Lyme Disease Serology
Problems and Opportunities

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SEROLOGIC ASSAYS FOR LYME DISEASE, FIRST USED IN 1983,[1] are widely ordered in the United States, with an estimated 2.8 million tests performed annually.[2] High demand undoubtedly has provided a potent stimulus for the development and marketing of a large number of assays for detection of antibody to *Borrelia burgdorferi*. As reported by Brown and colleagues[3] of the Food and Drug Administration (FDA) in this issue of THE JOURNAL, 45 first-step assays, including enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays (IFAs), or immunodot techniques, and 8 second-step (supplemental) immunoblot assays have been granted FDA approval. Given the tremendous interest in these tests, it is important for physicians, other health care professionals, and the public to appreciate the strengths and limitations of these tests so that they are used in a helpful way. Brown and colleagues have addressed many of these issues in their article.

Ten years ago, when Magnarelli[4] wrote an editorial in *JAMA* on the quality of Lyme disease tests, many commercially available and in-house tests did not appear to meet desirable performance standards. Lack of test specificity in particular has resulted in lingering misconceptions about the protean nature of this illness. Virtually any constellation of symptoms was attributed to *B burgdorferi* infection because of the large number of inaccurate test results.[5] This, in turn, created an inappropriate demand for more serologic testing, which led to even more false-positive results, causing a vicious cycle.

In the intervening decade, a number of positive events have occurred. In 1995, the Centers for Disease Control and Prevention (CDC), in conjunction with the Association of State and Territorial Public Health Laboratory Directors, instigated a fundamental change in serologic testing for Lyme disease.[6] Conditional 2-step serologic testing was recommended, in which a serum specimen with a positive or equivocal first-step test result (eg, ELISA) is further tested by an immunoblot assay. Seropositivity requires reactivity by both test methods. Evidence-based recommendations for immunoblot interpretation were suggested. An important component of the testing recommendations, too often overlooked by practitioners, is the requirement for IgG immunoblot positivity for patients with illness of longer than 30 days' duration. This recommendation reflected the natural evolution of antibody production and the lower specificity of the IgM immunoblot. Conditional serologic testing, while not addressing concerns about the performance of individual test kits, has improved specificity (G.P.W., unpublished data, 1999).[7,8]

See also p 62.

The semantics of diagnostic testing also have begun to change to reflect more precisely test limitations. None of the serologic tests should be termed a "screening" test, because each lacks sufficient specificity to be used in this fashion. Immunoblot is a "supplementary" test rather than a "confirmatory" assay for first-step tests because it shares common antigens and is therefore not an independent test (G.P.W., unpublished data, 1999).

The FDA now recommends that manufacturers of diagnostic assays for Lyme disease evaluate their tests using a CDC-generated panel of serum specimens and requires that the results be included in the product labeling. The panel consists of approximately 40 serum specimens from patients with various stages of Lyme disease; many of the patients with early Lyme disease and erythema migrans had positive cultures. However, serum specimens were collected at various time intervals, ranging from days to months, after antibiotic treatment, and the patients were not necessarily symptomatic at the time of serum collection. Also included in this panel are serum specimens from 5 healthy negative controls (Martin E. Schriefer, PhD, and Grant L. Campbell, MD, PhD, written and oral communication, May 1999). It is clear from the results reported by Brown et al[3] that test performance still varies substantially among the various tests, even among immunoblot assays.

This information can be used by laboratories and physicians to compare the performance of tests from different manufacturers,[3] although certain significant limitations exist. For reasons that are unclear, several test manufacturers have failed to participate in the voluntary testing program recommended by the FDA. Furthermore, the "manufacturers'" serum panel does not provide a complete picture of the accuracy and precision of a test. The panel might be of greater value if it included a larger number of serum specimens from well-characterized symptomatic patients with Lyme disease. The number of control specimens also should be increased and should include serum specimens from healthy volunteers from endemic areas in which testing for Lyme disease is most likely to be needed,[9] as well as from patients with a variety of other

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infectious and noninfectious diseases. The serum panel should be used in a blinded fashion and should include duplicate specimens to evaluate reproducibility of the assay. A sufficient number of serum specimens should be tested to allow a meaningful assessment of sensitivity, specificity, and precision. However, this testing should complement, not replace, regular proficiency testing programs for laboratories that eventually will perform these serologic assays.[10]

A recently reported, placebo-controlled efficacy study of a recombinant outer surface protein (OspA) vaccine preparation has provided some new perspectives on the role of serologic testing in the diagnosis of Lyme disease.[11] Among the approximately 5000 placebo recipients followed over 2 Lyme disease transmission seasons, 124 developed clinical manifestations meeting the CDC surveillance definition of Lyme disease.[12] Of these, nearly 98% had erythema migrans. Serologic testing is not needed and not recommended for patients with erythema migrans, due to low sensitivity.[2,13] Therefore, for the overwhelming majority of patients with Lyme disease who have objective findings, serologic testing is unnecessary to support the clinical diagnosis.

For patients without erythema migrans, but with other objective findings, such as a swollen knee, serologic tests have high enough sensitivity to warrant their use.[2] The confusion that arises most often is the failure to appreciate a generic limitation of indirect tests; namely, that serologic results do not equate with medical diagnoses. A positive serologic test means that the probability of Lyme disease has increased, and a negative result implies that the probability has decreased, in each instance by an amount that is a function of the sensitivity and specificity of the particular test performed.[2] Whether that probability reaches an appropriate threshold to initiate therapy (>50%)[2] depends on the likelihood of Lyme disease prior to doing the test. The American College of Physicians guidelines on laboratory evaluation in the diagnosis of Lyme disease published in December 1997 has provided some guidance on estimating pretest probability.[2,13] However, increased attention should be paid to standardizing methods of quantifying the pretest probability of infection.[8] It is noteworthy that the American College of Physicians guidelines suggested that persons with only the nonspecific symptoms of myalgias, fatigue, and arthralgias should not be tested or treated for Lyme disease, since a positive test result for these patients does not increase the probability of disease above the treatment threshold.[2,13]

Any gains that have been realized by conditional 2-step testing are threatened by a new array of problems anticipated to begin this year. Recent licensure of a recombinant OspA vaccine for prevention of Lyme disease carries profound implications for serologic testing. Even the most accurate of the first-step serologic tests using whole-cell antigen preparations is obsolete in vaccine recipients, because OspA antibodies uniformly cause reactivity in these tests (M.E.A-R., unpublished data, 1999). Accurate interpretation of immunoblots is still possible for vaccine recipients who develop Lyme disease, but familiarity with the diverse effects on immunoblot of high-titer OspA antibody is required. Use of immunoblot as a stand-alone antibody test method is associated with a reduction of specificity compared with 2-step conditional testing and will substantially increase costs (M.E.A-R., unpublished data, 1999).[7,8] It has been estimated that an additional expenditure of $77 million annually would be incurred by routinely performing immunoblot assays instead of conditional 2-step testing in the United States (M.E.A-R., unpublished data, 1999).

Reconfiguration of serologic tests for Lyme disease is possible with OspA-deficient strains of *B burgdorferi*[14] or by using tests composed of multiple recombinant antigens,[15] although patent ownership issues may make the latter approach prohibitively expensive. In any case, all potential new commercial tests will undergo review by the FDA prior to approval. This may provide a valuable opportunity to obtain important data on test accuracy in a standardized manner so that this second generation of tests can be meaningfully compared (for the first time) with each other. This approach should enable laboratories to make an informed decision when choosing among the available tests for *B burgdorferi* antibody and improve serologic testing performance. Such a welcome development, however, will not obviate the need for practitioners to order and interpret testing for *B burgdorferi* antibody in an appropriate clinical context.

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