Development of an Antigen-Detection Diagnostic Assay for Lyme Disease

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Lyme disease, the most prevalent tick-borne illness in the United States, is transmitted through the bite of ticks carrying the bacterium, Borrelia burgdorferi (B. b.). Current serological diagnosis assays commonly misdiagnose the critical early stages of the disease due to the late or slow build-up of antibodies raised against the bacteria that the tests rely on. This study aimed to purify a protein that was found to be associated with B. b. from urine samples of patients with confirmed Lyme disease and to determine its reactivity against antibodies raised against the protein with the goal of developing a more noninvasive and direct form of diagnostic test. A recurring antigen associated with B. b. was chosen as a biomarker candidate. Its gene was cloned into the pET17b expression vector and the protein of interest was over-expressed in BL21 pLysS E. coli hosts. Each stage was confirmed by a double digestion with corresponding restriction enzymes and a western blot to confirm a polyhistidine tag, respectively. The protein was purified by metal-affinity chromatography and antibodies to this antigen were raised and tested against the purified antigen for sensitivity using an immunological assay (ELISA). The resulting tests show detectable reactivity between antigen and antibody even at a 1/51,200 dilution factor. In the future, the antibodies will also be tested against clinical urine samples from patients with Lyme or other diseases to further test its sensitivity and specificity.

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