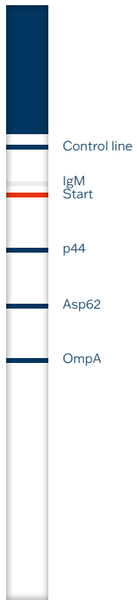
Test characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Anaplasma IgG	92.0%	94.0%
BLOT-LINE Anaplasma IgM	91.4%	99.0%

Antigens



BLOT-LINE Anaplasma IgG



BLOT-LINE Anaplasma IgM

p44 – main antigen of antibody response to HGA

Asp62 – surface protein; works as a membrane transporter

OmpA – surface protein of outer membrane; lipoprotein associated with peptidoglycans; significant marker of virulence

* In case of positive results within the Anaplasma screening it is recommended to perform further examination to confirm the HGA

IMMUNOBLOT kits for the diagnostics of ***pertussis*** and ***parapertussis***

Introduction

B. pertussis and ***B. parapertussis*** are very closely related species, producing similar virulence factors.

Bordetella pertussis is considered to be the main cause of whooping cough. Before the introduction of vaccination, the disease had been one of the most serious diseases of infants and children.

B. pertussis causes severe forms of the disease, while *B. parapertussis* causes milder forms. This is due to the fact that the bacteria Bordetella parapertussis do not produce a pertussis toxin. The milder infection of *B. parapertussis* can be the main cause of prolonged bronchitis.

Postvaccination and postinfection immunity acquired after the disease caused by *B. pertussis* do not protect from the disease caused by *B. parapertussis*!

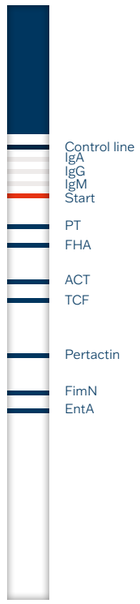
Clinical application

- Method for differentiation of postinfection and postvaccination antibodies (due to IgA antibodies presence)
- Method for proof of acute infection
- Method for differential diagnostics of *B. pertussis* and *B. parapertussis*

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
Bordetella pertussis IgA	95.5%	99.9%
Bordetella pertussis IgG	99.9%	99.9%
Bordetella parapertussis IgA	99.9%	99.9%
Bordetella parapertussis IgG	88.9%	99.9%

Antigens



BLOT-LINE Bordetella

B. pertussis

PT – Pertussis toxin (45 kDa) – basic virulence factor, specific only for *B. pertussis*; the most important pertussis antigen

FHA – *B. pertussis* filamentous hemagglutinin – adhesive protein, important immunogen; selected part of the sequence with high specificity

ACT – Adenylate cyclase toxin (CyaA)
– important virulence factor of *B. pertussis*; antiphagocytic factor during infection

TCF – Tracheal colonization factor
– protein produced only by *B. pertussis* strain, not by *B. parapertussis*; protein adhesin, that binds to ciliated epithelial cells of respiratory tract

B. parapertussis

Pertactin – Outer membrane protein (75 kDa) of virulent *B. parapertussis* strains

FimN – Fimbriae N – protein adhesin; it is not produced by *B. pertussis*

EntA – Entericidin A – membrane lipoprotein

IMMUNOBLOT kits for the diagnostics of **Lyme Borreliosis**

Introduction

Lyme borreliosis is a multisystem disease caused by the bacterial spirochete *Borrelia burgdorferi*.

The specific clinical sign of **early localised stage** is erythema migrans (EM), the characteristic lesion spreading from the site of a tick bite. **Early disseminated stage** may include nonspecific symptoms as for example lymphadenopatia, later arthritis, carditis and early neuroborreliosis, EM multiple, borrelial lymphocytoma and inflammatory changes of other organs.

Untreated borreliosis develops to the **late stage**. Specific presentation seen at this stage can be acrodermatitis chronica atrophicans that involves various organ manifestations. The symptoms can persist for years. In later stages of the disease antibiotic treatment becomes sometimes ineffective.

Human granulocytic anaplasmosis (HGA) is a disease caused by *Anaplasma phagocytophila* bacteria.

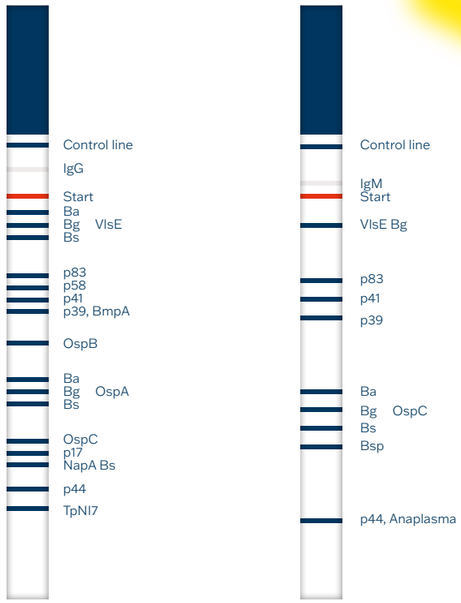
Clinically, the disease can manifest itself in various ways – from entirely asymptomatic to rather serious forms. Characteristic pain may include: fever, headache, muscle and joint aches, skin alterations (erythema migrans similar to Lyme borreliosis, mostly accompanied by maculopapular rash and hemorrhage), hepatosplenomegaly and lymphadenopathy.

Antibodies are detectable approximately two weeks after the outbreak of the infection, which is why acute infections (30–60% positivity) are not always diagnosed correctly. During the convalescence time we usually find positivity in 70–90% of samples.

Clinical application

- Immunodominant antigens from individual Borrelia species – *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto
- Recombinant antigen p44 from Anaplasma phagocytophila – useful for differential diagnosis of HGA
- Recombinant antigen TpN17 for exclusion of cross-reactivity with Treponema pallidum
- Possible to detect Borrelia antibodies in cerebrospinal fluid

Antigens



BLOT-LINE Borrelia/HGA IgG

BLOT-LINE Borrelia/HGA IgM

Test characteristics

	Borrelia		Anaplasma	
Immunoblot	**Diagnostic Sensitivity**	**Diagnostic Specificity**	**Diagnostic Sensitivity**	**Diagnostic Specificity**
BLOT-LINE Borrelia/HGA IgG	99.3%	99.4%	92.3	96.9%
BLOT-LINE Borrelia/HGA IgM	99.2%	99.3%	94.7%	97.1%

Antigens summary

Diagnostic importance of BLOT-LINE Borrelia/HGA IgG

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
p 58 Bg OppA-2 (Oligopeptide permease 2)	Membrane transporter, is considered a marker of disseminated stage of Lyme disease
p41	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 OspA Bg OspA Bs	Outer surface protein A | Antigen of late antibody response, typical of neuroborreliosis
OspC	Outer surface protein C | Antigen of early antibody response
p17	DbpA (Decorin-Binding protein A) | Antigen of early and late antibody response, typical of neuroborreliosis
NapA Bs	Neutrphil activating protein A - strong immunogen, main marker of Lyme arthritis pathogenesis
p44	Main antigen of antibody response to HGA
TpN17	Highly specific membrane protein of Treponema pallidum

Diagnostic importance of BLOT-LINE Borrelia/HGA IgM

VlsE Bg	Expressed part of variable major protein-like sequence, significant for IgG antibody response, species-specific antigen
p83	Main extracellular protein (product of p100 degradation)
p41	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
OspC Ba OspC Bg OspC Bs OspC Bsp	Outer surface protein C – main antigen of early antibody response, immunodominant marker of IgM antibody response; species-specific antigen (Ba – *B. afzelii*, Bg – *B. garinii*, Bs – *B. burgdorferi* sensu stricto, Bsp – *B. spielmanii*)
p44	Main antigen of antibody response to HGA

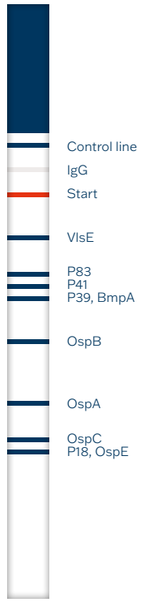
Monospecific IMMUNOBLOT kits for the diagnostics of **Lyme Borreliosis**

Advantages

- Immunodominant antigens from individual Borrelia species
- Possible to detect Borrelia antibodies in cerebrospinal fluid

Antigens

BLOT-LINE
Borrelia garinii IgG



BLOT-LINE
Borrelia b.
sensu stricto IgG

BLOT-LINE Borrelia afzelii IgG
BLOT-LINE Borrelia garinii IgG
– recombinant antigens:
VlsE, p83, p41, p39 (BmpA), OspA, OspC, p17 (DbpA)

BLOT-LINE Borrelia b. sensu stricto IgG
– recombinant antigens:
VlsE, p83, p41, p39 (BmpA), OspB, OspA, OspC, p18 (OspE)

BLOT-LINE Borrelia afzelii IgM
BLOT-LINE Borrelia garinii IgM
BLOT-LINE Borrelia b. sensu stricto IgM
– recombinant antigens: VlsE, p83, p41, p39 (BmpA), OspC

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Borrelia afzelii IgG	97.3%	99.9%
BLOT-LINE Borrelia afzelii IgM	96.6%	98.0%
BLOT-LINE Borrelia garinii IgG	97.1%	99.9%
BLOT-LINE Borrelia garinii IgM	97.6%	99.9%
BLOT-LINE Borrelia b. sensu stricto IgG	96.3%	99.9%
BLOT-LINE Borrelia b. sensu stricto IgM	97.4%	99.9%

IMMUNOBLOT kit for the diagnostics of **CMV infection**

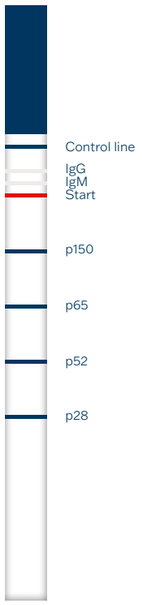
Introduction

Human cytomegalovirus (CMV, Human Herpesvirus 5, HHV 5) is a member of the Herpetoviridae family. Primary infection of CMV occurs mainly in childhood or adolescence. The infection can be transmitted in different ways (e.g. respiratory apparatus, digestive or urogenital tract). Clinically, the disease process is usually asymptomatic or mild (fever, fatigue, mononucleosis symptoms). Dormant infections where the virus survives can be reactivated – generally by changes in host-virus relations (pregnancy, serious disease, stress, immunosuppressive treatment). Reinfection is possible with a different strain of CMV. During the acute stage of infection, convalescence and reactivation of the disease, the virus is exuded in saliva and urine. CMV infection during pregnancy causes developmental defects (virus is easily transmitted through the placenta and affects the fetus). During primary infection mother-to-infant transmission of the virus (i.e. via placenta) occurs in 1/3 to 1/2 of cases; however, during the reactivation stage, transplacental transmission occurs in only 1% of cases.

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
CMV IgG	95.9%	99.0%
CMV IgM	96.5%	99.0%

Antigens



BLOT-LINE
CMV

p150 Tegument protein UL32
A strong immunogen of the late stage of infection (late antigen), it does not develop in the early stage. Detectable in the IgG class in higher titres even in reactivation.

p65 Tegument protein UL83
In the IgM class – one of the markers of the early stage of infection
In the IgG class – rather typical for the late stage or infection reactivation

p52 CM2 protein; UL44
In the IgM class – an important marker of the early stage of primary infection
In the IgG class – reactivity rather in the late stage, or infection reactivation

p28 Tegument protein UL99
A strong immunogen; it may develop in late stages of infection.

IMMUNOBLOT kits for the diagnostics of ***Chlamydia sp.***

Introduction

In terms of human health, the most important *Chlamydia* pathogens are *Chlamydia trachomatis* and *Chlamydia pneumoniae*. *Chlamydia psittaci* is primarily an animal pathogen, which can be transmitted to humans.

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen, causing venereal diseases in humans worldwide. The most vulnerable group is young people between 15 and 30 years of age. Urogenital chlamydia infections often occur in the form of "post-gonococcal inflammation". Cervical chlamydia infection is currently considered to be one of the risk factors for uterine cervix carcinoma. Chlamydia trachomatis is also the most frequent cause of sterility in both men and women.

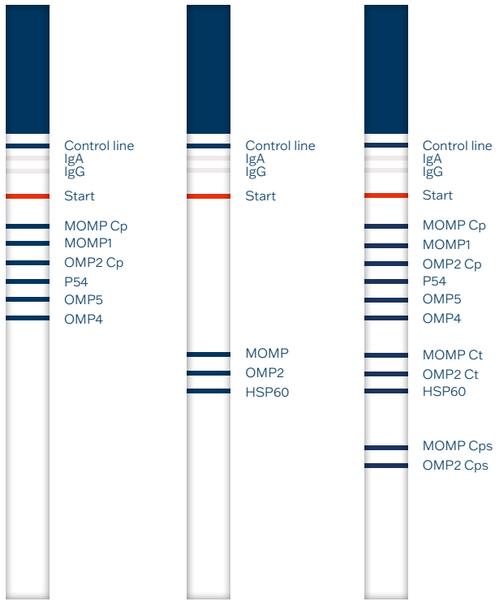
Chlamydia pneumoniae is the most widely spread Chlamydiaceae species in the human population. In recent years, the number of acute and chronic infections has increased. Primary infection generally occurs between 5 and 18 years of age. Major clinical symptoms include: rhinitis, sinusitis, otitis media, pharyngitis, bronchitis, atypical pneumonia with non-productive cough and indistinctive auscultatory findings.

Chlamydia psittaci can cause human diseases with atypical pneumonia-like (avian strains) or placentitis-like (mammal strains) manifestation.

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
Chlamydia pneumoniae IgA	98.8%	99.9%
Chlamydia pneumoniae IgG	97.7%	98.2%
Chlamydia pneumoniae IgM	95.0%	99.0%
Chlamydia trachomatis IgA	97.4%	98.2%
Chlamydia trachomatis IgG	98.2%	98.0%
Chlamydia psittaci IgA	99.9%	99.9%
Chlamydia psittaci IgG	99.9%	99.9%

Antigens



BLOT-LINE Chlamydia pneumoniae

BLOT-LINE Chlamydia trachomatis

BLOT-LINE Chlamydia sp.

Chlamydia pneumoniae

MOMP Cp – dominant major outer membrane protein (species specific) – structural protein; metabolic function

MOMP1 – isoform, produced by posttranslational modification

OMP2 Cp – outer membrane protein (species specific) – structural protein of Chlamydia outer membrane complex

OMP4 – outer membrane protein

OMP5 – outer membrane protein

P54 – immunodominant outer antigen, highly specific to *Ch. pneumoniae* – sensitive marker for diagnosis of acute infection

Chlamydia trachomatis

MOMP Ct – dominant major outer membrane protein (species specific) – structural protein; metabolic function

OMP2 Ct – outer membrane protein (species specific) – structural protein of Chlamydia outer membrane complex

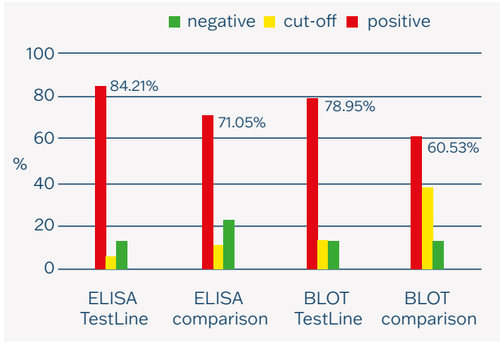
HSP60 – heat shock protein (GroEL); marker of chronic infection

Chlamydia psittaci

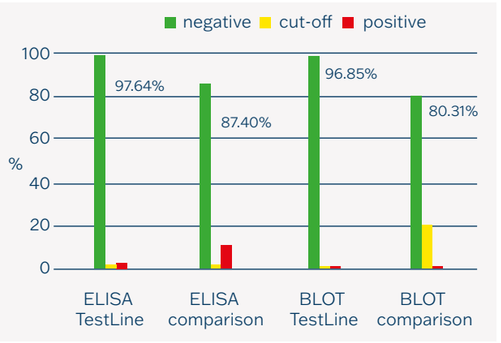
MOMP Cps – dominant major outer membrane protein (species specific) – structural protein; metabolic function

OMP2 Cps – outer membrane protein (species specific) – structural protein of Chlamydia outer membrane complex

Reactivity of different diagnostic kits in a group of positive samples



Reactivity of different diagnostic kits in a group of negative samples



IMMUNOBLOT kits for the diagnostics of **Epstein-Barr virus infection**

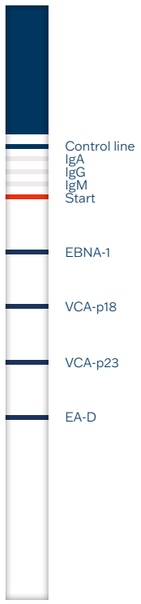
Introduction

Epstein-Barr virus (EBV) belongs to a group of herpesviruses (HHV4). The source of the infection is an infected person. The virus is transmitted via droplet infection or direct contact. EB virus is an aetiological agent of infectious mononucleosis (IM) and is also related to Burkitt's lymphoma and nasopharyngeal carcinoma. 90% of the infections will occur in childhood. IM develops after an incubation period (1-2 months). EBV does not disappear completely from the body after the primary infection, it remains in latent state in the body and can be reactivated.

Test Characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE EBV IgA	95.4%	98.2%
BLOT-LINE EBV IgG	99.9%	98.6%
BLOT-LINE EBV IgM	92.7%	99.0%

Antigens



Recombinat antigens:

EBNA-1 – Epstein-Barr nuclear antigen 1; in the IgG class, an important diagnostic marker of late phase or reactivation of the infection; in IgM class detectable antibodies for 2-4 months after primary EBV infection, may also appear also in reactivation

VCA-p18 – Viral Capsid Antigen p18; an important marker of late phase of infection; IgG antibodies do not appear in primary infections; IgM antibodies can be detected in early phase of the infection

VCA-p23 – Viral Capsid Antigen p23; antibodies to this antigen can be detected at all stages of infection in IgG and IgM class, IgG antibodies persist in the body for a long time

EA-D – Early Antigen Diffuse p54; BMRF1; an additional marker of acute EBV infection, antibodies detectable in IgG and IgM class, even in the latent phase of primary infection

BLOT-LINE
EBV

IMMUNOBLOT kits for the diagnostics of **Helicobacter infection**

Introduction

Helicobacter pylori belongs in the genus *Helicobacter*. Morphologically, it is a Gram-negative, microaerophilic bacterium. It is found as a pathogen in patients with infection of the gastric mucosa, particularly in the area of pyloric antrum and duodenum. It is a causative agent of B-type chronic gastritis, which is linked to the development of gastric ulcers. In this case, *H. pylori* is detected in 100% of individuals. *H. pylori* infection is often associated with dyspepsia. Active chronic gastritis can further develop in the atrophy of stomach lining and increase the risk of gastric carcinoma.

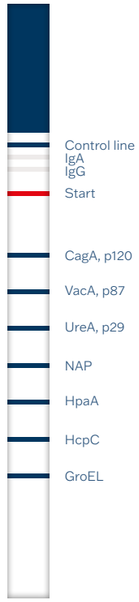
The factors of *H. pylori* pathogenicity are based on both the morphologic structure of the bacteria cells (helix-shaped curved rod, flagella) and its ability to produce extracellular enzymes and cytotoxins (e.g. urease, catalase, protease, VacA and CagA).

Bacterial strains can be pathogenic or facultatively pathogenic. Their virulence depends on the qualitative and quantitative representation of the above mentioned factors. Their pathogenesis is also influenced by a host response. Resistant strains are isolated mainly from unsuccessfully treated patients.

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Helicobacter pylori IgA	95.2%	99.9%
BLOT-LINE Helicobacter pylori IgG	96.9%	99.9%

Antigens



CagA, p120 – Cytotoxin associated gene A, highly specific, virulence factor

VacA, p87 – Vacuolating cytotoxin A, highly specific, virulence factor

UreA, p29 – Light subunit of urease, specific, virulence factor

NAP – Neutrophil-activating protein, virulence factor, potential biomarker of gastritis

HpaA – Helicobacter pylori adhesin A, surface lipoprotein, potential biomarker of gastritis and gastric ulcer

HcpC – Helicobacter cystein-rich protein, virulence factor

GroEL – Chaperonin, heat shock protein (Hsp 60), virulence factor, is considered a marker of chronic infection

BLOT-LINE
Helicobacter pylori

IMMUNOBLOT kits for the diagnostics of **Mycoplasma infection**

Introduction

Mycoplasma pneumoniae is a primary pathogenic agent of the human respiratory tract. It causes pneumonia accompanied by fever, nausea, ague, cough and fatigue. The disease is prolonged but well curable with antibiotics. The pathogen is airborne, spread especially in dense gatherings of children, particularly during spring and autumn months.

Primary infection is indicated by **IgM** antibody increase (1-2 weeks after the infection). The antibody level reaches its maximum after 1 month from the beginning of the infection and antibodies may persist for more than 1 year.

IgA antibodies are usually produced later than IgM and their level often decreases earlier. IgA antibodies determination is relevant when IgM antibodies are absent or in case of reinfection.

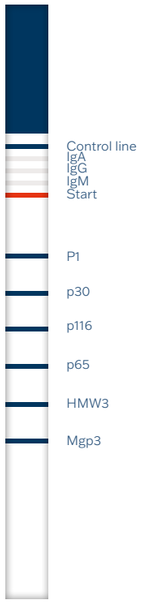
IgG antibodies may rise already 2-3 weeks after appearance of symptoms. The maximum is reached after a longer period (even 6 months). Antibodies persist more than one year. Some cases of antibody persistence longer than 4 years were reported. In case of reinfection, it is necessary to evaluate dynamics of antibodies in paired samples collected after 1 - 2 weeks.

It is crucial to examine all three classes of antibodies and sometimes even perform reinvestigation of paired samples for proper evaluation of serological findings.

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Mycoplasma IgA	93.3%	99.5%
BLOT-LINE Mycoplasma IgG	96.2%	99.0%
BLOT-LINE Mycoplasma IgM	96.7%	99.2%

Antigens



P1 – Adhesin; the most important protein, a major virulence factor
p30 – Cytadhesin p30; the second most important protein, a major virulence factor
p116 – Adhesin, a major virulence factor
p65 – Surface protein; proline-rich P65 protein
HMW3 – Cytadherence high molecular weigh 3; adhesion-promoting protein
Mgp3 – Adhesion-promoting protein

BLOT-LINE
Mycoplasma

IMMUNOBLOT kit for the diagnostics of **Toxocariasis**

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.

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

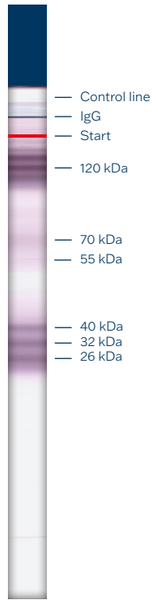
Clinical application

- 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.

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT Toxocara IgG	90.9%	99.0%

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

BLOT-LINE
Toxocara

IMMUNOBLOT kit for the diagnostics of **Toxoplasmosis**

Introduction

Toxoplasmosis is a widespread parasitic disease caused by protozoan Toxoplasma gondii. 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 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.

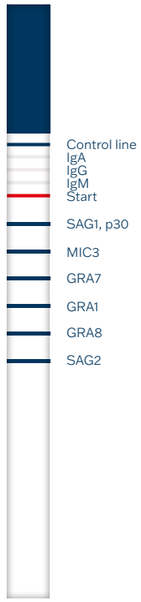
Clinical application

- Useful method to trace immune profile of mothers' and new-borns' sera – determination of congenital toxoplasmosis

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%

Antigens



BLOT-LINE
Toxoplasma

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.

IMMUNOBLOT kits for the diagnostics of **Syphilis**

Introduction

Syphilis (lues) is a sexually transmitted disease caused by spirochaeta *Treponema pallidum subsp. pallidum*. The disease is spread predominantly by sexual intercourse with an infected individual, however in approximately 5-10% of cases the infection is transmitted in another way (from mother to child, rarely by contact with infected blood or by dermal manifestations).

In case that an expectant mother suffers from an untreated primary or secondary acquired form of syphilis, the disease can be passed to the unborn infant which results in abortion or infection of the foetus and its severe damages (congenital syphilis).

In some cases the asymptomatic congenital form of syphilis can appear with manifestation between 7 and 19 year of life.

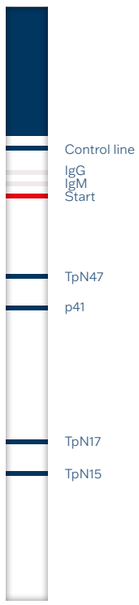
Clinical application

- Confirmation of treponemal and non-treponemal tests
- Determination of IgG and IgM antibodies enables to distinguish between new and previous infection

Test characteristics

Pathogens	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Treponema IgG	98.0%	99.9%
BLOT-LINE Treponema IgM	97.6%	99.9%

Antigens



BLOT-LINE
Treponema pallidum

Highly specific antigens:
TpN47
TpN17
TpN15
Specific antigen:
TpN41

IMMUNOBLOT kits for the diagnostics of **Yersinia infections**

Introduction

Yersinia are pathogenic gram-negative bacteria of the Enterobacteriaceae family and their representatives *Y. enterocolitica* and *Y. pseudotuberculosis* are known as human enteropathogens. The carriers are latently infected warm-blooded animals.

The infection occurs orally after the ingestion of contaminated water or food.

The clinical signs of *Y. enterocolitica* and *Y. pseudotuberculosis* infection are very similar. Differences are mostly observed in intestinal complaints, pseudoappendicitis and sepsis. The most widespread Y. enterocolitica causes diarrhoea in humans, accompanied by diarrhoea of the small intestine, colon or appendix. It can also cause joint inflammation and enlargement of the lymph nodes. Complications such as acute reactive arthritis, erythema nodosum, acute glomerulonephritis and myocarditis may develop during the infection.

Skin symptoms appear about 1–6 weeks after the infection. In some cases, *Y. enterocolitica* may also persist for years in the intestinal mucosa and in the lymphatic tissues.

Antibody response

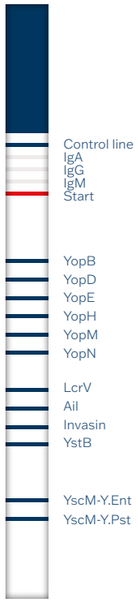
IgA, IgG and IgM antibodies can be detected in the initial phase after contact with virulent Yersinia factors. IgA and IgM titres will decrease after several months.

IgG class antibodies persist longer and can be detected for longer than one year. IgG antibodies may persist in some cases throughout the live.

Test characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Yersinia IgA	94.2%	99.9%
Yersinia IgG	97.8%	99.9%

Antigens



BLOT-LINE Yersinia

YopB – YopB Yersinia outer protein, transmembrane protein
YopD – Yersinia outer protein, transmembrane protein
YopE – Yersinia outer protein
YopH – Yersinia outer protein
YopM – Yersinia outer protein
YopN – Yersinia outer protein
LcrV – Low calcium response Virulence, important for YopD a YopB secretion
Ail – Attachment-invasion locus protein early phase, involved in the adhesion and invasion process, and allows yersinia to survive outside the host cell, a significant virulence factor
Invasin – surface adhesin that binds to β1 integrins on the surface of target cells and is important particularly in the first stage of infection, a virulence factor
YstB – heat-stable enterotoxin B, responsible for the virulence and pathogenicity of Y. Enterocolitica strains, biotype 1A
YscM-Y.ent – Yop proteins translocation protein M (specific for Y. enterocolitica)
YscM-Y.pst – Yop proteins translocation protein M (specific for Y. pseudotuberculosis)

IMMUNOBLOT kits for the diagnosis of **antibodies against neutrophil cytoplasm and GBM**

Introduction

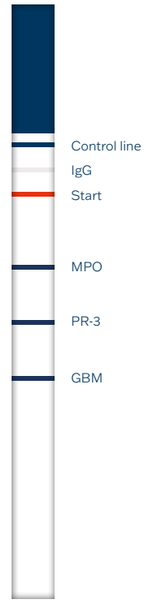
Antineutrophil cytoplasmic antibodies (ANCA) are a group of antibodies directed against cytoplasm antigens of neutrophilic granulocytes and monocytes. ANCA examinations are considered basic tests in immunological laboratories. The determination of ANCA is of great importance, in particular in case of suspected acute vasculitis of small vessels, with severe pulmonary impairment or renal failure, but also in some non-vasculitic clinical syndromes such as inflammatory bowel diseases, e.g. ulcerative colitis. The most common target antigens of ANCA-associated vasculitis are proteinase 3 or myeloperoxidase.

Antibodies against **proteinase 3 (PR3)** are referred to as c-ANCA fluorescent subtype, namely cytoplasmic antibodies (granular cytoplasmic fluorescence). PR3 is a neutral serine proteinase 3, also known as Wegener's autoantigen. Antibodies against PR3 are a highly specific marker in diagnosing Wegener's granulomatosis.

Antibodies against **myeloperoxidase (MPO)** are referred to as p-ANCA subtype, since they form a perinuclear fluorescence pattern. This ANCA fluorescent subtype includes other antibodies such as antibodies against lactoferrin, cathepsin G, or elastase. However, in at least 60% of p-ANCA reactivity cases, the main antigen is MPO. Anti-MPO antibodies are primarily considered an important indicator for progressing nephritis; they are largely present in patients with severe renal impairment. They are also important for diagnosing Churg-Strauss syndrome and microscopic polyangiitis. The presence or absence of antibodies against MPO and PR3 in combination with the positivity of antinuclear antibodies may be regarded as a differentiating marker between ANCA-associated vasculitis and SLE--induced vasculitis.

Anti-GBM antibodies (glomerular basal membrane; Goodpeasteure's antigen) are important for the diagnosis of glomerulonephritis, which may be accompanied by pulmonary haemorrhage (Goodpasteure's pulmo-renal syndrome). Due to clinical symptoms similar with systemic vasculitis, it is appropriate to concurrently carry out the tests for anti-GBM and ANCA antibodies. In all cases, the activity and severity of the disease closely correlates with the concentration of antibodies. Thus, the information about the determined levels of antibodies can also be used in monitoring disease progression.

Antigens



BLOT-LINE ANCA

Myeloperoxidase (MPO)
– a highly specific sign for the diagnosis of rapidly progressive nephritis, necrotising glomerulonephritis; a positive response in 70-90% of patients with severe renal impairment.
Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA) and other vasculitis.

Proteinase 3 (PR3)
– a highly specific serological sign for the diagnosis of Wegener's granulomatosis. Microscopic polyarteritis, Churg-Strauss syndrome, mild systemic sclerosis, ulcerative colitis.

Basal membrane of glomeruli (Goodpasture's antigen, GBM)
– diagnostically significant antibodies in glomerulonephritis; Pulmo-renal Goodpasture's syndrome (rapidly progressive glomerulonephritis, in 2/3 of patients with pulmonary haemorrhage.

Test characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
MPO	91.1%	91.9%
PR3	93.5%	96.7%
GBM	100.0%	95.5%

IMMUNOBLOT kits for the diagnosis of **Systemic autoimmune diseases**

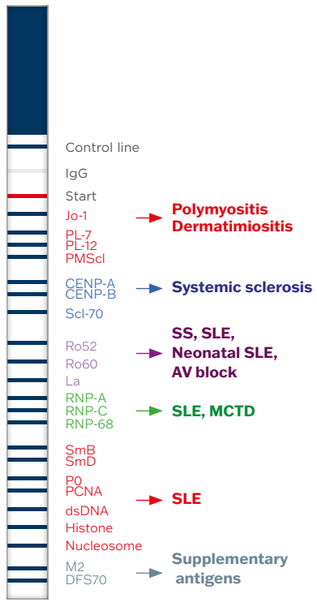
Introduction

Determination of antinuclear antibodies is important for diagnosis of systemic auto-immune diseases. These organ non-specific autoantibodies are directed to intracellular antigens located mainly in the nucleus of the cell. Their detection can indicate the presence of some systemic autoimmunopathologic process – especially: systemic lupus erythematodes (SLE), Sjögren's syndrome (SS), sclerodermia, mixed connective tissue disease (MCTD), systemic sclerosis, polymyositis and dermatomyositis.

An important group of antinuclear antibodies represent antibodies against ENA (extractable nuclear antigens: SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1). They are mainly ribonucleoproteins and nuclear enzymes.

The group of antinuclear antibodies also includes antibodies against nucleic acids (ssDNA, dsDNA), complexes of nuclear proteins (DNP, RNP) and histones.

Antigens



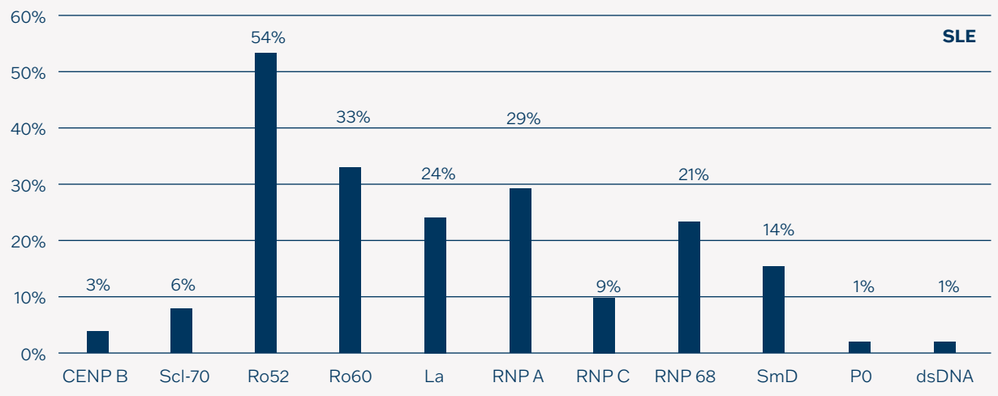
BLOT-LINE ANA

Test characteristics

Immunoblot	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE ANA	96.6%	97.4%

Clinical Data

Detection of individual antigens for group of patients with SLE – results of BLOT-LINE ANA (n=65)



Ordering information

BLOT-LINE

Infectious serology

Cat. No.	Product	No. of Tests
ApGL10	BLOT-LINE Anaplasma IgG	10
ApML10	BLOT-LINE Anaplasma IgM	10
BpAL20	BLOT-LINE Bordetella IgA	20
BpGL20	BLOT-LINE Bordetella IgG	20
BGL020	BLOT-LINE Borrelia/HGA IgG	20
BML020	BLOT-LINE Borrelia/HGA IgM	20
BaGL20	BLOT-LINE Borrelia afzelii IgG	20
BaML20	BLOT-LINE Borrelia afzelii IgM	20
BgGL20	BLOT-LINE Borrelia garinii IgG	20
BgML20	BLOT-LINE Borrelia garinii IgM	20
BsGL20	BLOT-LINE Borrelia b. sensu stricto IgG	20
BsML20	BLOT-LINE Borrelia b. sensu stricto IgM	20
CAL020	BLOT-LINE Chlamydia IgA	20
CGL020	BLOT-LINE Chlamydia IgG	20
CpAL20	BLOT-LINE Chlamydia pneumoniae IgA	20
CpAG20	BLOT-LINE Chlamydia pneumoniae IgG	20
CpAM20	BLOT-LINE Chlamydia pneumoniae IgM	20
CtAL20	BLOT-LINE Chlamydia trachomatis IgA	20
CtGL20	BLOT-LINE Chlamydia trachomatis IgG	20
CMGL20	BLOT-LINE CMV IgG	20
CMML20	BLOT-LINE CMV IgM	20
EBAL20	BLOT-LINE EBV IgA	20
EBGL20	BLOT-LINE EBV IgG	20
EBML20	BLOT-LINE EBV IgM	20
HpAL20	BLOT-LINE Helicobacter IgA	20
HpGL20	BLOT-LINE Helicobacter IgG	20
MyAL20	BLOT-LINE Mycoplasma IgA	20
MyGL20	BLOT-LINE Mycoplasma IgG	20
MyML20	BLOT-LINE Mycoplasma IgM	20
TpGL20	BLOT-LINE Treponema IgG	20
TgAL20	BLOT-LINE Toxoplasma IgA	20
TgGL20	BLOT-LINE Toxoplasma IgG	20

TgML20	BLOT-LINE Toxoplasma IgM	20
TpML20	BLOT-LINE Treponema IgM	20
YAL020	BLOT-LINE Yersinia IgA	20
YGL020	BLOT-LINE Yersinia IgG	20

Autoimmunity

Cat. No.	Product	No. of Tests
ANC3L20	BLOT-LINE ANCA-3	20
ANAL20	BLOT-LINE ANA	20

RELATED PRODUCTS

Cat. No.	Product
SwIm03	Immunoblot Software

PRODUCER

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