Deep learning-based peptide panel vertical flow assay for serodiagnosis of Lyme disease

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Introduction: Lyme disease (LD) is the most prevalent tick-borne disease caused by the bacteria *Borrelia burgdorferi.* The two-tier serology test is a gold standard laboratory-based method used for the diagnosis of LD. However, this method is expensive, requires a centralized hospital and has limited sensitivity and specificity, especially at the early stages when LD treatment is most efficient. Here, we present a point-of-care peptide-based multiplexed vertical flow assay (xVFA) for LD, overcoming typical limitations of laboratory-based methods. The assay can be easily operated in <20 minutes using only 20 µL of serum sample. The peptide panel was optimized using a deep learning-based diagnostic model to achieve high specificity and sensitivity to LD, including early-stage cases. Blind testing results on patient sera showed sensitivity and specificity of 61% and 100%, respectively, closely matching the performance of the standard two-tier test.

Materials and Methods: xVFA is a paper-based assay that contains a sensing membrane functionalized with a peptide panel optimized for specific detection of LD-associated IgM and IgG antibodies (Figure 1a-b). The assay operation consists of two steps, including the loading of a 20 µL sample at the first step, followed by the loading of gold nanoparticles (AuNPs) for signal generation at the second step. Total assay operation time takes <20 minutes, and after the operation, the activated assay is captured by a mobile phone-based reader, and immunoreaction signals are processed by a fully connected neural network model trained to output LD diagnosis (Figure 1a). The peptide panel was computationally optimized to achieve the highest sensitivity and specificity. The optimized xVFA was blindly tested on samples from the Disease Control & Prevention (CDC) LD repository.

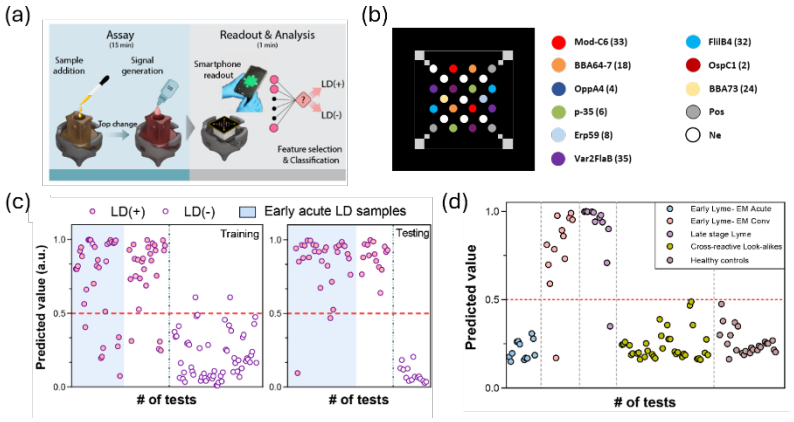

Figure 1. (a) Operation of peptide-based xVFA (b) xVFA spot map (c) xVFA performance on LDB sample set; (d) xVFA performance on CDC sample set.

Results and Discussion: At first, 9 out of 18 candidate peptides were downselected based on their variance in reactivity against the set of six LD control samples. These peptides were spotted on the xVFA sensing membrane, and the assay was further computationally optimized on 60 patient samples collected by the Lyme disease biobank (LDB) (Figure 2c). The optimal peptide subset was determined as the peptide combination with the highest area under the curve (AUC) score and contained 3 peptides, i.e., modVlsE-FlaB, Var2FlaB and OppA4. The optimized assay was blindly tested on 32 patient samples from the CDC and showed 61% sensitivity and 100% specificity despite 12 cross-reactive samples from look-alike diseases present in this cohort (Figure 2d). In addition, the assay achieved 90.9 % sensitivity in detecting early-stage acute LD samples (Figure 2c).

Conclusions: In conclusion, we developed a multiplexed paper-based peptide panel assay for early-stage diagnostics of LD with minimal cross-reactivity to other diseases. The assay achieved 100% specificity for LD detection, mitigating cross-reactivity with look-alike diseases, and showed >90% sensitivity in LD detection in early-stage acute LD samples. Competitive LD diagnostics performance, along with rapid operation and low-cost design, make our peptide-based xVFA a promising point-of-care sensor for early-stage LD diagnostics.