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Bio:  
Mark Kopnitsky has been with Zeus Scientific (R&D), Inc. for 29 years. Mark’s B.S. degree (Shippensburg University of Pennsylvania) is in Biology, Secondary Education and he taught for approximately three years in the public school system. Mark also possesses a M.S. (Clarion University of Pennsylvania) in Microbiology with emphasis in Molecular Genetics. At ZEUS Scientific, Mark provides technical support to all areas of the company, and oversees the following departments: Research & Development, Quality Systems, Regulatory Affairs, Quality Assurance and Quality Control. The current product line of in vitro diagnostics includes approximately 150 tests for various autoimmune disorders and infectious diseases. Because of his responsibilities, he contributes to, and is therefore involved in product development from assay conceptualization through regulatory clearance. At the present, he has submitted and cleared more than 120 IVD products through the Food and Drug Administration using the 510(k) process.


Background: Since 1994, serology testing for Lyme disease in the United States has consisted of a two-tiered algorithm, referred to as standard two-tiered testing (STTT). In the STTT algorithm, specimens equivocal or positive by first tier screening methodologies are subsequently tested via IgG and/or IgM immunoblots. While relatively specific, the immunoblotting portion of the STTT algorithm contains drawbacks such as insensitivity for detecting acute infection, subjectivity of result interpretation, and technically challenging procedures. Consequently, studies have been published that describe the replacement of immunoblotting with a more sensitive and automatable methodology such as ELISA; referred to as modified two-tiered testing (MTTT). The goal of these studies was to validate the performance of several FDA-cleared Borrelia burgdorferi ELISAs manufactured by ZEUS Scientific within two different MTTT algorithms.

Methods: [Retrospective Cohort, n = 356] 280 clinically-characterized and blind-coded serum samples (termed Premarketing Panel), were obtained from the Centers for Disease Control and Prevention (CDC). Additional banked Stage 2 and Stage 3 Lyme samples were obtained from Dr. Allen Steere. [Prospective Cohort, n = 2932] Routine Lyme serology specimens were collected prospectively at three locations: Mayo Clinic, Rochester, MN (1042 samples); Marshfield Clinic, Marshfield, WI (990 samples); Massachusetts General Hospital, Boston, MA (900 samples). The retrospective and prospective sample cohorts were tested and analyzed according to the following algorithms: [STTT] 1st Tier – ZEUS VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier – Marblot™ IgG and IgM Immunoblots; [MTTT-1] 1st Tier – ZEUS VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier – ZEUS Borrelia burgdorferi IgG/IgM ELISA; [MTTT-
2] 1st Tier – ZEUS VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier – ZEUS Borrelia burgdorferi IgG and IgM ELISAs.

**Results:** [Retrospective Cohort - Stage 1 Lyme (Acute): STTT (15/30, 50.0%), MTTT-1 (22/30, 73.3%), MTTT-2 (23/30, 76.7%); Stage 1 Lyme Convalescent: STTT (23/30, 76.7%), MTTT-1 (25/30, 83.3%), MTTT-2 (27/30, 90.0%); Stage 2 Lyme: STTT (34/56, 60.7%), MTTT-1 (37/56, 66.1%), MTTT-2 (47/56, 83.9%); Stage 3 Lyme: STTT (50/50, 100.0%), MTTT-1 (50/50, 100.0%), MTTT-2 (50/50, 100.0%); Healthy Controls: STTT (0/100, 0.0%), MTTT-1 (0/100, 0.0%), MTTT-2 (0/100, 0.0%); Other Disease Controls: STTT (0/90, 0.0%), MTTT-1 (2/90, 2.2%), MTTT-2 (2/90, 2.2%); [Prospective Cohort – MTTT-1 vs. STTT (PPA = 98.8%, NPA = 97.8%, TPA = 97.9%); MTTT-2 vs. STTT (PPA = 100.0%, NPA = 96.3%, TPA = 96.6%).

**Conclusion:** These studies represent the first multi-center validation of several ZEUS ELISAs within MTTT algorithms. The novel data presented herein are consistent with previously published literature and demonstrate that our MTTT algorithms yield improved sensitivity for detection of early Lyme disease, while maintaining acceptable specificity. Additionally, the ZEUS ELISA MTTT algorithms may offer other benefits to the clinical laboratory such as increased automation compatibility, workflow improvement, and reduced costs relative to the existing STTT algorithm. Furthermore, both MTTT algorithms have recently been cleared by the U.S. FDA, representing the first true paradigm shift in Lyme serology testing for over 25 years. The U.S. Centers for Disease Control and Prevention also recently updated their Lyme serology testing guidelines to include MTTT algorithms.