Tick-borne diseases (TBDs) pose a major health burden in the US, particularly as many cases go undiagnosed. Lyme disease (LD), caused by *Borrelia burgdorferi*, is the most prevalent, however, additional TBDs, including *Anaplasma phagocytophilum, Ehrlichia chaffeensis*, and *Mycoplasma pneumoniae*, may also occur alone or in co-infections. Many TBDs have similar and large, non-specific signs and symptoms, yet most people are treated solely for LD. Recommended therapy varies among patients based on disease severity and presentation, yet two different tests to achieve suboptimal specificity. This however is the cost of sensitivity: Brazilian patients with tick-borne diseases (TBDs) primarily rely on indirect immunofluorescence assays (IFA) to detect specific proteins, which are not subject to interpretation, requiring trained technologists and may cross-react with antibodies to other closely related proteins.

Serimmune has developed a serum epitope repertoire profiling technology (SERA), which can detect antibodies to any combination of pathogens in a single assay, without increased cost or complexity. Using SERA, we developed an expandable multiplex assay for *B. burgdorferi*, *A. phagocytophilum*, *E. chaffeensis*, *Ehrlichia* spp., and *B. microti*. SERA allows a highly diverse tandem peptide library for next-generation sequencing (NGS) and information to profile autoantibody repertoire, identify epitope "hotspots" and map these to specific disease antigens, enabling light on proteins that are targeted during generation of a humoral immune response. In initial validation studies, the SERA TBD panel exhibited superior sensitivities and specificities compared to the standard STTT algorithm, and the modified two-tiered testing algorithm for US using a set of 1500 specimens from individuals undergoing routine testing for TBDs at Mayo Clinic.

The negative predictive value of the SERA TBD assay exhibited superior sensitivity and specificity (76% and 98%, respectively) when compared to the STTT algorithm (66% and 96%, respectively). Also, roughly a quarter of these "motifs" and map these to specific disease antigens, shedding light on proteins that are targeted during generation of a humoral immune response. In initial validation studies, the SERA TBD panel exhibited superior sensitivities and specificities compared to the standard STTT algorithm, and the modified two-tiered testing algorithm for US using a set of 1500 specimens from individuals undergoing routine testing for TBDs at Mayo Clinic.

Additionally, in a set of clinically defined LD samples from the CDC repository and Lyme Disease Biobank, we performed blinded testing on 1500 samples with clinician assigned Lyme disease (LD), and results were compared to IFA test results. 

**Conclusions**

- SERA results suggest that patients with Lyme disease are more likely to be seropositive for non-tick-borne tick disease or other illnesses.
- Broad disease testing via SERA could increase diagnostic yield from ~10% to 30%.
- The epitope repertoire technology can be used for serological testing for TBDs.
- For non-LD TBD, SERA was strongly positive for some IFA negative cases and epitope mapped to known LD antigens, suggesting that SERA may detect cases that are false negative by IFA.
- SERA multiple disease testing may be useful for screening suspected Lyme patients that present with non-specific symptoms.

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