# Microbiology Focus



## rapidSTRIPE Test System for Detection of Disease Carrying Ticks

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Disease-carrying ticks are becoming more common as global climate change alters our weather. HybriScan Kits for Detection and Identification.....p 4 Legionella Detection ....p 5 Lactobacilli......p 6

SIGMA-ALDRICH®

## Detecting Disease Carrying Ticks with a New Microbiological rapidSTRIPE Test System

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Disease-carrying ticks are becoming more common as global climate change alters our weather; therefore, there is greater concern about the increased potential for parasitic diseases infecting humans after bites from these organisms. But today, detection systems are available for the rapid identification of disease-causing microorganisms found in ticks.

Scanbec GmbH is developing and producing rapid innovative molecular test systems for the detection of microorganisms. In 2003, they developed a test system called HybriScan®, which is now distributed by Sigma-Aldrich. Applications for the HybriScan system are in the food and beverage industry, water analysis, and medical diagnostics. The adaptability of the HybriScan technology makes possible the development of beneficial universal methods and is a good base for developing new testing systems. Scanbec, an accredited laboratory, also provides micro- and molecular-biological services.

Due to increasing temperatures and changing climatic conditions, ticks have become more and more dangerous. These organisms are able to transmit parasitic diseases, the most prominently known of which is called Borreliosis or Lyme disease.

Removing and then analyzing the tick itself, rather than testing the human who has been bitten, for evidence of pathogens is of extraordinary value to both the attending doctor and the concerned person. Everyone receives fast and reliable results, allaying fears about infection faster than ever before. Scanbec uses a new rapid test system called rapidSTRIPE assay that is able to detect and identify borreliosis (Lyme disease) pathogens in ticks within two hours; the test is conducted in collaboration with the company Analytik Jena AG bio solutions.

> There has been a proven increase in tick populations within the

> > last



Figure 1: Borrelia lateral flow assay (Analytik Jena)

few years; thus the risk of being bitten by a tick has drastically increased as well. Before the new system, people who were bitten by ticks had to wait four to six weeks after being bitten to be tested; the waiting time served to allow the person's immune system time to produce antibodies against the pathogens, antibodies that would then be detected using common blood tests. The direct detection of pathogens in the tick enables diagnosis and treatment of infection in its earliest stages.

Using this new test method, the removed tick can be fixed on a strip of tape and sent to Scanbec's laboratory by regular mail.

The detection of *Borrelia* that are transmitted via ticks occurs by a direct amplification of target DNA isolated from the tick sample. The analysis is performed in three steps: First, the tick is mechanically destroyed in order to isolate total DNA from the tick tissue (rapid-STRIPE Tick DNA Exytraction Kit; Analytik Jena). Then the *Borrelia* DNA is specifically amplified using rapid PCR (SpeedCycler; Analytik Jena). During PCR, *Borrelia*-specific primers are used to amplify the pathogen's DNA, detecting and amplifying all different *Borrelia* species. The last step entails the detection of the amplified *Borrelia* DNA using a stripe assay (Lateral Flow Assay; Analytik Jena). The amplified *Borrelia* DNA is applied to the test strip and the results become visible after a few minutes. Two violet lines indicate the presence of *Borrelia* species bacteria in the tick. One violet line indicates that the tick was free of all *Borrelia* species (Figure 1).

The accuracy of the rapid tick test for the detection and identification of *Borrelia* species was validated by the company Analytik Jena AG. Four hundred ticks were tested, and all positive results were then sequenced to verify the obtained results and to classify the detected *Borrelia* species. The sample pool of 400 included ticks of the following types: *Ixodes ricinus, Dermacentor reticulatus, Ixodes hexagonus, and Haemaphysalis concinna.* 

Tick species	Number		Borrelia species
	Total	<i>Borrelia</i> positive	(determined via sequencing)
Dermacentor reticulatus	36	2	B. afzelii
Ixodes hexagonus	1	-	
Haemaphysalis concinna	5	1	n.n.
Ixodes ricinus	358	56	B. afzelii, B. garinii
Total	400	59	

Table 1: Results of the validation study performed by Analytik Jena AG

After the detection of *Borrelia* in a tick, the identification stage of the test system begins and the particular species of *Borrelia* is determined by sequencing. With these results, the attending physician can predict which symptoms are likely to manifest in the patient. For example, *B. garinii* mediates neuroborreliosis (a disorder of the central nervous system), and *B. afzelii* is known to mediate *Erythema migrans* (causes areas of reddened skin 5 to 6.8 cm in diameter). As treatment of borreliosis is complicated and difficult, knowledge of which *Borrelia* species has infected the patient can be of great help.

It is important to note that a positive result for the presence and identification of *Borrelia* species is only the first part of a larger process to deal with the infection. Scanbec recommends in all cases of positive *Borrelia* test results that the person in question follow up promptly with a doctor for examination and further testing, including early-stage blood or serum sample collection to monitor the patient's serological processes (*Borrelia* antibody status), to see whether symptoms of borreliosis emerge and to ensure effective treatment with the appropriate antibiotics.

Scanbec also offers test systems (Analytik Jena AG) for the detection and identification of pathogenic *Rickettsia, Babesia,* and *Anaplasma* species in ticks. These tests work on

the same principle as the *Borrelia* test system, differing only in the species-specific PCR primers used for rapidPCR (SpeedCycler; Analytik Jena) amplification of the DNA of the respective microorganisms.

Like *Borrelia*, all three of these pathogens can be transmitted by ticks to humans. A growing tick population, caused by global climate change, increases the likelihood of tick-borne infections like borreliosis/Lyme disease, tick-borne meningoencephalitis (TBEVirus), and rickettsiosis in humans.

*Rickettsia* species are found in the intestine and intestinal epithelia of arthropods like ticks. With this disease, detection during the early stages of infection means an easy treatment with antibiotics, thus the new rapid test time is key to a better outcome for the patient.

A recent survey on the proliferation of pathogens in ticks revealed that *Rickettsia* species are occurring with unexpected frequency in ticks. *Rickettsia* are able to survive only in endothelial cells; therefore, traditional testing for these bacteria involved proliferation by means of cell culture, which is highly time- and costintensive. Further, reliable confirmation of the presence of *Rickettsia* is only possible by nucleic acid based detection by PCR. This new system provides a faster and more cost-effective alternative to these traditional tests. The new tick test for detection of *Rickettsia* was also validated by Analytik Jena AG. Again, a sample pool of 400 ticks was examined for the occurrence of *Rickettsia* to evaluate the specificity and accuracy of *Rickettsia* detection results.

*Rickettsia* positive samples were sequenced to verify the obtained results and to classify the detected *Rickettsia* species. The following tick species were examined: *Ixodes ricinus*, *Dermacentor reticulatus, Ixodes hexagonus*, and *Haemaphysalis concinna*.

Ticks are also able to transmit animal pathogens like *Babesia bovis* and *Babesia bigemenia* (tick fever or cattle fever). Both *Babesia* species are animal pathogens that usually affect mice, cattle, horses, and dogs; in exceptional cases, however, humans can be infected after being bitten by a tick carrying the protozoa. *Babesia divergens* and *Babesia microti* are also widespread through Europe and North America and can cause influenza-like symptoms such as fever, ague, arthralgia, thrombopenia, haemolytic anaemia, and haemoglobinuria in humans.

Ticks feeding on infected animals absorb the *Babesia* with erythrocytes. Within the intestine of the tick, the *Babesia* bacteria are released from the erythrocytes and start to proliferate. The *Babesia* then divide into thousands of sporozoites, the infectious stage of the parasite, in the saliva of the tick and are transmitted to a new host when the tick moves on.

Anaplasma phagocytophila is able to proliferate in leukocytes, especially in granulocytes. Transmission of anaplasmosis occurs, according to the pertinent literature, mainly via ticks. Anaplasma phagocytophila (formally known as Ehrlichia phagocytophila) causes human granulozytic anaplasmosis (HGA) and is prominent in southern and eastern Europe. Humans infected with anaplasmosis exhibit symptoms like fever, head and limb pain, gastrointestinal and pulmonary symptoms, and exanthema.

Tick species	Number		Rickettsia species
	Total	<i>Rickettsia</i> positive	(determined via sequencing)
Dermacentor reticulatus	36	16	<i>R. slovaca, R.sibirica sibirica</i> (Subtype Chabarowsk)
Ixodes hexagonus	1	-	
Haemaphysalis concinna	5	-	
Ixodes ricinus	358	39	R. helvetica
Total	400	55	

Table 2: Results of the validation study performed by Analytik Jena AG

# HybriScan Rapid Detection and Identification Kits

Jvo Siegrist, Product Manager Microbiology.... ivo.siegrist@sial.com

#### For detection and identification of microorganisms in food, beverages and waste water

The HybriScan<sup>®</sup> method has many advantages over conventional methods and give microbiologist an exciting new tool for microbial detection and identification. The kits can be used for a broad range of routine microbial diagnostics in food and beverage, medicine and pharmaceutical industries.

- Rapid Time-savings compared to cultivation-based systems
- Sensitive Many ribosomes in one cell
- Easy handling
- Reliable rRNA method
- Does not detect dead cells
- Economical No PCR, uses 96-well microplate format
- Minimal interference by sample matrix
- Detects many nonculturable microbes
- Quantitative as well as qualitative

#### **Application range:**

Genus	Species	<b>Groups/Applications</b>
Lactobacilli Legionella Listeria (validated to LFGB § 64) Salmonella (validated to LFGB § 64) Campylobacter (validated to LFGB § 64) Megasphaera Leuconostoc	E. coli Legionella pneumophila Listeria monocytogenes (validated to LFGB § 64) Microthrix parvicella Brettanomyces Candida albicans Lactobacillus brevis Lactobacillus brevis Lactobacillus buchneri Lactobacillus lindneri Pectinatus cerevisiiphilus Pectinatus cerevisiiphilus Pediococcus damnosus Enterobactar sakarakii	Waste Water Index Beer Spoilage Organisms Beverage Spoilage Organisms Total Bacterial Count Yeasts

Table 1: Application range of the HybriScan kits



Figure 1: Principle of test system

The HybriScan method is based on the detection of rRNA via hybridization events and specific capture and detection probes (Figure 1). Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labelled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin-labelled detection probe provides an enzyme-linked optical signal read out. Detection is via application of anti-DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB (3,3',5,5'-tetramethylbenzidine). Measurement is via absorbance at 450 nm compared with standard solutions.

Cat. no.	Brand	Description Re	actions
62533	Fluka	HybriScan <b>D</b> Beer	96
56917	Fluka	HybriScan <b>D</b> Campylobacter	96
68301	Fluka	HybriScan <b>D</b> Drinks	96
96343	Fluka	HybriScan <b>D</b> E. coli	96
12838	Fluka	HybriScan <b>D</b> Enterobacter sakazaki	i 96
59744	Fluka	HybriScan <b>D</b> Lactobac	96
16593	Fluka	HybriScan <b>D</b> Legionella	96
07190	Fluka	HybriScan <b>D</b> Legionella pneumophila	96
55661	Fluka	HybriScan <b>D</b> Listeria	96
49699	Fluka	HybriScan <b>D</b> Listeria monocytogenes	96
55662	Fluka	HybriScan <b>D</b> Salmonella	96
02349	Fluka	HybriScan <b>D</b> Total Bacterial Count	96
04447	Fluka	HybriScan <b>D</b> Waste Water Microthrix parvicella	96
78436	Fluka	HybriScan <b>D</b> Waste Water Total Bacterial Count	96
61397	Fluka	HybriScan <b>D</b> Yeast	96
79742	Fluka	HybriScan <b>l</b> Brettanomyces	48
19503	Fluka	HybriScan <b>l</b> Candida albicans	48
76545	Fluka	HybriScan <b>l</b> E. coli	48
75724	Fluka	HybriScan I Lactobacillus brevis	48
80065	Fluka	HybriScan <b>l</b> Lactobacillus buchneri	48
86827	Fluka	HybriScan <b>l</b> Lactobacillus lindneri	48
49417	Fluka	HybriScan <b>l</b> Legionella pneumophila	48
77007	Fluka	HybriScan <b>l</b> Leuconostoc	48
49712	Fluka	HybriScan <b>I</b> Listeria monocytogenes	48
42875	Fluka	HybriScan <b>l</b> Megasphaera	48
89384	Fluka	HybriScan <b>l</b> Pectinatus cerevisiiphilus	48
73582	Fluka	HybriScan <b>l</b> Pectinatus frisingensis	48
67289	Fluka	HybriScan <b>l</b> Pediococcus damnosus	48
44492	Fluka	HybriScan Software	

**Table 2:** HybriScan<sup>®</sup> products (HybriScan<sup>®</sup>**D** = detection kit; HybriScan<sup>®</sup>**I** = identification kit)

## Legionella Detection

By Jvo Siegrist, Product Manager Microbiology.... ivo.siegrist@sial.com

*Legionella* contamination in air conditioners and water supply systems poses a serious health concern. Sigma-Aldrich offers traditional media and innovative, award-winning kits for the specific, rapid and reliable detection of this pathogen.

*Legionella* is a Gram negative, rod-shaped bacterium commonly found in aquatic environments. Some species have been isolated from soil. The organism can survive a wide range of conditions, including temperatures from 0 to 63°C, and pH values from 5.0 to 8.5. Because *Legionella* has cysteine as a growth requirement, it does not grow on common blood agar media.

Legionella species cause Pontiac fever (named from a 1968 outbreak in Pontiac, Michigan) and Legionnaires disease (named from a 1976 outbreak in Philadelphia, Pennsylvania at an American Legion convention). In 1977 the new pathogen was identified and named *Legionella* (see Figure 1). The disease is contracted by inhaling water vapour contaminated by the *Legionella* bacteria. Symptoms are flu-like, with chills, muscle aches and fever. Death can result from severe pneumonia. The Philadelphia outbreak sickened hundreds and resulted in 34 deaths.

The traditional method to detect *Legionella* is based on buffered charcoal yeast extract (BCYE) agar. For growth, the organism requires the presence of cysteine and therefore does not grow on common blood agar media. Common laboratory procedures recommend concentrating the sample by centrifugation and/or filtration through 0.2  $\mu$ m filters before inoculation. For selective isolation, antibiotics like polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple are added. Additionally, selectivity can be attained by applying heat or acid.



**Figure 1:** A single strain LPO2 *Legionella* pneumophila bacterium isolated from the 1976 Philadelphia outbreak. The bacteria is just under 1 µm in width.

Source: Maria Ericsson, Russel Vance (Harvard Medical School Electron Microscopy Facility); modified by Sigma-Aldrich: R. Freuler.

Brand	Cat. No.	Name	Description
Fluka	86558	Buffered Charcoal Yeast Extract Agar (Base)	For selective cultivation of Legionella species with Legionella Supplement (Fluka 92394) and Legionella Selective Supplement IV (Fluka 94029)
Fluka	05598	Feeley Gorman Agar	Feeley Gorman Agar is recom- mended for isolation and presumptive identification of <i>Legionella</i> species. To make the media selective optional <i>Legionella</i> Selective Supplement (Fluka 18284) can be added

 Table 1: Media for detection isolation and differentiation of Legionella species, specially Legionella pneumophila



1. Sample filtration (100-1000 mL water, 15 min)



**3. Cell lysis / rRNA recovery** (2 mL sample, 13'000 rpm, 37°C, 45-60 min)



2. (optional) Enrichment (BCYE-medium, 36°C)



4. HybriScan®- Test Solution (Forming of sandwich complexes between specific probes and the sample, 10 min)



5. Immobilisation (Binding of the "sandwiches" to the binding plates)



6. Enzyme coupling (Coupling of an enzyme to the "sandwhiches", 10min)



**7. Washing** (Removel of unbound components, 2 x 1 min)



9. Meassures / Calculations (HybriScan<sup>®</sup> Sofware)

Figure 2: Workflow for the HybriScan kits



8. Detection reaction (chromogenic Enzyme reaction, 15 min)



## Did you know...

#### that spa pools could be dangerous?

The temperature and the circulation of water and air, along with dirt, hair, dead skin cells, etc. from bathers provide optimal growth conditions for Legionella bacteria.

In June 2004, the BBC reported that an elderly man died after inhaling Legionella while shopping in a spa bath showroom.

### New Kits for Legionella Detection

Sigma-Aldrich has developed a new kit based on the detection of *Legion-ella* rRNA. The test is performed on a microtitre plate and takes less than 2.5 hours. Cell count quantification is possible with this kit using photometric methods. Compared to PCR, our system does not count dead cells, is much easier to use (see work flow in Figure 2), is less expensive, and is not affected by the sample matrix. Confirming its utility, our new test system won several innovation awards, and will surely become a routine method to detect *Legionella*. The rapidity, sensitivity, reliability, robustness, adaptability to sample matrix, and time-savings meet today's analytical microbiology demands. For more details of our new *Legionella* detection kit, please visit *www.sigmaaldrich.com/hybriscan*.

Brand	Cat. No.	Name	Description
Fluka	16593	HybriScan <b>D</b> <i>Legionella</i>	Detection of <i>Legionella</i> , including <i>L. pneumo phila</i> in water supplies and air- conditioning systems
Fluka	07190	HybriScan <b>D</b> Legionella pneumophila	Detection and identification of <i>L. pneumo phila</i> in water supply and air-conditioning systems
Fluka	49417	HybriScan <b>l</b> Legionella pneumophila	Identification of Legionella pneumophila

Table 2: HybriScan kits for detection and identification of Legionella species

## Lactobacilli

By Jvo Siegrist, Product Manager Microbiology.... ivo.siegrist@sial.com

## This large group plays an important and varied role in human health

#### **Structure and Metabolism**

Lactobacilli are rod-shaped, Gram-positive, fermentative, facultative anaerobic or microaerophilic organotrophs. Normally they form straight rods but under certain conditions spiral or coccobacillary forms have been observed. In most cases they form chains of varying length. Lactobacilli belong to the lactic acid bacteria and comprise the major part of this group. As their name implies, they produce lactic acid and derive energy from the fermentation of lactose, glucose and other sugars to lactate via homofermentative metabolism. About 85-90% of the sugar utilized in the fermentative process is converted to lactic acid. However, there are some heterofermentative lactobacilli that produce alcohol in addition to lactic acid from sugars. This acid-producing mechanism inhibits growth of other organisms and favours the growth of lactobacilli that thrive in low pH environments. ATP is generated during the process by non-oxidative substrate-level phoshorylation.

# *Lactobacillus* species can be divided into three groups according their metabolism (according Kandler and Weiss):

- Obligately homofermentative (Group I) L. acidophilus, L. delbrueckii, L. salivarius, L. helveticus
- Facultatively heterofermentative (Group II) L. casei, L. plantarum, L. curvatus, L. sakei
- Obligately heterofermentative (Group III) L. brevis, L. buchneri, L. fermentum, L. reuteri

Lactobacilli have a generation time ranging from 25 to several hundred minutes. The optimal growth temperature ranges from 30 to 40°C, although some thermophilic strains grow well and have highly activated metabolism at temperatures around 45°C.



Figure 1: rod-shaped Lactobacillus bacteria

#### Natural source

Lactobacilli are ubiquitous and normally harmless. In humans and animals they are found in the intestinal tract and perform many beneficial functions, including immunomodulation, suppression of enteric pathogens and maintenance of intestinal flora. On the negative side, Lactobacilli decompose plant material and are responsible for spoiling vegetables, fruits, beverage and other nutrients. *L. casei* and *L. brevis* are two of the most common beer-spoilage organisms.

#### Usage of lactobacilli

Life without Lactobacilli is unimaginable: They are used in the production of yogurt, cheese, chocolate, sourdough bread, sauerkraut, pickles, kimchi (a traditional Korean pickled dish), beer, wine, cider and many other fermented foods. Lactobacilli are also important in animal feed (silage) production. They produce lactic acid, lower the pH and thereby inhibit growth of bacteria.

Many studies have shown the beneficial effect of a healthy intestinal Lactobacilli-containing microflora. Their potential therapeutic roles in-

clude anti-inflammatory, anti-cancer, and boosting the immune system, among other benefits. As a result, Lactobacilli are often added to

foods as a probiotic supplement.

#### Identification of Lactobacilli and Bifidobacteria

Today it is standard practice to differentiate lactobacilli based on their phenotype using selective media. Classical phenotypic tests

for identification of lactobacilli are based on physiological characteristics, like motility, growth temperature, respiratory type and growth in sodium chloride, as well as on diverse biochemical characteristics, such as fermentation type, metabolism of carbohydrate substrates, production of lactic acid isomers, coagulation of milk and presence of specific enzymes like arginine dihydrolase. In Bergey's Manual, *Lactobacillus* is described as a Gram-positive rod, non-spore forming, acid fast negative and catalase negative. The colony morphology on certain media is taken for the presumptive identification. There are also three API tests (API 50 CH, LRA Zym and API Zym) for the identification, but the reliability of these tests has been questioned (1). Another interesting method is the protein fingerprint, where an SDS gel electrophoresis is made of the whole bacteria cell.

As modern alternative, molecular biology-based methods, like PCR, can be consulted. However, they are often quite expensive. Under our Fluka Brand, Sigma-Aldrich provides a revolutionary molecular biology method that is rapid, easy and cost effective. Based on the detection of rRNA, this method completely avoids the needs for PCR amplification. The sandwich hybridisation test, called HybriScan, is performed on a microtiter plate. The range of Lactobacilli detected by HybriScan tests is listed in **Table 2**.

More information about the test and the technical principles behind it are found in the article "HybriScan® Rapid Detection and identification Kits" found in this issue of Microbiology Focus, as well as on our web site *sigma-aldrich.com/hybriscan*.

Cat no.	Brand	Description
17123	Fluka	Elliker Broth
17154	Fluka	Lactobacillus bulgaricus Agar (Base)
17158	Fluka	Litmus Milk
17153	Fluka	LS Differential Agar
69964	Fluka	MRS Agar
41782	Fluka	MRS Agar, Vegitone
69966	Fluka	MRS Broth
38944	Fluka	MRS Broth modified, Vegitone
75405	Fluka	Orange-serum Agar
2538	Fluka	Raka Ray Agar, Base
83920	Fluka	Rogosa Agar
R1148	Fluka	Rogosa SL Agar
85515	Fluka	Sorbic acid Agar (Base)
17216	Fluka	Tomato Juice Agar
17218	Fluka	Tomato Juice Broth
T2188	Fluka	Tryptone Glucose Yeast Extract Agar
17215	Fluka	WL Differential Agar
W2136	Sigma	WL Differential Broth
17222	Fluka	WL Nutrient Agar
W2261	Fluka	WL Nutrient Broth
Y3127	Fluka	Yeast Malt Agar

 Table 1: A selection of media for Lactobacilli, for the complete list see

 www.sigma-aldrich.com/lactobacilli.

Cat. no.	Brand	Description	Specifity	Reactions
62533	Fluka	HybriScan <b>D</b> Beer	Detects all beer spoiling bacteria of the genera Lactobacillus, Pediococcus, Pectinatus and Megasphaera	96
68301	Fluka	HybriScan <b>D</b> Drinks	Detects yeast of the genera Saccharomyces, Zygosacchromyces, Brettanomyces, Torulaspora, Pichia, Candida and bacteria of the genera Lactobacillus, Acetobacteraceae and Alicyclobacillus	96
59744	Fluka	HybriScan <b>D</b> Lactobac	Detects bacteria of the genera Lactobacillus and Pediococcus in fruit juices and non-alcoholic beverage.	96
75724	Fluka	HybriScan <b>l</b> Lactobacillus brevis	Identification of Lactobacillus brevis	48
80065	Fluka	HybriScan <b>l</b> Lactobacillus buchneri	Identification of Lactobacillus buchneri	48
86827	Fluka	HybriScan <b>l</b> Lactobacillus lindneri	Identification of Lactobacillus lindneri	48

Table 2: HybriScan products (HybriScanD = detection kit; HybriScanI = identification kit)

#### **References:**

Figure 2: Probiotic yoghurt (lacto-

bacilli and their probiotic activities)

1. Coeuret, V.; Dubernet, S.; Bernardeau, M.; Gueguen, M.; Vernoux, J. P. Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products, Lait, 2003, 83, 269–306.

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