EPITOGEN

WHITE PAPER

Next-Generation Serological Testing for Lyme Disease: A Peptide Multiplex Approach

Key Takeaways

Current diagnostic tests for Lyme disease have various limitations, underscoring the **need for better diagnostics.**

Comparative studies demonstrate the **superior performance** of the **Epitogen® peptide-based test**.

The Epitogen® Lyme Detect™ IgG test is ready and available for evaluation and collaboration within the research community and its stakeholders.

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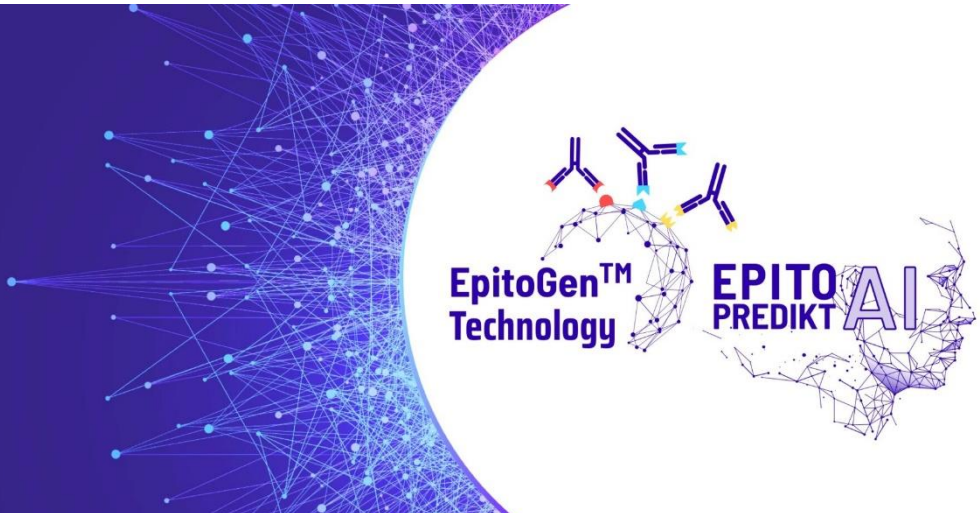

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Abstract

Lyme Borreliosis, commonly known as Lyme disease, poses a significant health threat worldwide. The condition stems from infection by the bacterial species known as *Borrelia,* which is transmitted to humans through tick bites. Left untreated this condition can lead to severe health problems and long-term damage. Despite current tests serving as valuable diagnostic tools they still suffer from technical limitations, such as suboptimal sensitivity during the early stages of infection, variability in interpretation, and a propensity for false-positive results due to cross-reactivity with other antigens. Furthermore, existing tests still lack the capability to differentiate between past and active/chronic forms of the infection which can lead to further clinical complications. These limitations mainly stem from a single tests inability to capture the antigenic complexity of *Borrelia* species and patients' heterogenous immune response to infection. This whitepaper introduces the "Epitogen® Lyme Detect™ IgG test", a peptide multiplex assay designed using 120 specific immunodominant peptides selected from 37 antigenic proteins covering the main pathogenic *Borrelia* species. This novel test demonstrates its significant potential in terms of sensitivity and specificity and thereby paves the way for the next generation of diagnostics based on peptide-precision.

Background

Lyme disease, caused by a diverse group of bacteria of the *Borrelia burgdorferi sensu lato* complex, represents a growing worldwide health concern, with approximately 700,000 cases reported annually in the United States and Europe. Existing diagnostic methods rely on symptom-based evaluation, serology testing and exposure risk assessments. The two-tiered serological testing approach is comprised of an enzyme immunoassay followed by either an immunoblot test or a second enzyme immunoassay. This serves as the cornerstone for Lyme disease diagnosis. While the current serology tests yield adequate outcomes for the later disseminated stages of infection, the tests often lack the necessary precision for early localized detection where timely intervention is crucial to full recovery from Lyme disease. Furthermore, the tests can lead to false-positive results due to cross-reactivity with antigens from related microorganisms or even autoimmune diseases.

Challenges

Shortcomings of the Current Two-Tiered Serology Tests for Lyme Disease Diagnostics

Limited sensitivity in the early stages of infection. The two-tiered serology testing method often fails to detect Lyme disease during its early stages, leading to delayed diagnosis and treatment. Despite Lyme disease patients developing antibodies against the pathogen in the early stages of infection, the levels remain below the detection threshold of current tests.

Cross-reactivity and false positives. The tests may produce false-positive results due to cross-reactivity with antigens from other microorganisms or medical conditions. This can lead to unnecessary treatment, delay due to misdiagnosis, and psychological distress for patients who are wrongly diagnosed with Lyme disease.

Limited antigen representation in Lyme tests. Existing tests utilise a single or restricted array of antigens which fail to capture the complex antigenicity profile of *Borrelia* and the heterogenous immune response of a population in a single test. In fact, many of the current tests rely solely on using antigens from a single *Borrelia* species, which potentially leads to the omission of numerous cases of individuals infected by other *Borrelia* species.

Lengthy and subjective interpretations. The two-tiered testing process is time-consuming and subject to variability in interpretations, particularly with supplemental immunoblot results. This variability can introduce uncertainty and delays in diagnosis thereby hindering timely intervention.

Resource intensive testing. Conducting two-tiered serology testing requires significant resources, including laboratory equipment, trained personnel, and time. This can strain healthcare systems and lead to delays in diagnosis and treatment.

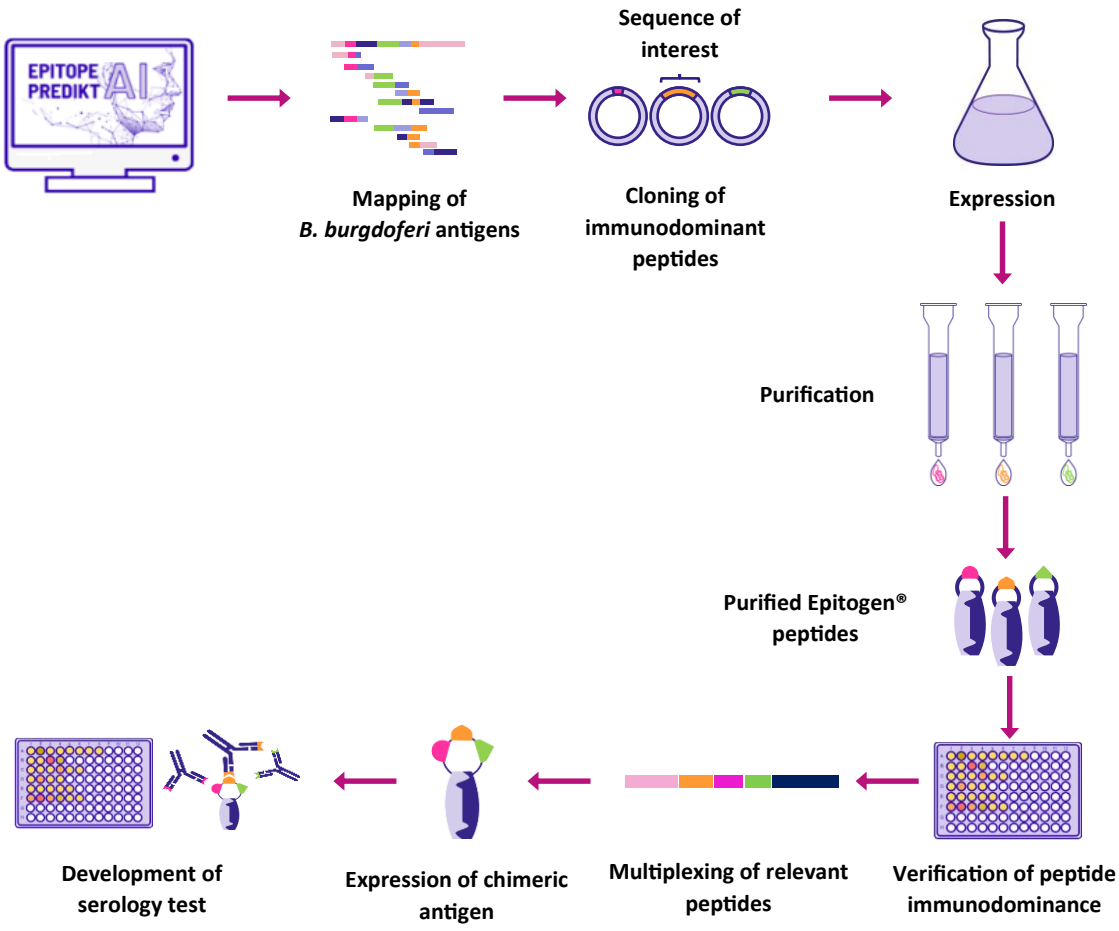
Solution

The Next-Generation of a Peptide-Based Lyme Disease Serology Test

To address the limitations in existing diagnostic technologies, EpitogenX has developed a Lyme test utilizing a peptide-based precision platform. This innovative strategy combines EpitoPredikt™ and Epitogen® technologies to identify immunodominant-peptides and seamlessly multiplex them into mosaic antigens. Employing this pioneering approach, we identified 120 specific and seropositive immunodominant peptides sourced from 37 *Borrelia* antigenic proteins corresponding to the main pathogenic species (*B. afzelii, B. garinii, B. mayonii, and B. burgdorferi*). These peptides were strategically utilised to make mosaic antigens that form the foundation of the Lyme Serology test. The peptides covered a range of sizes, spanning from 40 amino acids to full subunits of immunodominant antigens which enables them to capture linear and conformational epitopes. This new approach enhances test sensitivity, specificity, and its relevance for a global market.

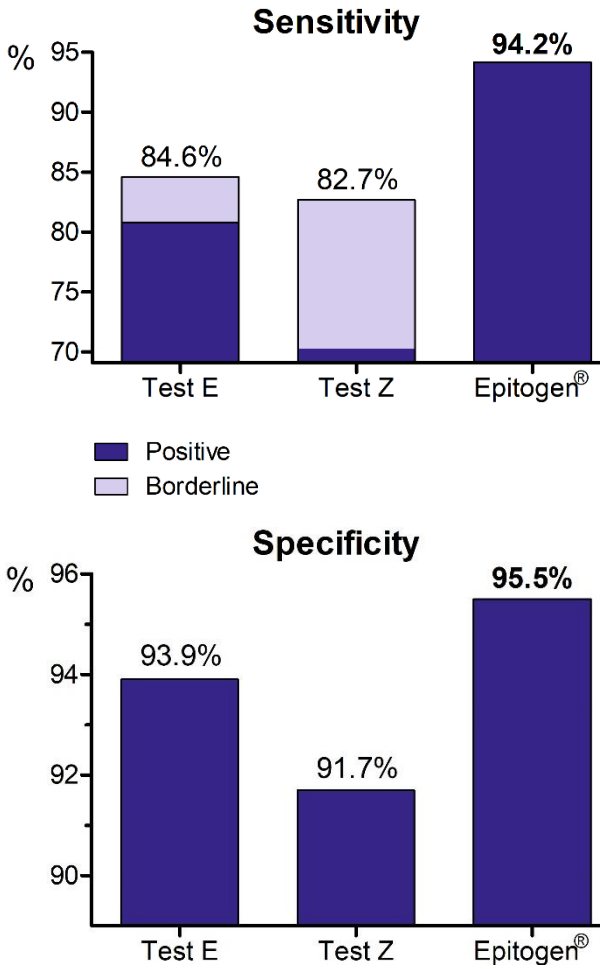
Epitogen® Serology Test Development

Immunodominant “hotspots” from the main pathogenic *Borrelia* species were mapped using EpitoPredikt™ with 500 peptides identified across 37 antigens. Each peptide was individually cloned into the Epitogen® plasmid construct, expressed in *E. coli*, and purified. The peptides were then screened using 175 Lyme-positive and 125 Lyme-negative serum samples, which included individuals with potential cross-reactive conditions. 120 seropositive peptides, with no cross-reactivity, were then fused together with the Epitogen® construct to form a multi-peptide antigen which served as the foundation for the test.



Benefits

Epitogen® Benchmark Against its Competition



Our peptide-based Epitogen® Lyme Detect™ IgG test demonstrates a marked improvement in sensitivity and specificity, outperforming commonly used commercial assays.

Performance of the newly developed Epitogen® test was compared against two currently available commercial Lyme tests (Test E and Test Z). Consequently, **the Epitogen® Lyme Detect™ IgG test was found to be at least 9.6% more sensitive and 1.6% more specific** than the best performing commercial test.

This leap forward in performance holds great promise for advancing Lyme disease diagnosis.

Assay sensitivity was evaluated using 52 standard two-tier tests from confirmed Lyme positive serum samples from NHS Grampian Biorepository. Assay specificity of the Epitogen® Lyme Detect™ IgG test was evaluated using samples from 198 negative controls. The specificity values used for Test E and Test Z were reported by the manufacturer.

In the Pipeline

Advancing Lyme Disease Testing and Product Portfolio

The Epitogen® Lyme Detect™ IgG test is currently available for the research community and its stakeholders for evaluation and further collaboration. As EpitogenX progresses, independent verification of the performance of its test is currently ongoing in Lyme reference laboratories and research institutes. Subsequently, we aim to achieve CE marking and FDA approval, marking a significant milestone in ensuring regulatory compliance and market readiness.

Our efforts have also extended to the development of the Epitogen® Lyme Detect™ IgM test, a crucial addition aimed at enhancing our ability to detect early Lyme disease. The IgM prototype Lyme test has already been developed and is ready for verification with well characterised early Lyme disease samples. Furthermore, the peptide-based nature of the assays creates an opportunity to develop differential tests that can identify different pathogenic species, thereby aiding in prognostic evaluations, understanding regional variations, and facilitating research and surveillance efforts.

EpitogenX's innovative peptide-based approach holds great promise beyond Lyme disease diagnostics. By leveraging the peptide-based technology, we seek to develop precise diagnostics for a spectrum of other diseases, thereby expanding our impact and relevance in the broader healthcare landscape.

Conclusion

Peptide-based serology assays have the potential to significantly enhance Lyme disease diagnostics by offering solutions to the limitations inherent in current diagnostic methods. Using a game-changing technology, we have developed a serology test with enhanced performance to ultimately improve detection of Lyme disease. This breakthrough promises to transform the diagnostic pathway for Lyme disease detection, enabling more effective and timely interventions, and potentially providing an alternative to the lengthy two-tiered testing system. **The Epitogen® Lyme Detect™ IgG test is complete and available for the research community and stakeholders for evaluation and further collaboration.** Moreover, our peptide-based approach can be further extrapolated to develop accurate diagnosis for other diseases. When independently verified and approved, this innovative product will play an important role in the battle against Lyme disease and mark a significant advancement in disease diagnostics and management.

About the Authors

Dr Ralfs Buks is an expert in pathophysiology with a strong background in clinical assay development. Dr Buks is an Innovation Officer at EpitogenX and Honorary Research Fellow at the University of Aberdeen.

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EpitogenX Info

EpitogenX is a forward-thinking biotech with innovative platforms backed by a world-leading scientific team.

Our **EpitoGen®** and AI **EpitoPredikt™ technologies** create a powerful game-changing platform that transforms:

diagnostics

vaccine design

biotherapeutics

antibody development

We offer *in silico* solutions that are validated *in vitro,* and applied to create clinical products using Epitogen® Technology.

Products developed using our technology are compatible with gold standard lab-based and point-of-care applications (i.e. ELISA, LFA, microfluidics), making them affordable and user friendly.

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