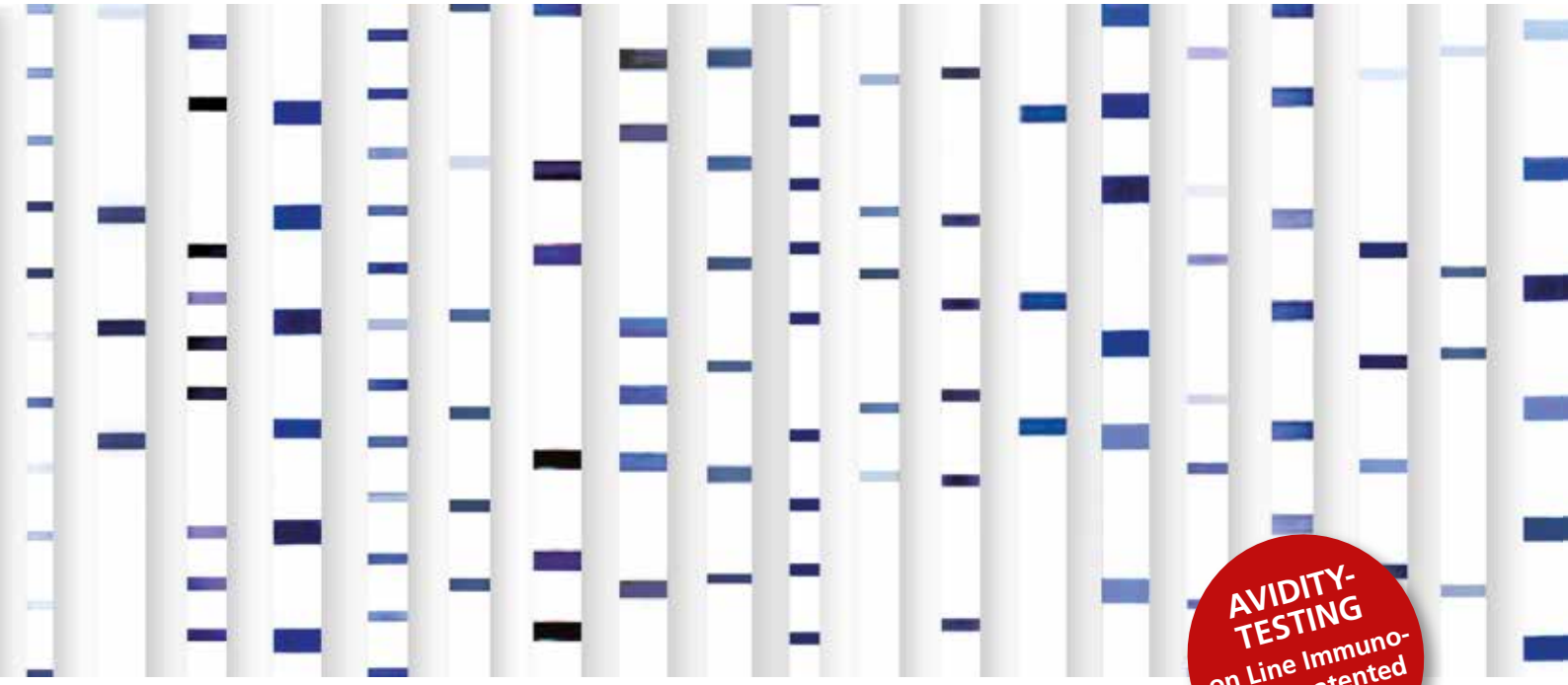
MIKROGEN
DIAGNOSTIK



*recom*Line

Comprehensive Line Immunoassay portfolio
for infectious and autoimmune diseases.
Innovative system solutions for
individual laboratory automatisation.

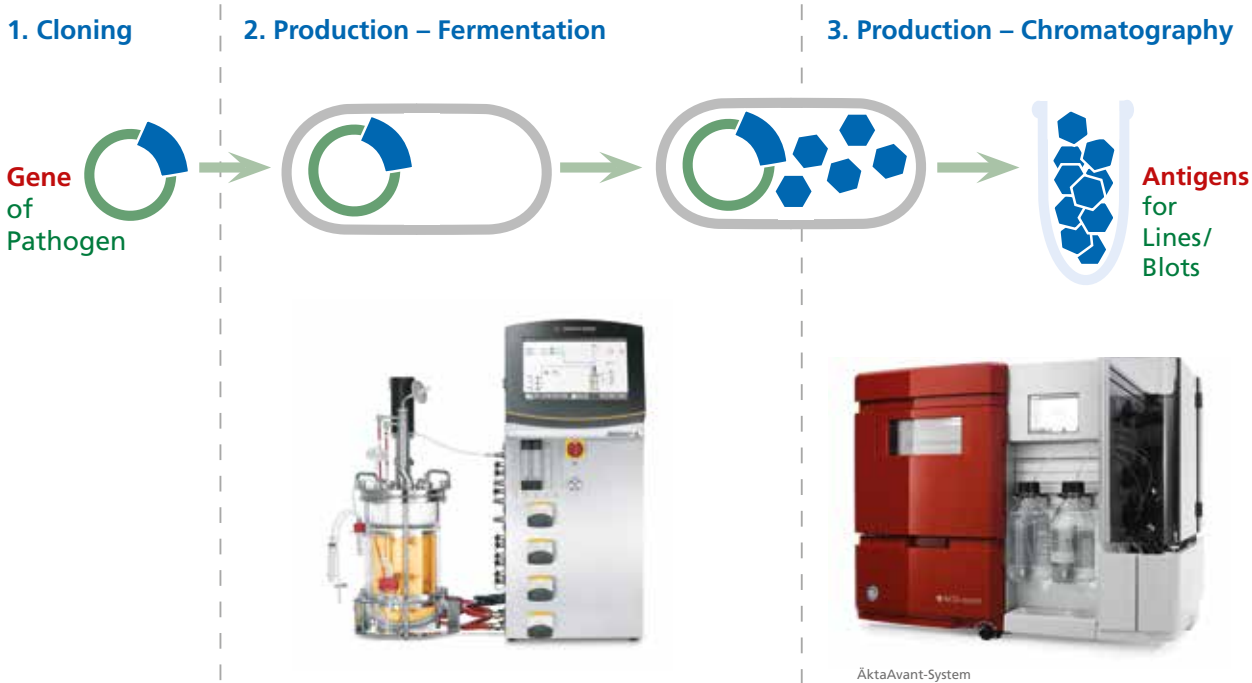
With more than 30 years of experience in the development of genetically engineered diagnostics, MIKROGEN belongs to the worldwide top specialists in the field of recombinant antigens. Recombinant antigens are selected pathogen proteins that are manufactured by genetical engineering. This high quality recombinant proteins are used in several MIKROGEN product lines such as LINE, BEAD and ELISA.

The MIKROGEN *recom*Line product family is a high quality test system for serological testing of infections and autoimmune diseases. It covers a broad range of infectious diseases, caused by e.g. Borrelia, HIV, HCV, HEV, Treponema or CMV. In order to provide tests for safe confirmatory diagnostics in different stages of infection, MIKROGEN offers tests for several classes of antibodies such as IgG, IgM and IgA. Additionally, avidity testing is available for selected parameters. *recom*Line assays are characterized by high quality, easy workflow and the possibility of full automatisation.

Benefits of MIKROGEN *recom*Line strip assays

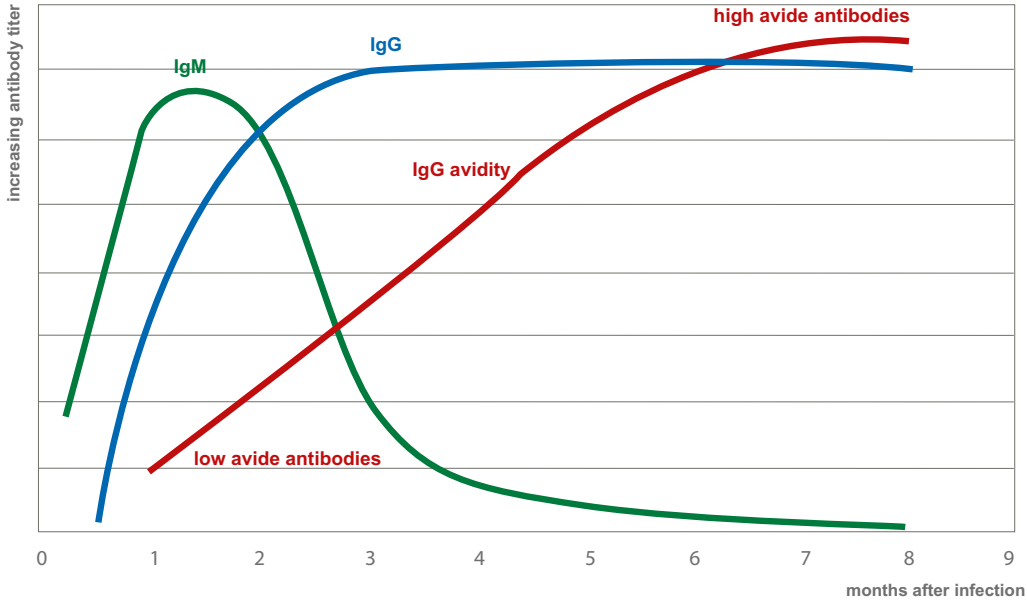
- High sensitivity and specificity
- Easy and clear interpretation
- Easy test procedure – semi and full automation possible
- Fast and objective evaluation and documentation by *recom*Scan software
- Test procedure and reagents identical in all MIKROGEN strip tests – reagents exchangeable
- Separate detection of different antibodies and antibody classes
- MIKROGEN patent for avidity testing on Line Immunoassays
- Phase specific antigens indicating time of infection
- Safe evaluation: strip specific internal controls (cut-off, conjugate and incubation control)
- CE label: Our *recom*Line tests meet the high standard of the EC directive 98/79/EC – IVD

From pathogen to antigen



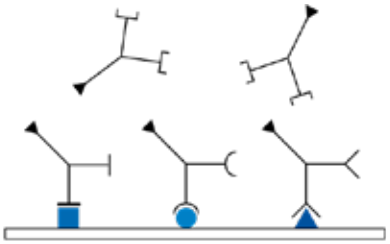
ÄktaAvant-System

Typical antibody response and avidity development during the course of infection



*recom*Line strip assay – Test Principle and Procedure

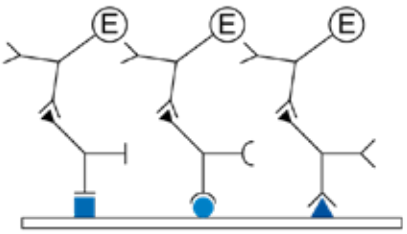
Sample incubation



A test strip loaded with recombinant antigens is incubated with diluted serum or plasma in a dish for **1 hour** or 3 hours for HIV, HCV and Treponema.

Washing step

Conjugate incubation



Peroxidase conjugated anti-human antibodies (IgG or IgM or IgA specific) are added. Incubate for **45 minutes**.

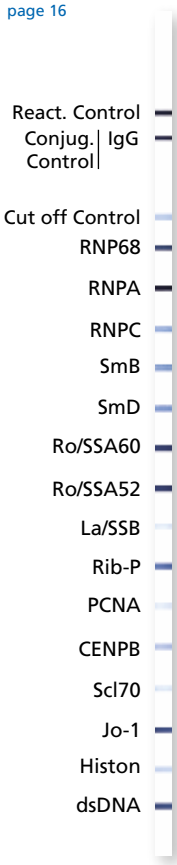
Washing step

Detection

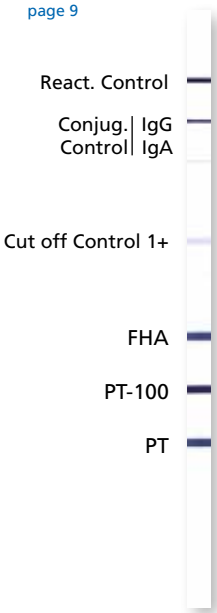
8 minutes after addition of the coloring solution, insoluble colored bands develop at the sites on the test strips occupied by antibodies.

Test Principle and Procedure for Avidity testing see page 10

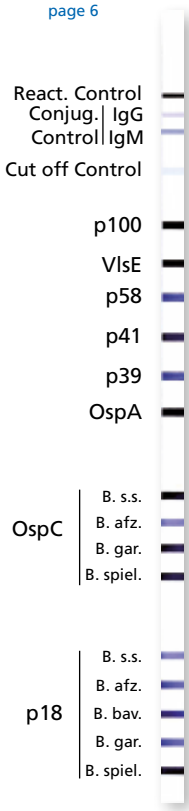
ANA/ENA
page 16



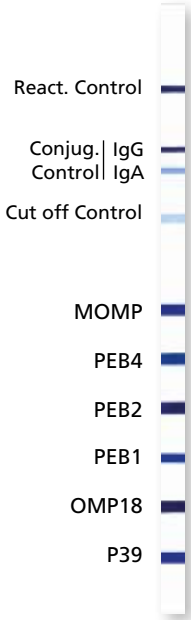
Bordetella
page 9



Borrelia
page 6



Campylobacter
page 14



Chlamydia
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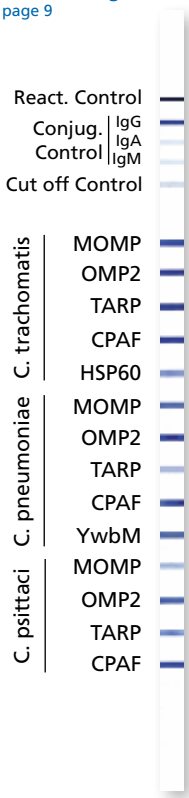

HEV
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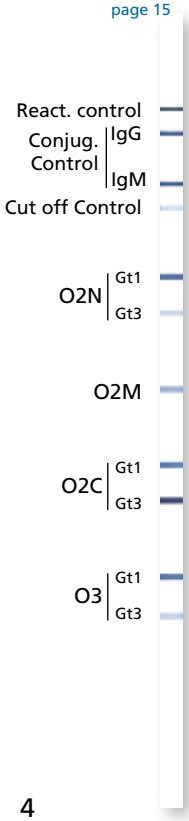
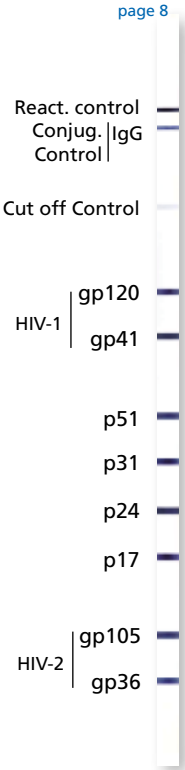
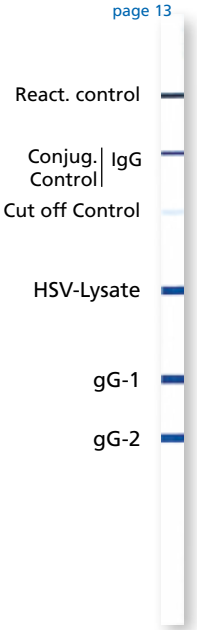


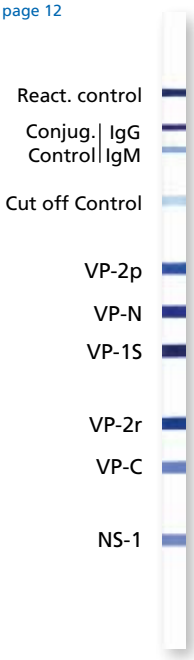
HIV
page 8



HSV
page 13



Parvovirus
page 12



TORCH
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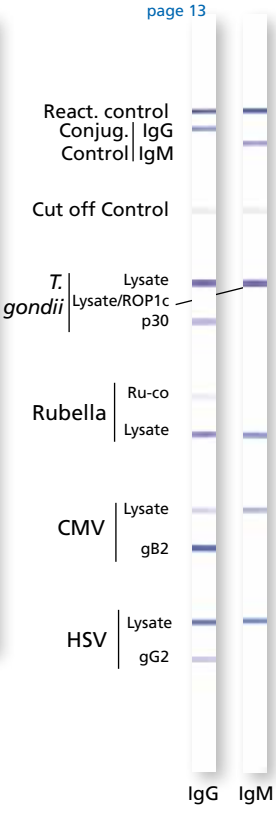
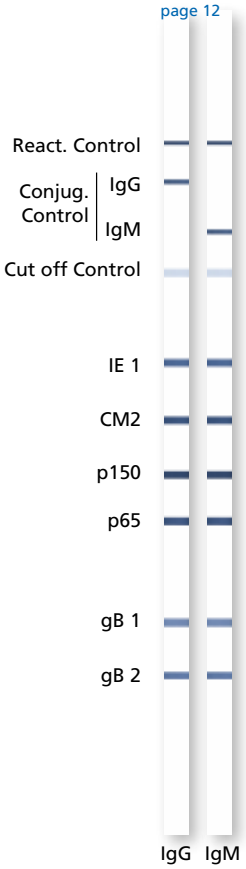
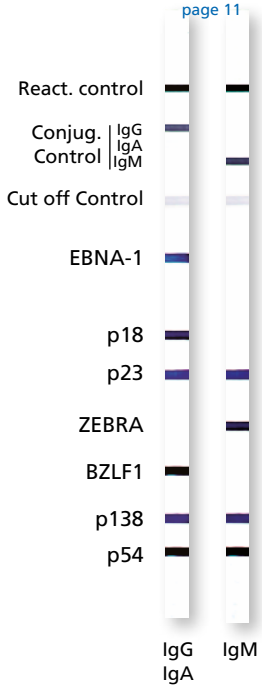


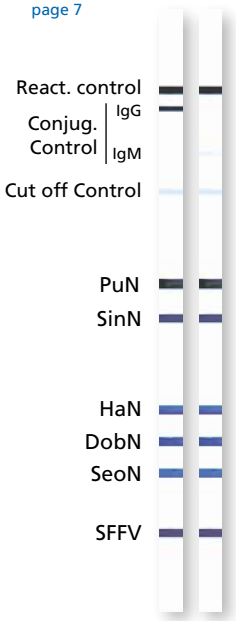
CMV
page 12



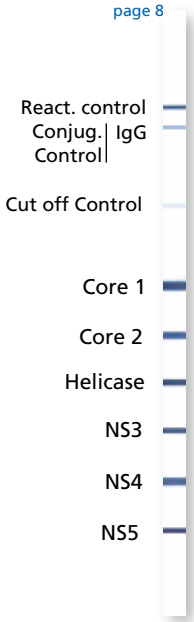
EBV
page 11



Hantavirus
page 7



HCV
page 8



Helicobacter
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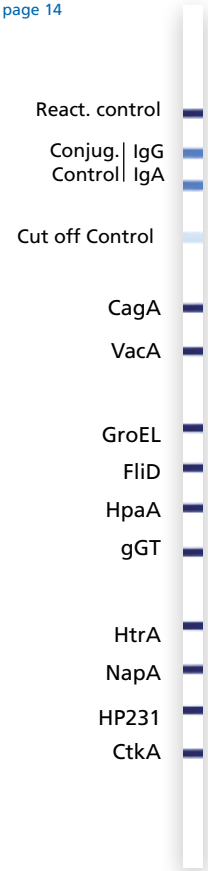

Toxoplasma
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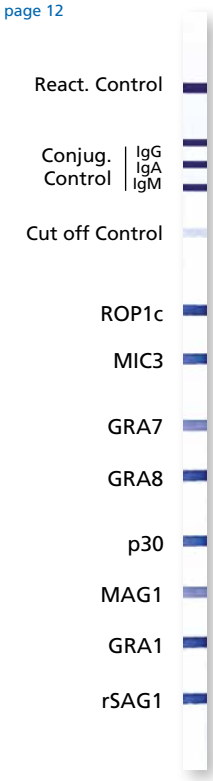
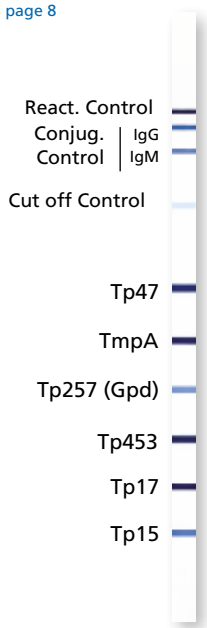


Treponema
page 8



Trop. Fever
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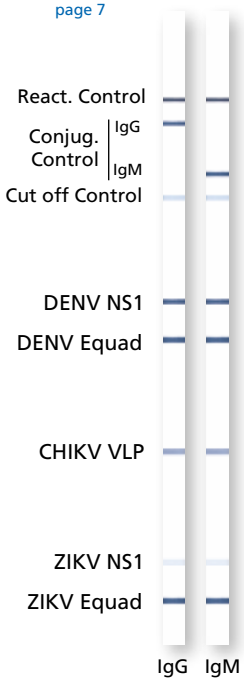
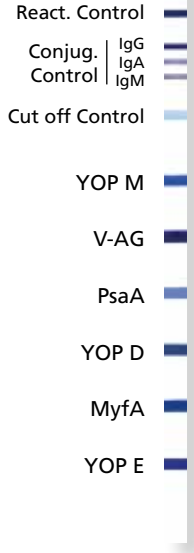
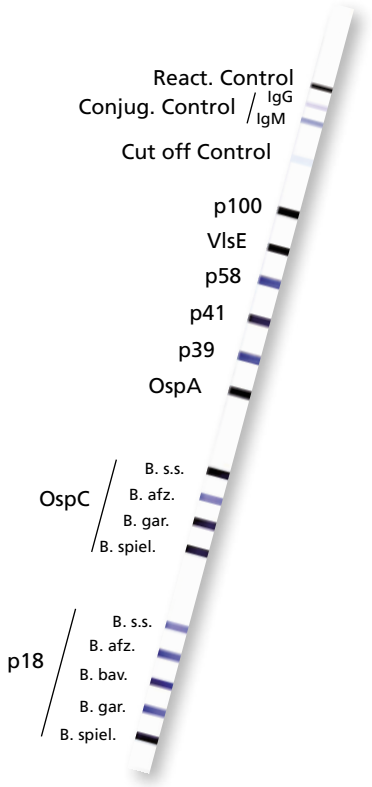


Yersinia
page 14



Borrelia burgdorferi

*recom*Line Borrelia IgG, IgM



- Contains all known antigens relevant for Borrelia diagnostics
- Reliably detects all five pathogenic Borrelia species common in Europe
- Identifies early and late infection status by different typical antigen band patterns
- Allows to analyse pairs of CSF and serum to detect antibody index in suspicious Lyme neuroborreliosis cases

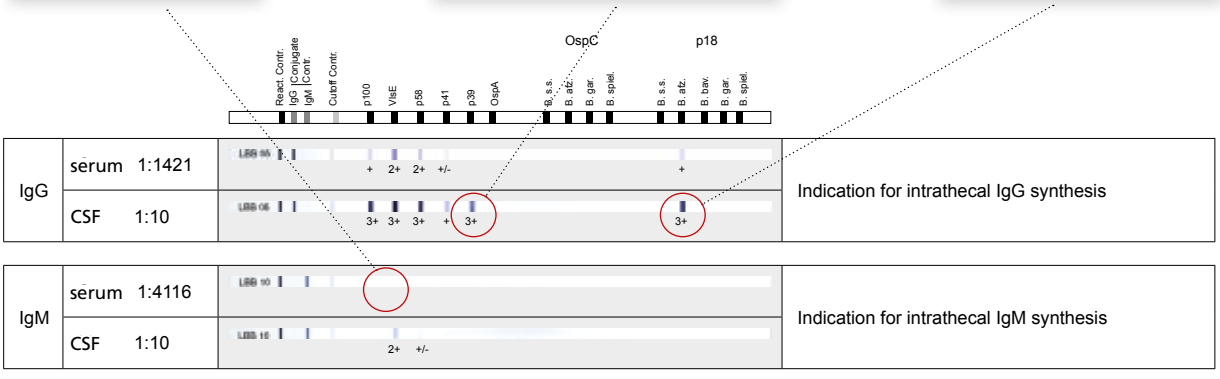
Lyme disease, the most common tick-borne disease, is caused by different Borrelia species belonging to the Borrelia burgdorferi sensu lato complex. In Europe five pathogenic Borrelia species are known: B. burgdorferi sensu stricto, B. garinii, B. afzelii, B. spielmanii, B. bavariensis, whereas in the United States B. burgdorferi sensu stricto, causing arthritis, is mainly present. Diagnosis of Lyme disease is based on clinical findings but to confirm infection status serological diagnostics is an important additional diagnostic tool.

Visualization of specific intrathecal antibodies in *recom*Line Borrelia

High dilutions may cause negative serum findings to appear

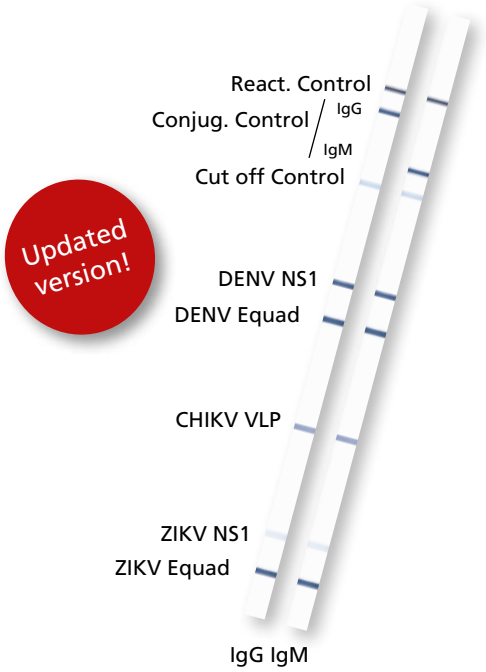
An additional positive antigen band in the CSF is a clear indication for intrathecal antibody production

Appearance of more intensive bands in the CSF than in the serum also indicates an intrathecal production



Dengue, Chikungunya and Zika Virus

*recom*Line Tropical Fever IgG, IgM

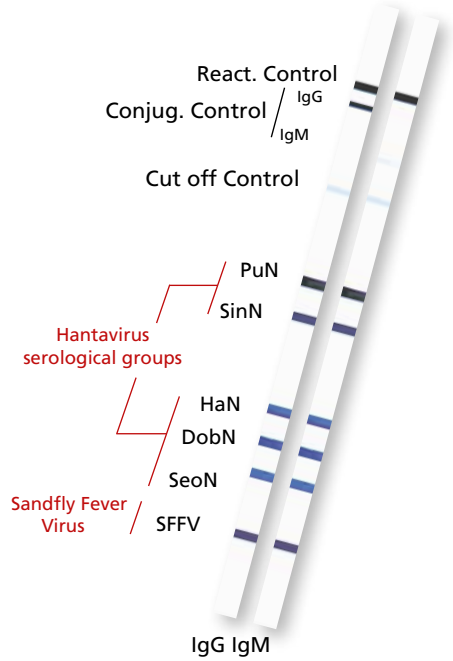


- Simultaneous detection and differentiation of Dengue, Chikungunya and Zika virus infections
- Parallel testing for IgG and IgM enables seroconversion monitoring and facilitates diagnosis of both primary and secondary flavivirus infections
- Patent-pending serological assay for the differentiation of arboviruses
- Updated: partly new antigens for higher sensitivity and improved differentiation

Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) viruses are primarily transmitted by mosquitoes from the Aedes genus and mainly found in tropical and subtropical regions. Though, DENV, ZIKV and CHIKV infections all show significant overlap in early clinical presentation and geographical distribution, the disease outcome and case management among them differs. Furthermore, DENV and ZIKV are genetically related flaviviruses. Therefore, reliable detection and differential diagnosis of these arboviral infections is critical.

Hantavirus

*recom*Line HantaPlus IgG, IgM

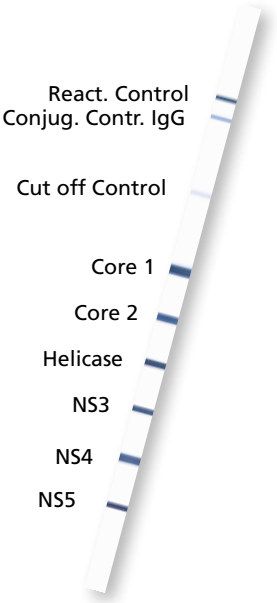


- Detection of different Hantavirus serotypes clustered in two serological groups due to homology
- Serotyping possible with IgG results in combination with travel history
- Detection of Sandfly Fever Virus (SFFV) using recombinant antigens of the serotypes Toscana and Sicilian
- Developed in cooperation with the German Reference Laboratory for Hantaviruses

Hanta- and Phleboviruses (incl. Sandfly Fever Virus) are Bunyaviruses which are present in Europe. Different rodent species are vectors for the different Hantavirus serotypes including Puumala and Dobrava virus in Europe, Hantaan and Seoul virus in Asia, Sin Nombre and Andes virus on the American continent. Hantaviruses are transmitted by inhaling dust, contaminated with droppings of the host animals. Infections, characterized by flu-like symptoms in the beginning, can induce diverse complications with different grades of severity.

Hepatitis C Virus

*recom*Line HCV IgG

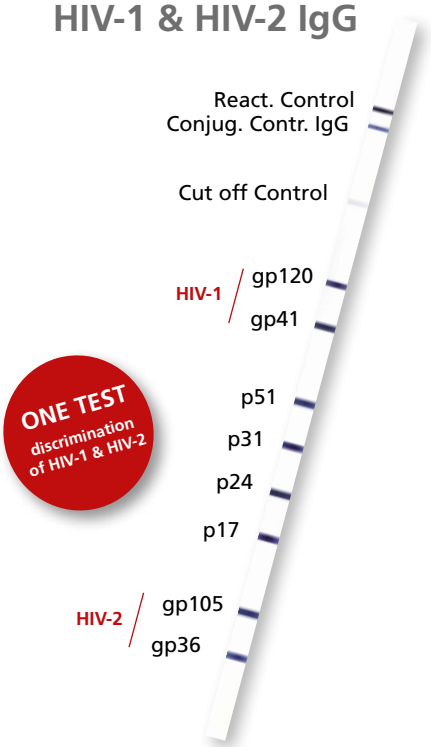


- Detection of antibodies for all important HCV genotypes (1–6)
- No cross reactivity with other viral hepatitis infections
- Very high specificity for blood donors and clinical samples

Hepatitis C virus (HCV) is the most important pathogen responsible for parenterally transmitted non-A, non-B hepatitis. This disease is characterised by an incubation period of 2 to 26 weeks and a mild to fulminant course in the acute phase. 50–70 % of the patients develop chronic hepatitis which leads to liver cirrhosis in 20% of the cases.

Human Immunodeficiency Virus

*recom*Line HIV-1 & HIV-2 IgG

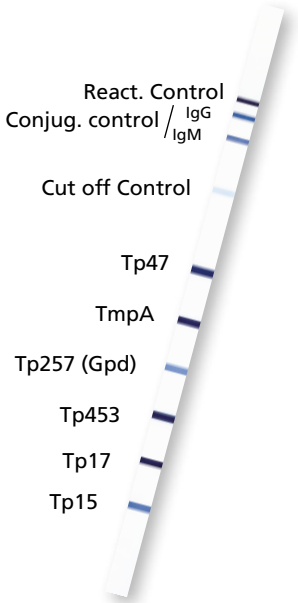


- Highest specificity – unmatched in comparison with corresponding HIV confirmatory tests
- Antibody detection of different subtypes of HIV-1 like group M and group O
- Solely uses recombinant HIV antigens
- HIV-1 & HIV-2 envelope antigens on one strip for fast discrimination

The Human Immunodeficiency Virus (HIV) was identified as the causative pathogen of the Acquired Immunodeficiency Syndrome (AIDS) in 1983. The virus is mainly transmitted through blood or sexual contact.

Treponema pallidum

*recom*Line Treponema IgG, IgM

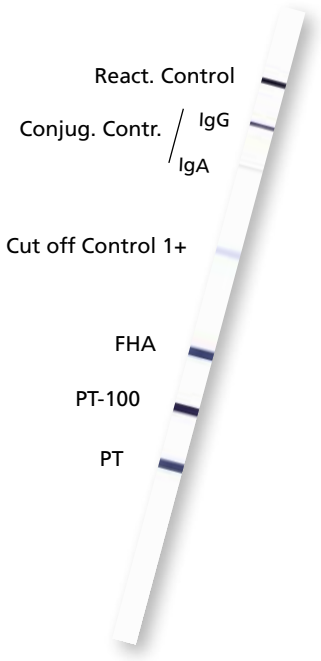


- Pathogen specific antigens Tp47, Tp17, Tp15 and TmpA
- Additional safety due to new antigens Tp257 and Tp453 with high specificity for Treponema pallidum
- Simple and quick interpretation by two-band criterion

Treponema pallidum subsp. pallidum is the causative agent of syphilis, a chronic disease occurring only in humans. The disease progresses in several stages. After the decline of acute symptoms T. pallidum can persist for years in the human organism (stage of latency) and then may cause late complications (tertiary syphilis and neurosyphilis).

Bordetella pertussis

*recom*Line Bordetella pertussis IgG, IgA

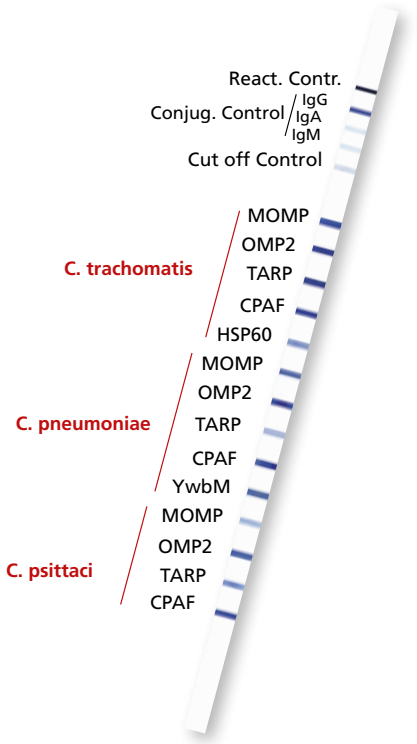


- Serological IgG and IgA antibody detection can show whether there was recent pathogen contact. IgG antibody values ≥100 IU/ml against the pertussis toxin (PT) correspond to a high probability of an acute infection when clinical symptoms are present. Vaccination management must be considered in the serological interpretation.
- PT-100 Antigen is calibrated on International WHO-Standard
- Clarification and confirmation of a Bordetella infection by *recom*Line Bordetella pertussis:
  - ▶ A reactive PT-100-IgG band confirms an acute pertussis infection
  - ▶ Evidence against a Bordetella species infection in presence of antibodies of the filamentous hemagglutinin antigen (FHA)

Bordetella pertussis is the main agent of whooping cough, a disease often lasting for weeks or even months. Milder courses can be caused by related agents, e.g. primarily Bordetella parapertussis.

Chlamydia

*recom*Line Chlamydia IgG, IgA [IgM]



- Serological differentiation of Chl. trachomatis, Chl. pneumoniae and Chl. psittaci in one test
- Supportive in in vitro fertilization diagnostics due to the species-specific antigens MOMP, TARP, CPAF and HSP60 of Chl. trachomatis
- High specific detection of Chl. pneumoniae due to the use of species-specific antigens, among others e.g. YwbM
- One and only serologic assay for the specific diagnostics of Chl. psittaci.

Three Chlamydia species are pathogenic for humans. Chl. trachomatis is among the most common sexually transmitted diseases and one of the risk factors for unwanted childlessness. Chl. pneumonia mainly infects the respiratory tract causing bronchitis, pneumonia and sinusitis amongst others. Chl. psittaci is the causative agent of ornithosis (psittacosis) affecting birds and humans (fever, headache, muscular pains and respiratory symptoms).

What is to be understood by the term „Avidity"?

Avidity (lat. avidus = avid, greedy)

- Avidity defines the entire binding strengh of a mixture of polyclonal IgG molecules to multiple antigen epitops of proteins (Fig. 1)
- It is important to discriminate between avidity and affinity:
  - ▶ Affinity means the binding strength of the particular antigen-antibody binding (given the dissociation constant)
  - ▶ Avidity defines the totality of all particular affinities
- Avidity „matures" over several month and reflects antigen mediated selection of B cells producing antibodies of increasing affinity

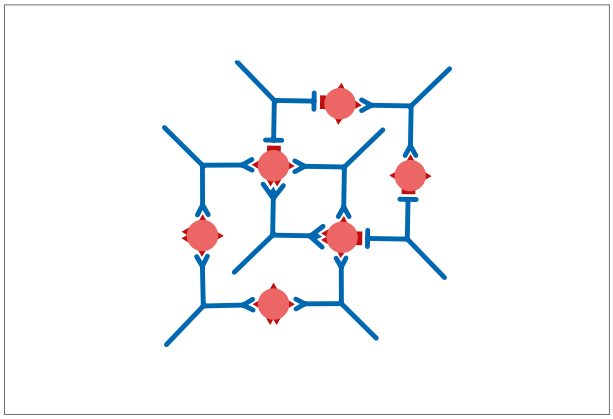


Fig. 1 Dense Network of antigen-antibody bindings

What is the benefit of Avidity testing?

Avidity testing is filling existing diagnostic gaps …

- IgG antibodies arising early after primary infection show low avidity – antibodies arising later during infection show high avidity
- Using determination of avidity the time of infection can be defined more precisely
- Uncertain screening results can be cleared
- Regarding pregnancy the ability to discriminate between acute and past infection permits a statement about meaning and relevance of the infection to the well-being of fetus and newborn

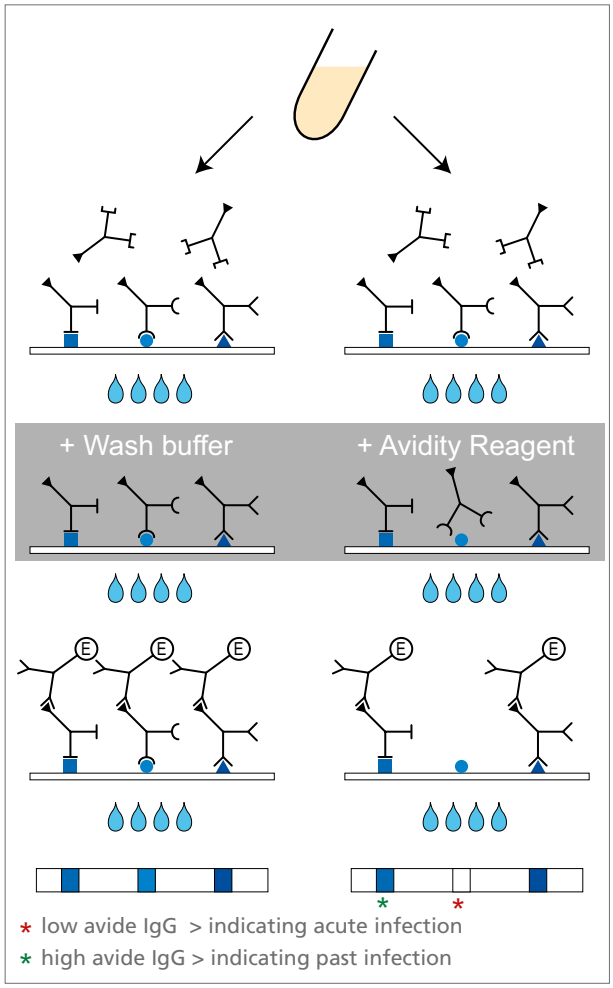


Fig. 2

Mikrogen holds the patent on Avidity

How to determinate avidity?

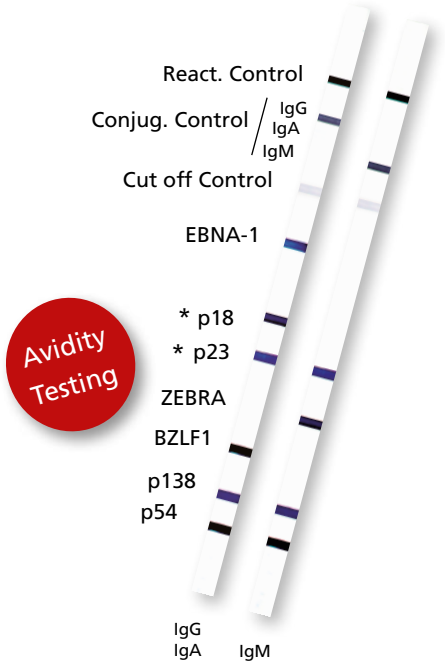
In the presence of substances having slightly denaturating effect on proteins (e.g. urea, potassium thiocyanat, guanidine chloride) only IgG of low avidity, but not IgG of high avidity will dissociate from antigen. We can analyse avidity using the Line Immunoassay technique.

- Two test strips (IgG) were incubated in parallel.
- Avidity reagent (can be ordered at Mikrogen) is added to one of the two strips approaches (3 minutes incubation). During this time, low avide antibodies are resolved from the corresponding antigen fixed on the test strip.
- Unbound antibodies are subsequently washed away.
- In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG) which are coupled to horseradish peroxidase (HRP).
- Unbound antibodies are subsequently washed away.
- Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. (Fig. 2)

In case of a fresh infection antigen-antibody binding will resolve and compared to the IgG strip the avidity strip will show reduced antigen reactivity.
In case of a long past infection antibodies of increased avidity will be present in the serum. Band reactivity on both strips will be nearly the same.

Epstein-Barr Virus

*recom*Line
EBV IgG [Avidity] [IgA], IgM



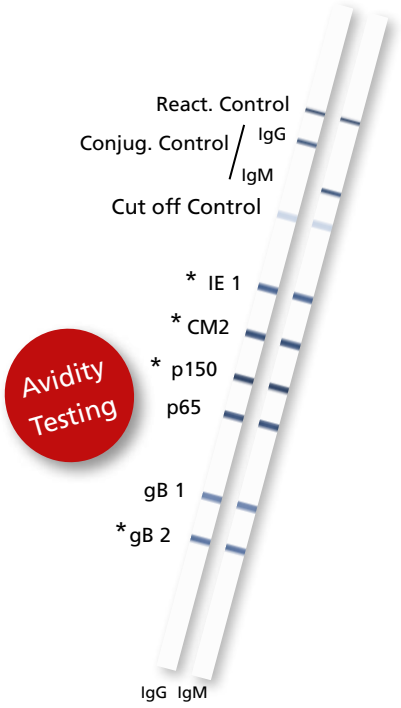
- Unique and patent protected p18 Mikrogen as additional IgG marker for past infections
- Maximum sensitivity by BZLF1 in IgG and ZEBRA in IgM in the early phase of acute infections
- Determination of avidity supports definition of infection phase
- More than 95% of the past EBV infections are correctly identified with the *recom*Line EBV IgG strip only

The Epstein-Barr virus, an ubiquitously occurring herpes virus, can cause the symptoms of infectious mononucleosis (Pfeiffer´s disease) on primary infection. Reactivations can occur, especially in immuno-incompetent persons. Due to the diversity of symptoms caused by primary infection or reactivation and their correspondence with the symptoms of other diseases, one of the main tasks in routine diagnosis is the serological detection of a primary infection, past infection or possible reactivation.

* antigens for avidity testing

Cytomegalovirus (CMV)

*recom*Line CMV IgG [Avidity], IgM

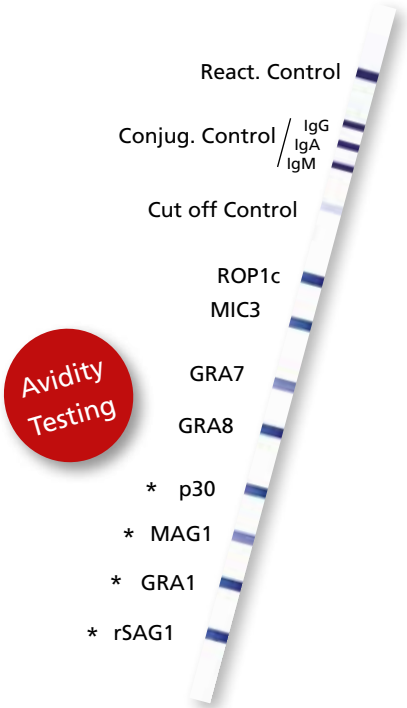


- Precise characterization of time of CMV infection
- Reliable detection of pregnancy relevant CMV infections
- No other commercially available CMV confirmatory assay based on recombinant antigens

CMV infection runs mild or asymptomatic. Immunocompromised patients and pregnant women/fetuses however are at high risk for health damage. About 10% of infected childs show severe damage at birth (microcephaly, seizures, petechiae, hepatosplenomegaly, hearing loss, vision impairment) or develop late effects (hearing, mental or coordination problems).

Toxoplasma gondii

*recom*Line Toxoplasma IgG [Avidity], IgM [IgA]

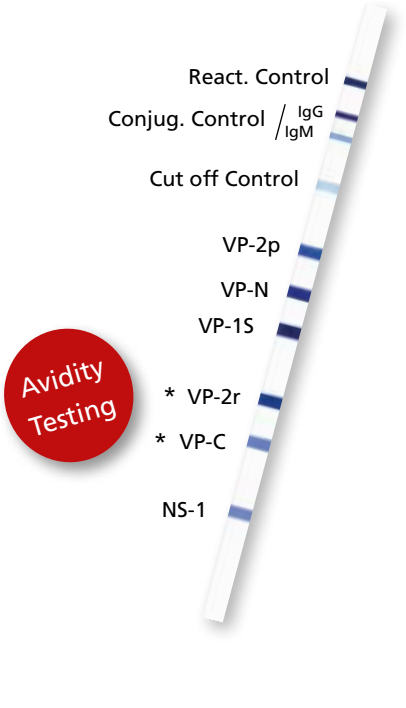


- Precise information about the time of Toxoplasma gondii infection
- Pregnancy relevant infections reliably can be identified
- In many cases superior to avidity test systems based on lysats

The course of a Toxoplasma gondii infection is asymptomatic or mild and confers lifelong immunity. Initial contact with the pathogen during pregnancy can result in transmission of the pathogens to the fetus, resulting in severe damage to it. The newborns might develop retinochoroiditis and neurologic deficits in childhood or early adulthood.

Parvovirus

*recom*Line Parvovirus B19 IgG [Avidity], IgM



- Precise information about the time of infection
- Parvovirus B19 infection within the last 4 weeks reliably can be excluded
- Excellent diagnostic quality due to internal controls and the use of recombinant VP2 particles

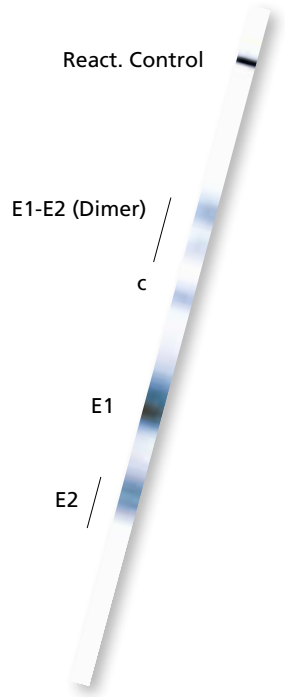
Parvovirus B19 causes the so-called Fifth disease (erythema infectiosum). The disease progresses in 20% of cases without symptoms or with flu-like symptoms. Apart from the exanthema, polyarthralgia and generalized swelling of lymph nodes may occur. After infection during pregnancy there is a high risk of abortion and/or development of hydrops fetalis.

* antigens for avidity testing

Rubella

*recom*Blot Rubella IgG

Western blot technique

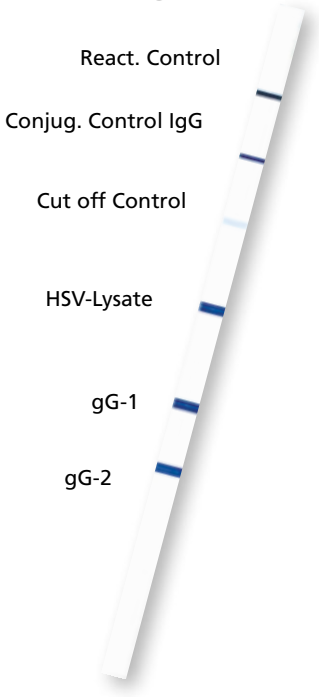


- Rubella virus infection within the last 3 months reliably can be excluded
- Enables to evaluate unclear IgM screening results

German measles caused by the Rubella virus typically appear during childhood and take a mild course in most cases. Primary infection in pregnancy however may result in abortion, premature birth or the "Rubella syndrome", the complete clinical picture for which is known as impairment of the heart, eye and inner ear (classic triad).

Herpes-simplex Virus

*recom*Line HSV-1 & HSV-2 IgG

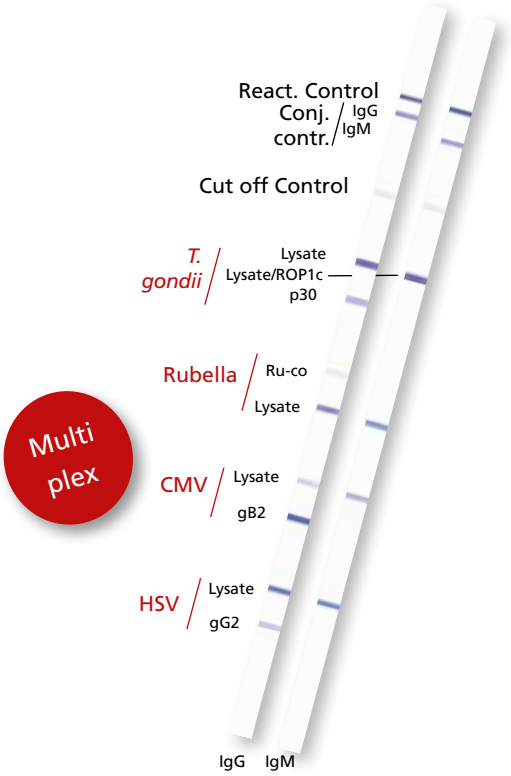


- Serological confirmation of suspicious HSV infections
- Reliable determination of subtype specific HSV serostatus in risk groups
- Risk estimation of primary HSV infection in pregnant women and the development of Herpes neonatorum in newborns

HSV infection of the newborn (Herpes neonatorum) may be caused perinatally during an ongoing primary Herpes genitales infection of the mother and lead to severe symptoms in the newborn. If infection of the pregnant is recognized at an early stage prophylactic measures may prevent transmission to the baby.

TORCH

*recom*Line TORCH Screening IgG, IgM

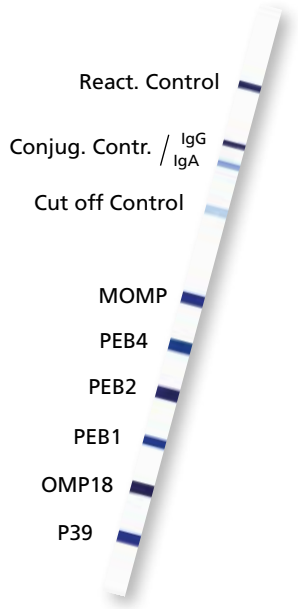


- Screening assay for detection of Toxoplasma, Rubella, CMV and HSV infections
- Additional markers to determine time of infection (CMV, Toxoplasma), protective vs. non-protective immunity (Rubella, WHO standard) or pathogen species (HSV)

TORCH screening is very important to identify pregnant women who are at risk to get an primary infection with potentially dangerous agents. In cases of seronegative pregnants prophylactic measures may protect fetuses and newborns against health damage.

Campylobacter

*recom*Line Campylobacter IgG, IgA

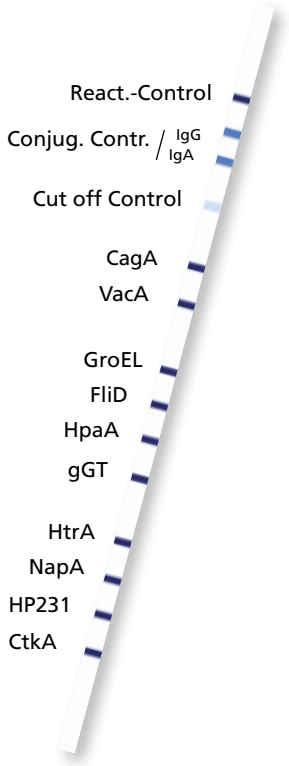


- Detection of IgG and IgA antibodies can be a very useful diagnostic tool if Campylobacter-induced arthritis is suspected

Human Campylobacter infections are mainly food associated intestinal infections with worldwide incidence. Post infectious complications such as Reactive Arthritis can occur a few weeks after the primary infection.

Helicobacter pylori

*recom*Line Helicobacter IgG 2.0, IgA 2.0

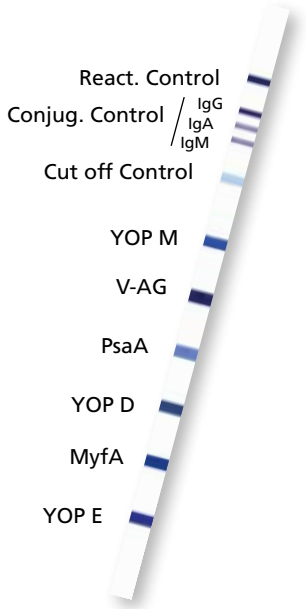


- Identification of highly virulent Helicobacter pylori type I infections by means of CagA
- Sensitivity: > 99% IgG-positive results in patients who are positive according to the gold standard
- Specificity: 100% IgG- and IgA-negative results in patients who are negative according to the gold standard

The bacteria Helicobacter pylori colonise the human stomach and the infection is believed to be linked to various gastric disorders, such as gastric ulcers, gastric adenocarcinomas and MALT lymphomas.

Yersinia

*recom*Line Yersinia IgG 2.0, IgA [IgM] 2.0

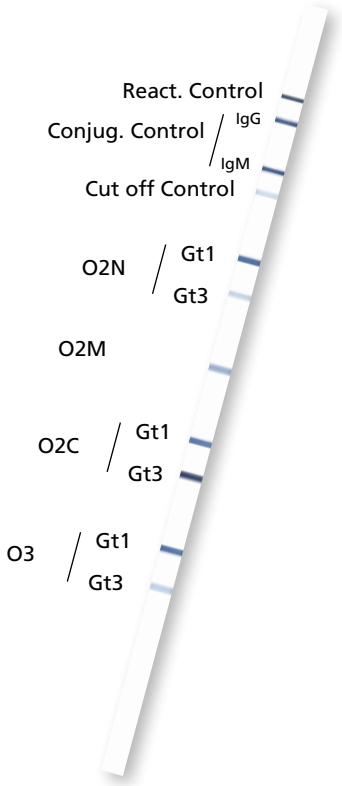


- Identification of all pathogenic Yersinia by means of Yersinia outer proteins (YOPs)
- Serological differentiation between Y. enterocolitica (MyfA) and Y. pseudotuberculosis (PsaA)
- Detection of IgG and IgA antibodies can be a very useful diagnostic tool if Yersinia-induced arthritis is suspected

These pathogens are transmitted orally either in food or in contaminated water. Post infectious complications such as Reactive Arthritis, erythema nodosum and other rheumatic diseases can occur.

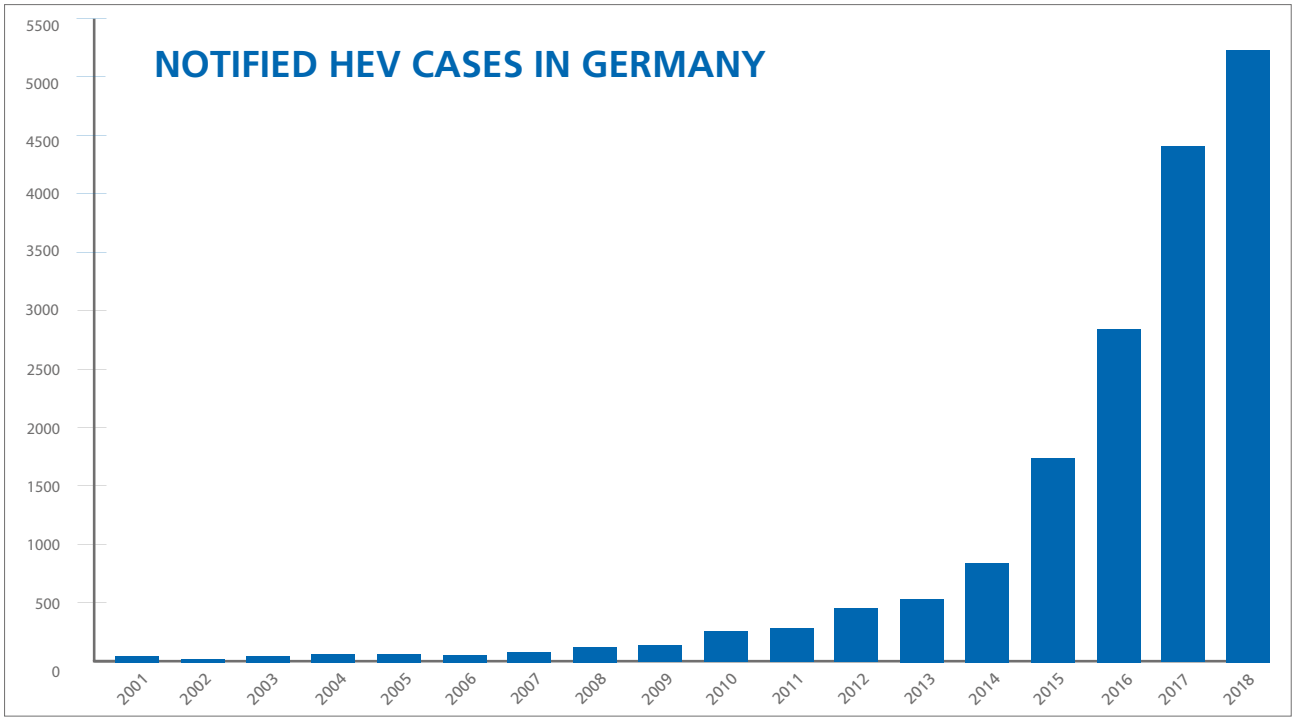
Hepatitis E Virus

*recom*Line HEV IgG / IgM



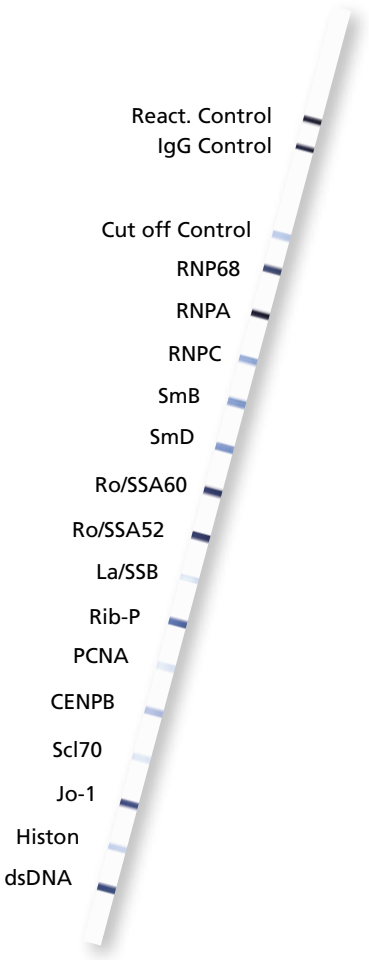
- Homologous antigens of two different genotypes: genotype 1 and genotype 3 ensure high sensitivity
- Detection of all four human pathogenic genotypes occurring worldwide
- Application for confirmation as well as for screening

Hepatitis E virus is one of the most common viral causes of acute hepatitis worldwide. An infection can have an inapparent to fulminant clinical manifestation. There are four human pathogenic genotypes (1–4) described that differ in their geographical distribution, transmission and possible complications. The HEV genotypes 1 and 2 occur primarily in developing countries and the transmission is usually faecal-oral via contaminated drinking water. In industrial countries the HEV genotypes 3 and 4 are widespread and are usually transmitted by infected pork that has not been adequately cooked.



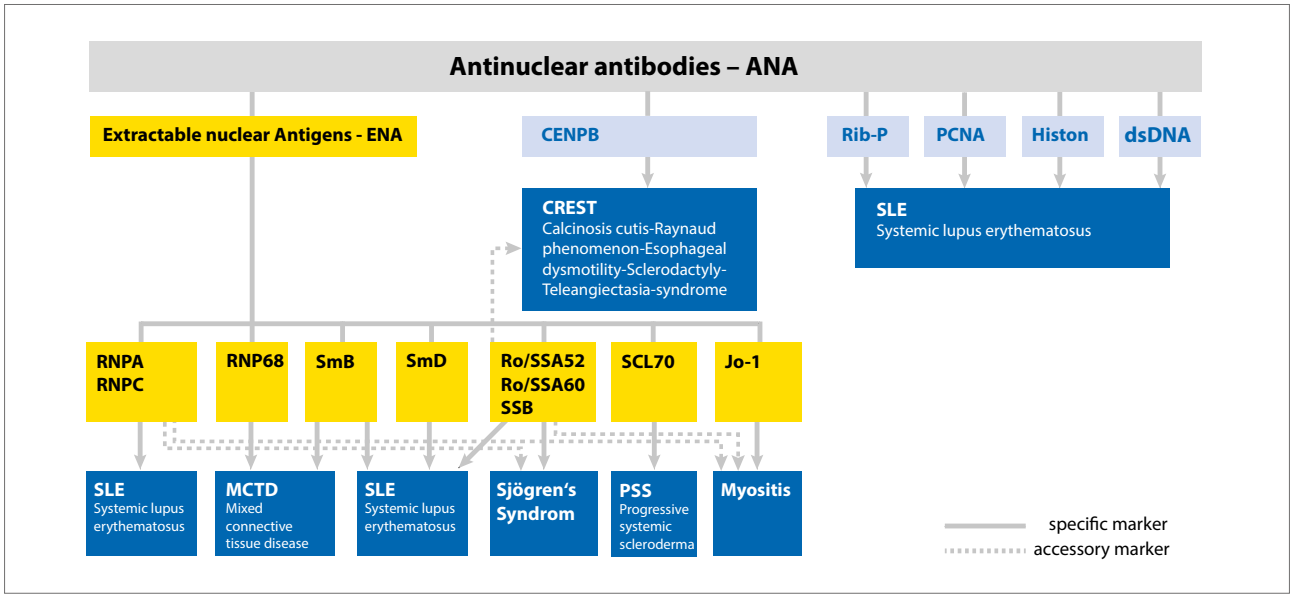
Connective Tissue Diseases

*recom*Line ANA/ENA IgG



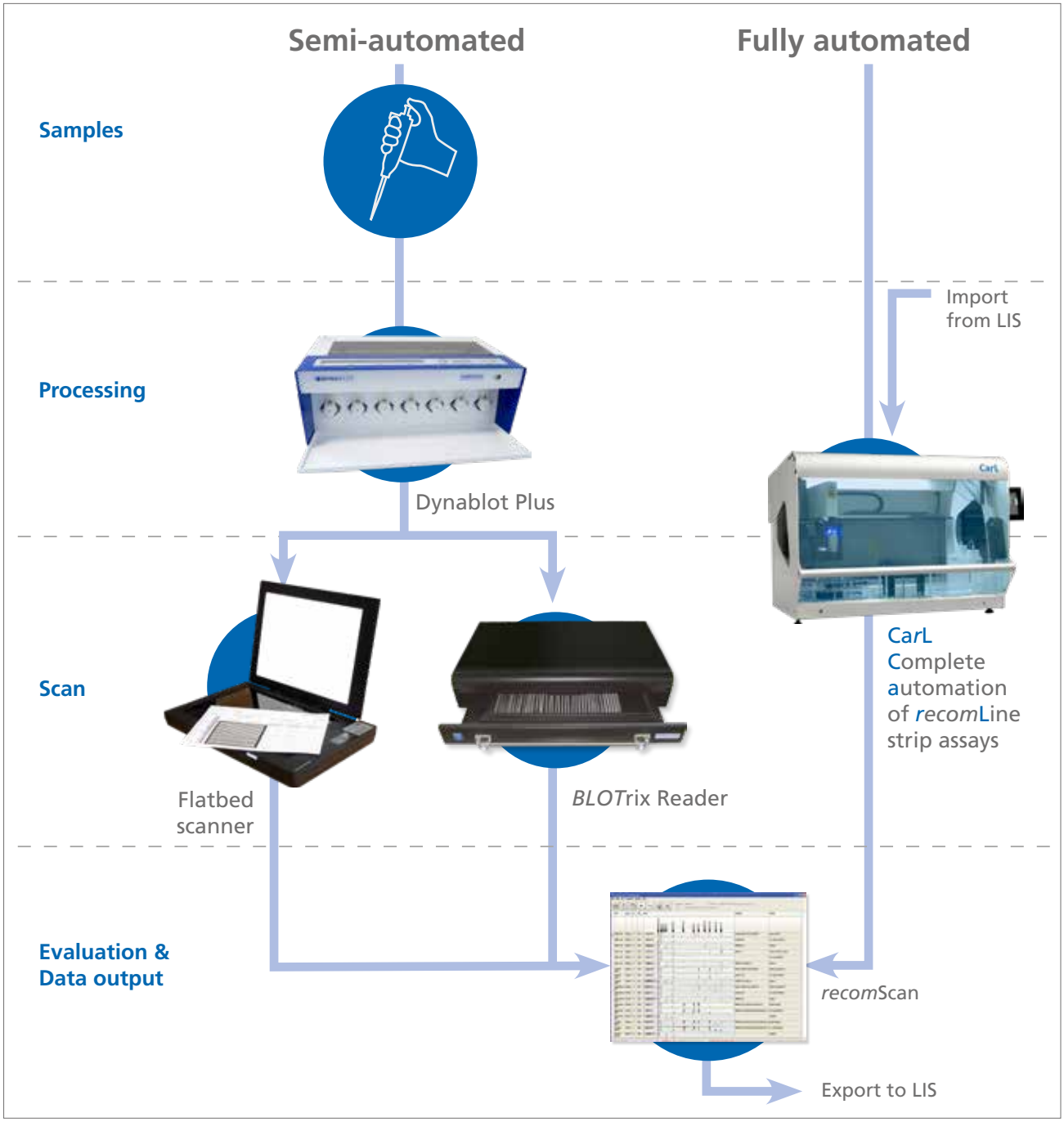
- Differentiation between the most frequent autoimmune collagenose diseases in a single approach by using 15 different antigens:
  - Systemic lupus erythematosus (SLE)
  - Sjögren`s syndrom (SjS)
  - Mixed connective tissue disease (MCTD)
  - Progressive systemic scleroderma (PSS)
  - Myositis
- Highly reliable diagnostic of SLE using subunits of Sm and RNP antigens and high specific SLE markers

Autoantibodies are immunoglobulines which are directed against endogenous structures. They are important diagnostic parameters with unclear pathogenetic significance, but of extraordinary relevance for differential diagnosis. In the case of CTD, the connective tissue is affected, which leads to inflammation, loss of elasticity and joint pain. According to their clinical symptoms, these diseases are assigned to the rheumatic disorders.



MIKROGEN offers two lines of automation solutions for every diagnostic laboratory:
a semi-automated and a fully automated approach. The semi-automated operational procedure includes automated processing of Line Immunoassays with the Dynablot Plus strip processor and scanning of strips with the *BLOT*rix Reader. Line Immunoassays are evaluated in a computer based manner by the MIKROGEN *recom*Scan software for optimal results.
The MIKROGEN gold standard is the fully automated Car*L* system, which combines processing, scanning and evaluation in one platform. Choose Car*L* for your diagnostic laboratory to receive the best performance in respect of security, efficiency and minimal hands-on time.

Workflow



Dynablot Plus

- Ideally suited and validated for the automatic processing of all MIKROGEN strip assays
- Combination of IgG / IgM / IgA and avidity possible in a single run
- **Flexible arrangement of strips:**
  - Sort by IgG / avidity / IgM / IgA per patient
  - Sort by Ig class per run
- **High reproducibility:** Variation dispensing accuracy is < 10%
- Minimal space requirement and low noise during operation
- Perfect addition to the automatic evaluation with *recom*Scan in combination with *BLOT*rix Reader / Flatbed Scanner
- **Easy and user friendly handling:**
  - **"Walk-Away" operation: I**nstrument operates fully automatic after addition of serum samples
  - Clear LCD display with current test status and remaining time
  - Audible indicators (for example, at the end of program)
  - Easy to load and easy accessibility of reagents
  - Built-in error detection and display
- **High safety and reliability:** The Dynablot Plus meets the highest standards according to EC-guidelines 98/79/EC for In-vitro diagnostics

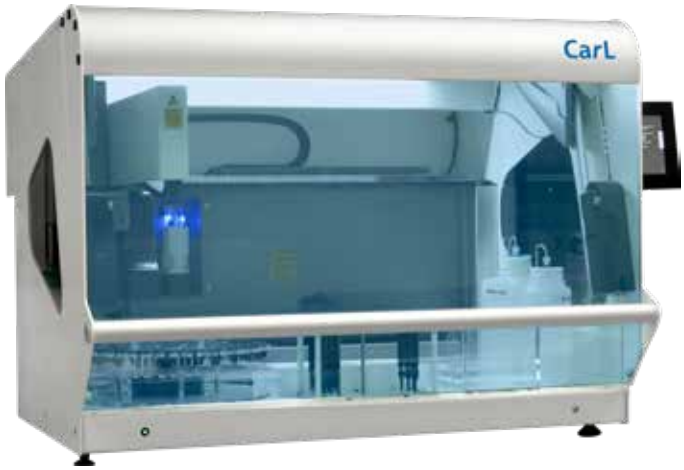
Capacity	Up to 44 strips
Memory space	20
Reagent channels	7
Pumps	7 peristaltic and 1 vac. pump
Rocking shaker	3 speed levels
Dimensions	52,5 x 31 x 25 cm
Weight	14,5 kg
Ports	Serial + USB
Power supply	100 - 240 V
Power consumption	Max. 30 W
Waste container	2 l with liquid level sensor

LINE – SOFTWARE *recom*Scan

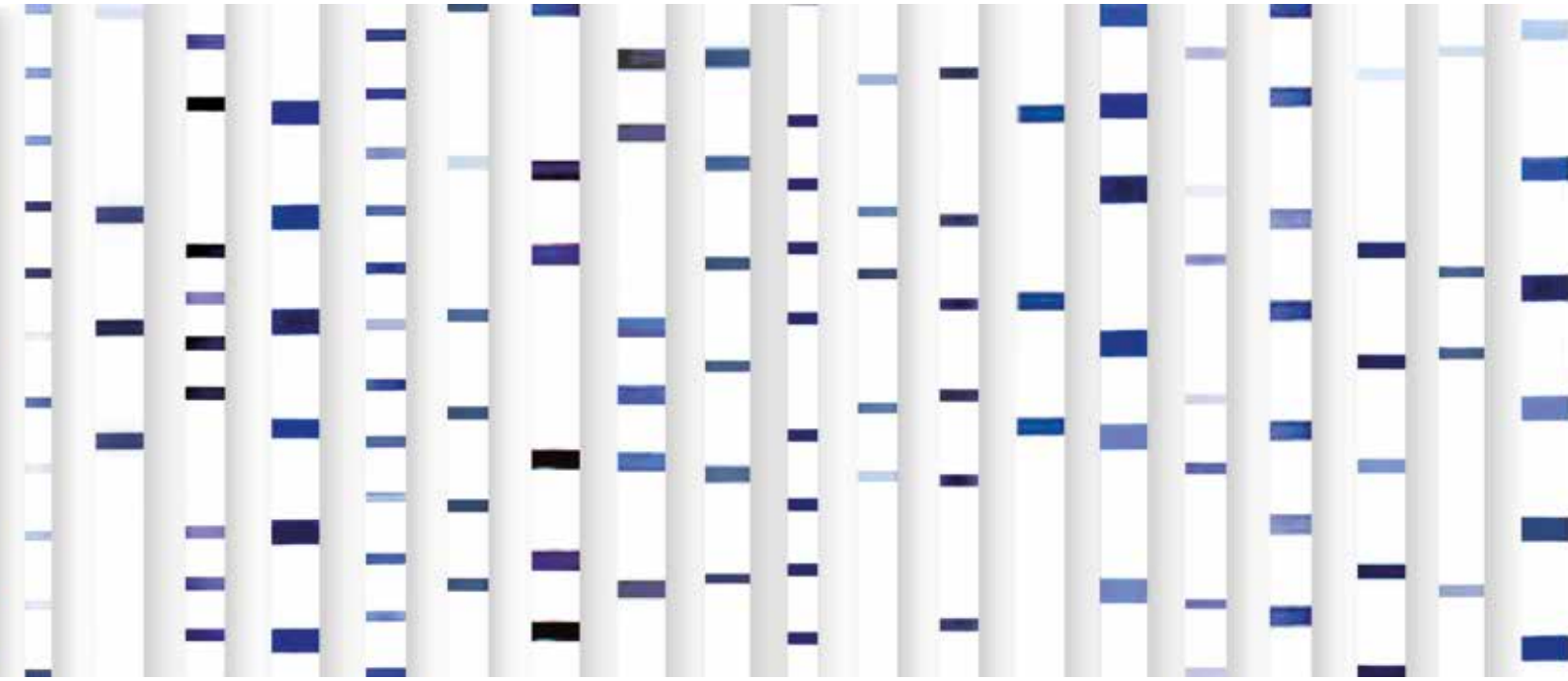
- Fast and simple handling
- Reproducible band analysis
- Interpretation of complex band patterns
- Extensive control and expedient correction options
- Histogram view
- Permanent storage of test strip images, easy data retrieval
- Meaningful reports in different detail variants
- Optional: standardised data exchange with laboratory information system
  - Prevention of reading and transmission errors
  - Import of worklist
  - Detailed export of analysis data
- *recom*Scan meets the highest standards according to EC-guidelines 98/79/EC for In-vitro diagnostics

CarL Complete automation for *recom*Line strip assays

- **Walk-Away:** Full automation from sample to scanning
- **Safe:** Barcode identification and LIS connectivity
- **Convenient:** Integrated drying, 44 strips in one run
- **Flexible:** Combination of all assays with the same sample incubation time
- **Space saving:** Integrated camera for scanning
- **Minimised dead volume:** To save precious reagents
- **No cross-contamination:** Disposable tips for sample pipetting
- **Minimal maintenance:** No daily startup routine and pump calibration required by user
- **Liquid level detection (LLD):** Pressure sensing technology for samples and reagents
- **User-friendly:**
  - ▶ Guided operation via large touch screen
  - ▶ Easy to load and easy accessibility of reagents
- CarL meets the highest standards according to EC-guidelines 98/79/EC for In-vitro diagnostics



Capacity	Up to 44 samples per run
Power	Universal input 100-240 V / 50 - 60 Hz
Sample ID	Integrated barcode reader for sample ID
Carousel for 48 primary tubes	Tube size ranging from 12 mm to 17 mm
Integrated Industrial PC	Integrated PC for connection to LIS and interpretation software
Piston Pump	Volume range 10 µl to 2.5 ml
Peristaltic Pumps	Aspiration and reagent addition
Liquid Level Detection	Pressure sensing technology for samples and reagents
Integrated Camera	Integral camera for image capture of developed strips
Disposable Tips	1 ml and 5 ml disposable tips can be used during the assay
Operator Interface	Integrated touch screen
Footprint	86 cm (W) x 61,5 cm (H) x 69 cm (D). Recommended footprint (incl. touch screen and PC): 182 cm (W) x 99 cm (H open) x 69 cm (D)



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