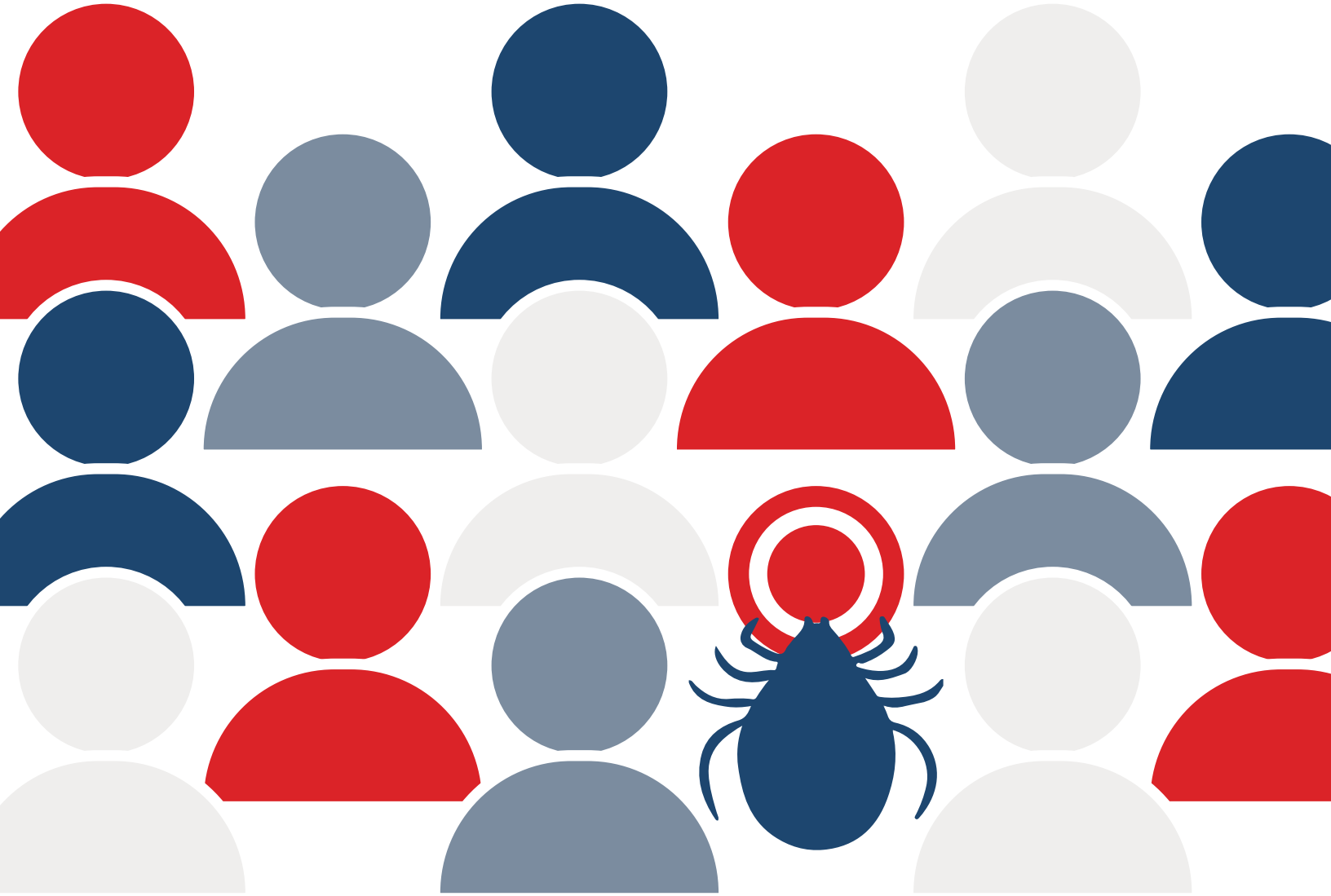
ROCKLAND



LYME DISEASE: THE SITUATION & SOLUTION

INTRODUCTION

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 roughly 30,000 confirmed cases of LD in the U.S. 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 which 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 false positive results. One drawback of the current method is that the *B. burgdorferi* protein cell lysates used for Western blotting contain numerous highly conserved proteins that often 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 a select few to detect circulating LD-specific antibodies present in the patient. 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:

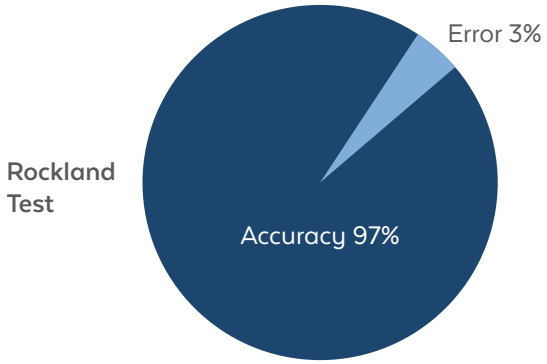
2-Tier is Gold Standard	Requires reveral days to complete
ELISA	Low throughput
Western Blot	Subjective interpretation

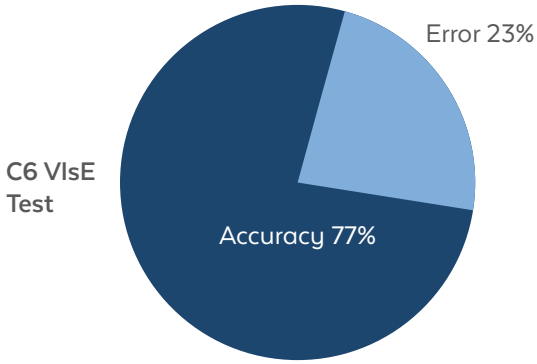
Complicating proper diagnosis, *B. burgdorferi* exists in several life cycle phases and different protein subsets are expressed in each phase. The LD-causing spirochete bacteria transitions from the deer tick vector to mammalian hosts, including pets, livestock, and humans. At each unique environment *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, such as VlsE. Detecting anti-*B. burgforferi* proteins is thus complicated.

OUR SOLUTION

Rockland's solution engages the evasive nature of *B. burgdorferi* and the different bacterial phases and allows for the detection of the unique response of each individual patient. To achieve this, Rockland has created a diagnostic matrix that specifically addresses the three known phases of infection; early, latent, and chronic. Rockland has cloned, expressed, and purified 14 *Borrelia*-specific antigens for use in the development of a highly sensitive, point-of-care, multiplex diagnostic device capable of 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.

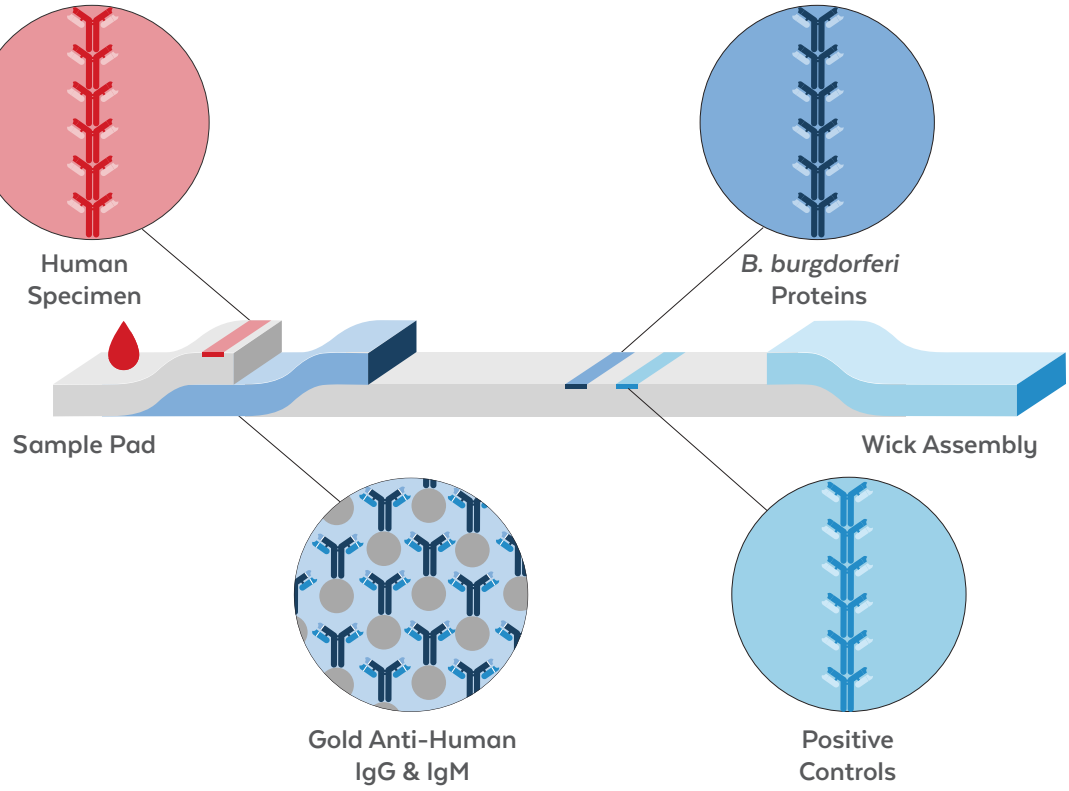
- The specificity of the **Rockland Test** suggests superior performance over the **C6 VlsE ELISA Test**.
- The **Rockland Test** correctly identifies 9 samples as LD negative whereas the currently used **C6 VlsE ELISA Test** falsely identified these samples as positive when compared with Western blot results and clinical findings.





LATERAL FLOW TECHNOLOGY

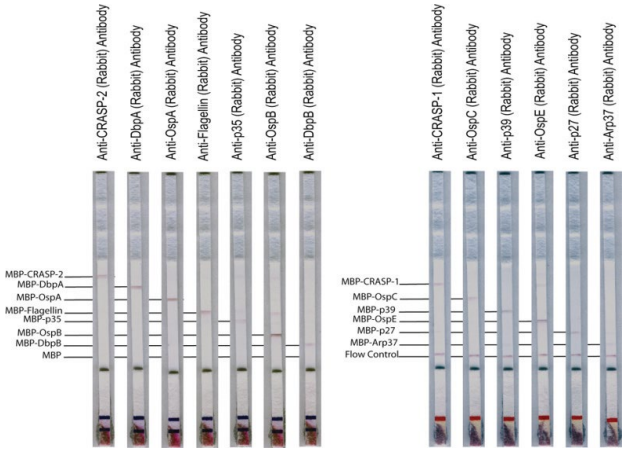
Lateral flow technology is well established and forms the basis of numerous strip-based tests, including consumer pregnancy tests and some HIV tests. Development of a point-of-care (POC) strip test for the detection of LD-specific antibodies would follow the layout presented below. Anti-LD antibodies 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

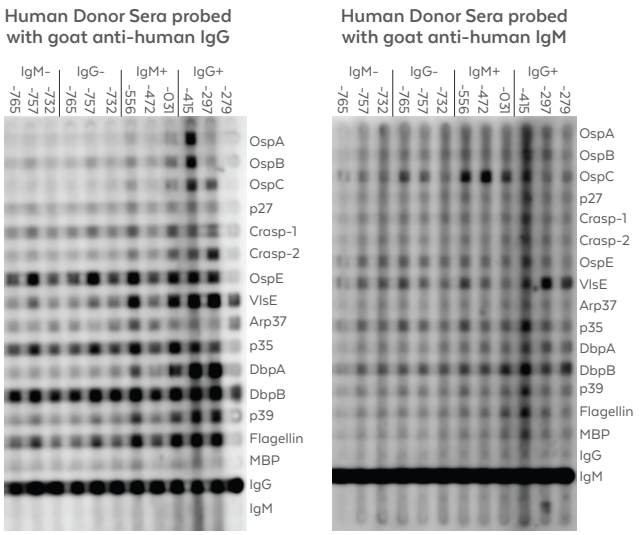
Rockland rabbit polyclonal antibodies raised against Bb proteins proved that the test strip configuration was functional.

1. *B. burgdorferi* antigens are properly immobilized on the strip
2. Gold cooloid particles are properly conjugated to respective antigens
3. Antibody/gold conjugated antigen complex is target specific
4. There is no non-specific cross-reactivity
5. The lateral flow assay performs correctly
6. Species independent



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.

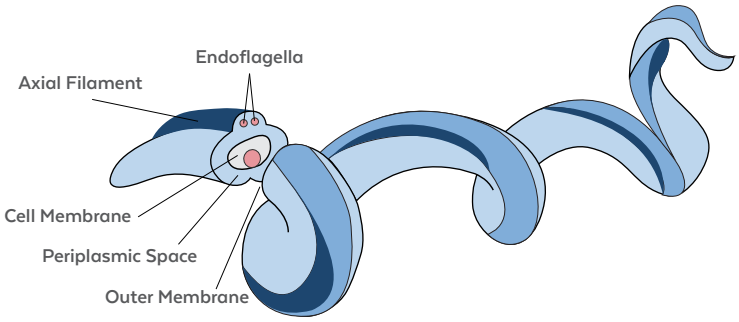


CORE TECHNOLOGY

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.

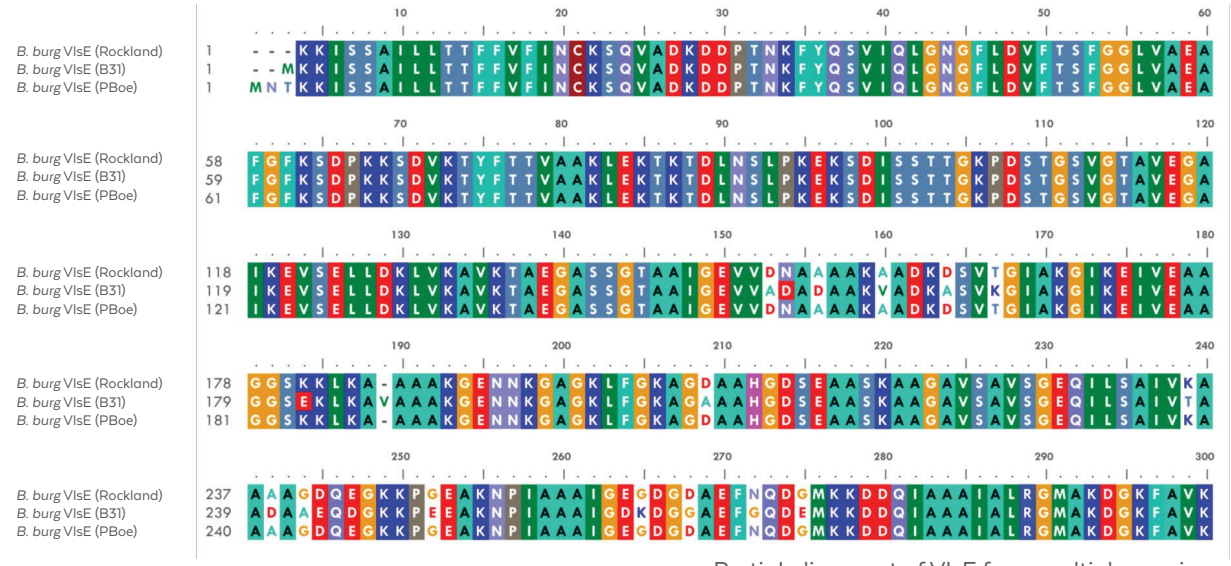
More Representative + BB. infection-stage independent = More Accurate

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 & 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.

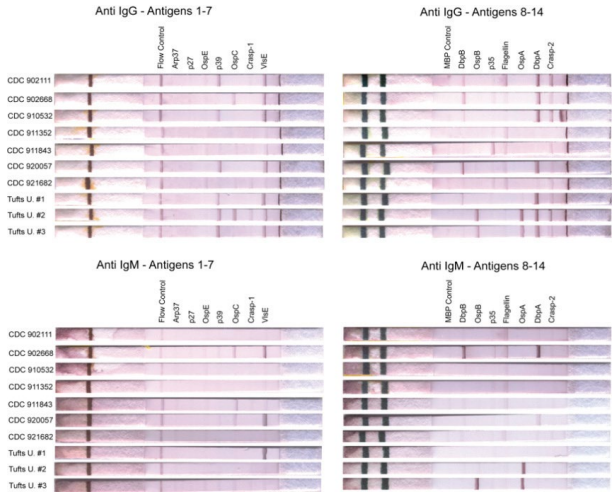


Partial alignment of VlsE from multiple species.

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.



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. Contact us to discuss development opportunities.

- **Alternative Assay Formats**: Lateral flow technologies and multiplex assay formats
- **Human Diagnostic Market**: Point of care, laboratory-based multiplex assays, and augmentation and verification of existing test methods and assay formats
- **Veterinary Market**: Point of care, cost effective, accurate results

BORRELIA ANTIGENS

Product Name	Applications	Item No.
p53 Control Protein	SDS-PAGE	000-001-C12
VlsE Control Protein	WB, SDS-PAGE	000-001-C33
Flagellin Control Protein	WB, SDS-PAGE	000-001-C14
OspA Control Protein	WB, SDS-PAGE	000-001-C13
p39 Control Protein	WB, SDS-PAGE	000-001-C17
DbpA Control Protein	WB, SDS-PAGE	000-001-B98
Crasp-1 Control Protein	WB, SDS-PAGE	000-001-C18
Crasp-2 Control Protein	WB, SDS-PAGE	000-001-C19
DbpB Control Protein	SDS-PAGE	000-001-C16
ErpD/Arp37 Control Protein	WB, SDS-PAGE	000-001-C09
ErpN/OspE Control Protein	WB, SDS-PAGE	000-001-C10
Surface Lipoprotein p27 Control Protein	WB, SDS-PAGE	000-001-C30
OspB Control Protein	SDS-PAGE	000-001-C15

BORRELIA ANTIBODIES

Product Name	Reactivity	Applications	Item No.
p35 Antibody	*Borrelia*	WB, ELISA	200-401-C12
Flagellin Antibody	*Borrelia B31*	WB, ELISA	200-401-C14
VlsE Antibody	*Borrelia*	WB, ELISA	200-401-C33
OspA Antibody	*Borrelia*	WB, ELISA	200-401-C13
p39 Antibody	*Borrelia, B. afzelii*	WB, ELISA	200-401-C17
DbpA Antibody	*Borrelia*	WB, ELISA	200-401-B98
ErpD/Arp37 Antibody	*Borrelia*	WB, ELISA	200-401-C09
ErpN/OspE Antibody	*Borrelia*	WB, ELISA	200-401-C10
DbpB Antibody	*Borrelia*	WB, ELISA	200-401-C16
Crasp-1 Antibody	*Borrelia*	WB, ELISA	200-401-C18
Crasp-2 Antibody	*Borrelia*	WB, ELISA	200-401-C19
Surface Lipoprotein p27 Antibody	*Borrelia*	WB, ELISA	200-401-C30

ROCKLAND IMMUNOCHEMICALS, INC.

For 55 years, Rockland has supported the research, diagnostic, and biopharma communities by providing the highest quality antibodies, assays, and research services including primary and secondary antibodies, chemiluminescent substrates, custom polyclonal and monoclonal antibody production, and assay development.

Rockland antibodies, substrates, buffers, and services adhere to QSR / cGMP with full reporting and traceability (CFR:21H part 820), as well as optional analysis options that include ELISA, WB, IF, IHC, HPLC, and SDS-PAGE (1-D, 2-D).

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