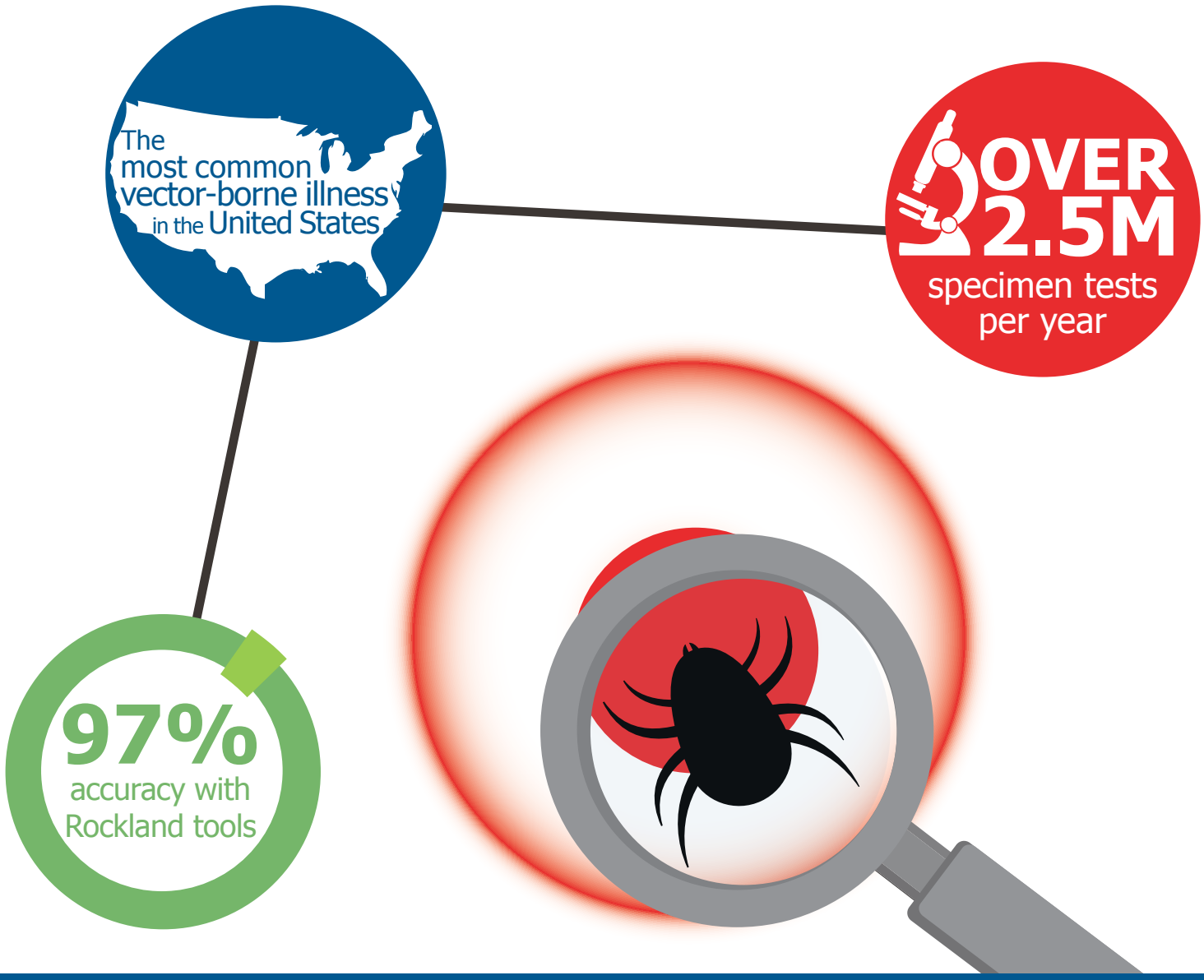
ROCKLAND™
antibodies & assays

LYME DISEASE

THE CURRENT SITUATION & OUR SOLUTIONS

Lateral Flow Data • Proof of Concept Studies • Core Technology
Sequence Analysis & Protein Expression



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Lyme Disease: Overview

Current Situation:

Lyme disease (LD) is caused by spirochetal bacteria from the genus *Borrelia*. *Borrelia burgdorferi* is the predominant cause of Lyme disease in the United States, whereas *Borrelia afzelii* and *Borrelia garinii* are predominantly implicated in most European incidences. The CDC estimates about 30,000 confirmed US cases of LD per year since 2008, but suggests the number may be closer to 300,000. Each year 2.5 million serological LD assays are performed, many of these are repeat tests. The recommendations by the CDC for the diagnosis of LD include a two-tiered approach: an initial ELISA-based screening test in conjunction with western blotting to establish the presence of anti-Borrelia antibodies.

The Problem:

Presently available LD tests are not only time consuming, but also display a high probability of false negative and/or positive results. One drawback of the current method is that *B. burgdorferi* protein cell lysates used for western blotting contain numerous highly conserved proteins that can result in false positive results when detected by antibodies against other bacterial infections. Another drawback is that individuals vary in the content of circulating anti-LD antibodies, and thus may not have the reactive-antibodies that are needed for a functional Lyme diagnostic test using the current ELISA and other testing platforms. Currently used LD assays utilize either a single Lyme disease antigen or perhaps a select few Lyme disease antigens to detect circulating LD-specific antibodies present in the patient samples. However the complex life cycle of *B. burgdorferi* confounds the use of a single protein antigen as a diagnostic marker, and the existing assays often do not properly diagnose the presence or absence of Lyme disease.

CDC Recommended Lyme Disease Testing	
2-Tier is Gold Standard	2-Tier requires several days to complete
ELISA	Low Throughput
Western Blot	Subjective Interpretation

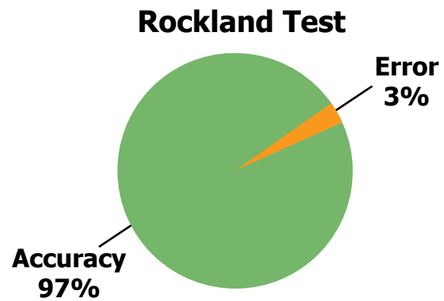
Complicating proper diagnosis, *B. burgdorferi* exists in several life cycle phases, and different protein subsets are expressed in each phase. The LD-causing spirochete bacteria transitions from the deer tick vector to mammalian hosts, including pets, livestock, and humans. At each unique environment *B. burgdorferi* undergoes rapid adaptive gene expression in response to environmental signals encountered during the different stages of its life cycle. During these transitions, the bacterial protein signature is modulated to allow for persistent infection. The host will only make antibodies to the proteins being expressed or the strongest antigens like VlsE. Detecting anti-B. burgforferi proteins is thus complicated.

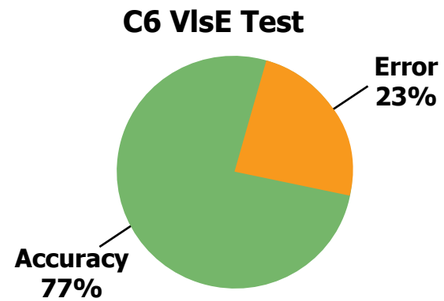
Lateral Flow Data

Rockland's Solution:

Rockland's solution engages the evasive nature of *B. burgdorferi* and its life-cycle changes and allows for the detection of the unique response of each individual patient. To achieve this Rockland has created a research toolbox matrix that specifically addresses the 3 known phases of infection; early, latent, and chronic. Rockland has cloned, expressed and purified 14 *Borrelia*-specific antigens that have shown promise for use in the development of hand held point-of-care (POC) and multiplex diagnostic detecting anti-Borrelia-specific IgM and IgG antibodies in human serum. These 14 antigens, expressed during different stages of the disease allow for detection of LD in patients from any stage of LD.

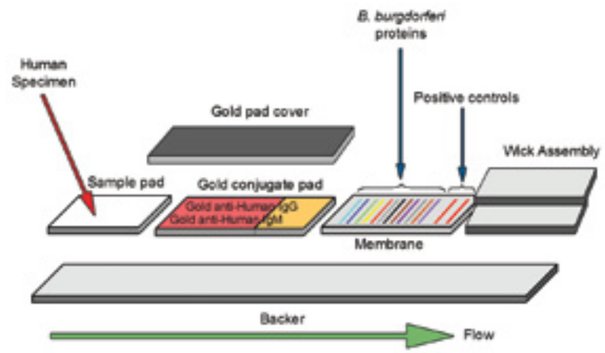
- The specificity of the Rockland test suggests superior performance over other LD assays
- The Rockland test correctly identifies patient samples as LD negative whereas the currently used C6 VlsE ELISA test falsely identifies these samples as positive to LD.





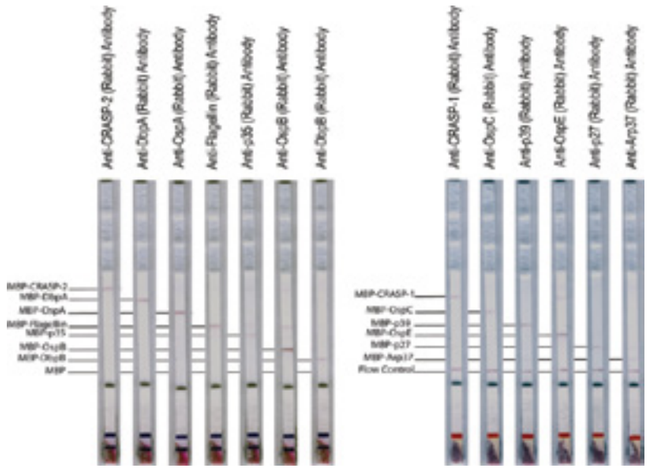
Lateral Flow Technology

Lateral Flow Technology is well established and forms the basis of numerous strip-based tests including consumer pregnancy tests and some HIV tests. Development of a Point-of-Care (POC) strip test for the detection of LD-specific antibodies would follow the layout presented below. Anti-LD antibodies present in human whole blood or serum are applied to the sample pad and migrate by capillary action over the gold conjugate pad binding to gold anti-human Ig antibodies present in the pad forming a complex. The complex flows over and binds to respective *B. burgdorferi* proteins imprinted on the membrane yielding a band indicating the presence of specific circulating antibodies in the specimen. Lateral movement of the antibody complex by capillary action is further promoted by the wick assembly found at the bottom end of the POC testing device.



Proof of Concept

Rockland rabbit polyclonal antibodies raised against Bb proteins proved that the test strip configuration was functional.



i. *B. burgdorferi* antigens are properly immobilized on the strip

ii. Gold cooloid particles are properly conjugated to respective antigens

iii. Antibody/gold conjugated antigen complex is target specific

iv. There is no non-specific cross-reactivity

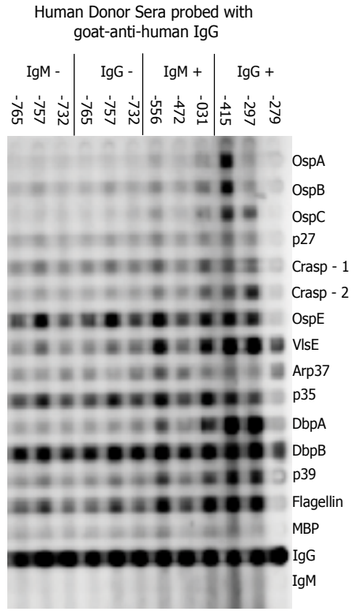
v. The lateral flow assay performs correctly

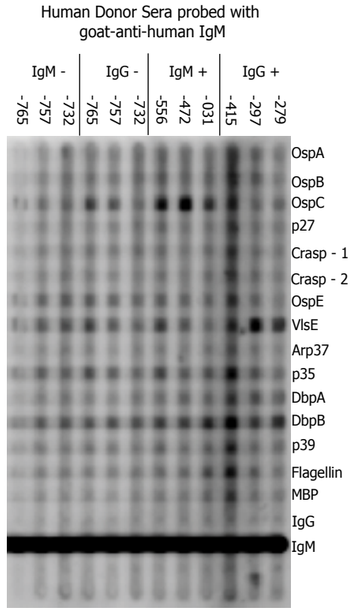
vi. Species independent

Polyclonal antibody reactivity to specific membrane immobilized *B. burgdorferi* proteins in the order shown to the left.

Proof-of-Concept Studies:

To determine the functionality of a strip-based diagnostic test for the detection of circulating LD-specific antibodies, 14 recombinant LD proteins were imprinted onto a nitrocellulose membrane using a slot blotter. In addition, secondary antibody-specific controls (IgG and IgM) as well as MBP were absorbed onto the membrane prior to blocking. Confirmed positive and negative LD human sera were used to bind recombinant proteins followed by the addition of either peroxidase (HRP) conjugated anti-human IgG F(c) or anti-human IgM Fc5m secondary antibodies.



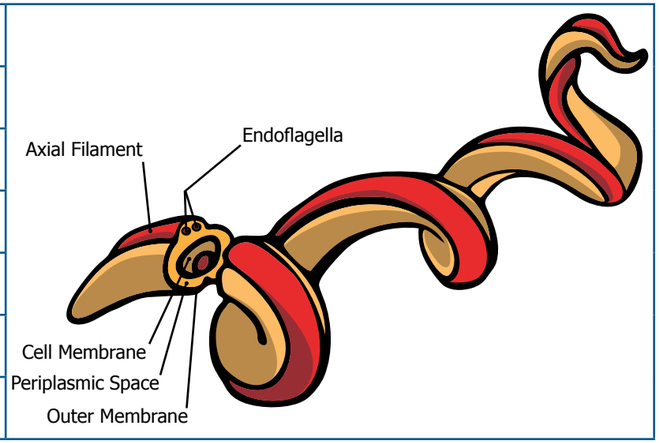


Core Technology

Cloning of *B. burgdorferi* genes into *E. coli* expression vectors

Screen using multiple (>14) relevant *B. burgdorferi*-specific proteins representative of each critical transition during the *in vivo** life cycle post infection:

OspA	Crasp-2
OspB	DbpA
OspC	DbpB
OspE	Arp37
Flagellin	P35
VlsE	P39
Crasp-1	P27



*For example OspE and the decorin binding proteins (Dbp) A and B are expressed during natural mammalian infection but not in culture.

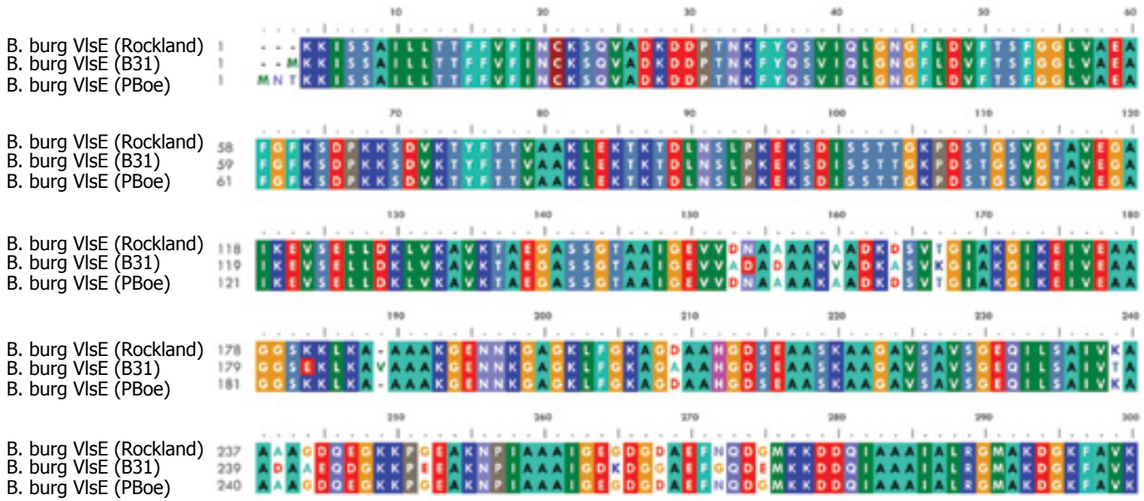
MORE REPRESENTATIVE + **BB. INFECTION-STAGE INDEPENDENT** = **MORE ACCURATE**

The technology is compatible with the current test methods like ELISA, Western Blot and Immunoblots but can also be used in the following formats:

- Lateral flow strip test – a rapid immunochromatographic assay in a simple device that can detect the presence of the target analyte with no need for specialized laboratory equipment and can return results in just minutes versus days or weeks with conventional methods.
- Multiplex platforms - allow for rapid high throughput evaluation of the multiple analytes in a single test sample while minimizing potential sample handling and processing.

Sequence Analysis and Protein Expression

All 14 proteins were expressed in the *E. coli* expression host and recombinant MBP fusion protein were extracted and purified using amylose resin.

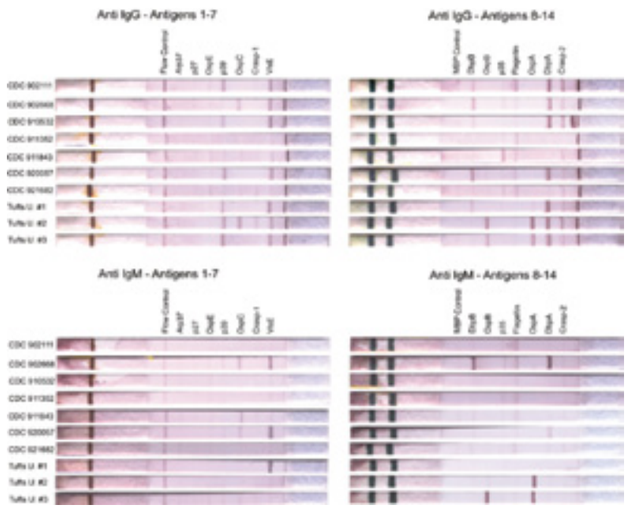


Partial alignment of VlsE from multiple species.

Patient Specific LF-LDA

IgG and IgM tests showing patient serum reactivity:

- Band visualization patterns for imprinted *B. burgdorferi* antigens.
- Diluted serum samples were pre-mixed with liquid gold-labeled recombinant LD proteins prior to application onto the sample pad.
- The flow control band indicates proper performance of the test.
- MBP negative control band indicates Bb protein specific reaction by patient sera.



Borrelia Products

Borrelia Antigens

Product Name	Catalog #	Size
P35 Control Protein	000-001-C12	100 µg
VIsE Control Protein	000-001-C33	100 µg
Flagellin Control Protein	000-001-C14	100 µg
OspA Control Protein	000-001-C13	100 µg
P39 Control Protein	000-001-C17	100 µg
DbpA Control Protein	000-001-B98	100 µg
Crasp1 Control Protein	000-001-C18	100 µg
Crasp2 Control Protein	000-001-C19	100 µg
DbpB Control Protein	000-001-C16	100 µg
Erpd/Arp37 Control Protein	000-001-C09	100 µg
ErpN/OspE Control Protein	000-001-C10	100 µg
Surface Lipoprotein p27 Control Protein	000-001-C30	100 µg
OspB Control Protein	000-001-C15	100 µg

Available in Bulk Quantities

Borrelia Antibodies

Product Name	Catalog #	Reactivity
Anti-p53 (rabbit) Antibody	200-401-C12	*Borrelia*
Anti-Flagellin (rabbit) Antibody	200-401-C14	*Borrelia B31*
Anti-VIsE (rabbit) Antibody	200-401-C33	*Borrelia*
Anti-OspA (rabbit) Antibody	200-401-C13	*Borrelia*
Anti-p39 (rabbit) Antibody	200-401-C17	*Borrelia, B. afzelii*
Anti-DPB A (rabbit) Antibody	200-401-B98	*Borrelia*
Anti-Erpd/Arp37 (rabbit) Antibody	200-401-C09	*Borrelia*
Anti-Erpn/Ospe (rabbit) Antibody	200-401-C10	*Borrelia*
Anti-DbpB (rabbit) Antibody	200-401-C16	*Borrelia*
Anti-CRASP-1 (rabbit) Antibody	200-401-C18	*Borrelia*
Anti-CRASP-2 (rabbit) Antibody	200-401-C19	*Borrelia*
Anti-Surface Lipoprotein p27 (rabbit) Antibody	200-401-C30	*Borrelia*

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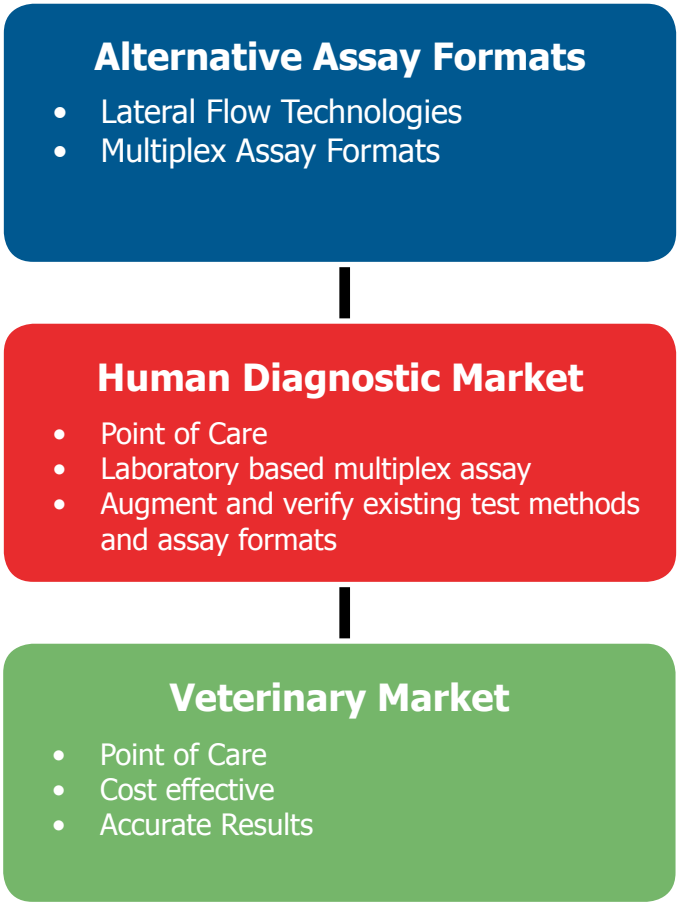
Development Opportunities

Rockland has developed the necessary reagents to move Lyme disease testing to the next level. Rockland provides accurate, timely and cost effective results. We are looking for collaborators to further develop these reagents into the next generation of Lyme disease testing. Please contact us at 1-800-656-7625 to discuss development opportunities.



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