Lyme Disease Diagnosis
Serology

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KEYWORDS

• Lyme disease • *Borrelia burgdorferi* • Serologic testing • Standard 2-tier testing • Pitfalls • Predictive value serology

KEY POINTS

- Serology has been the mainstay of laboratory confirmation of Lyme borreliosis because direct detection of *Borrelia burgdorferi* has limited application.
- Although standardized 2-tier testing (STTT) detection of early, localized infection is poor, that of late disease is very good.
- The best indicator of stage 1 infection, erythema migrans, is presented in the majority of US cases; when recognized in a relevant epidemiologic setting, it should prompt treatment without testing.
- Clinical and epidemiologic correlates of infection should be carefully assessed before ordering STTT and for evaluation of its results.
- Efforts to simplify serologic testing include use of recombinant antigens and alternatives to STTT; these developments promise to improve performance, particularly in early disease detection.

INTRODUCTION

In 1982, the spirochetal agent of Lyme disease, later named *Borrelia burgdorferi*, was identified in and cultured from *Ixodes scapularis* ticks.[1] Shortly thereafter, it was cultured from blood, erythema migrans (EM) skin rash biopsies, and the cerebrospinal fluid of patients with differing stages of Lyme borreliosis in the United States and Europe using a modified media that had been developed in 1971 for propagation of relapsing fever spirochetes.[2–4] Although culture isolation from patients and animal model infection studies solidified the etiology of Lyme disease, this gold standard diagnostic has not proven to be a particularly sensitive approach for laboratory confirmation of infection in the United States.[5–7] This shortcoming for Lyme disease, although not uncommon in the field of bacterial etiologic agent recovery from clinical samples, is owing to the vanishingly small number of cultivable spirochetes in any

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human tissue or fluid (often on the order <1/mL of blood in early disease) and that these numbers most often diminish with duration and dissemination of infection.[6] Differences in spirochete loads, persistence, and cultivability between vector ticks (certain *Ixodes* spp.), reservoir animals (such as *Peromyscus leucopus*) and dead-end hosts (such as *Homo sapiens*) are notable and prompt intriguing questions regarding the biology and survival mechanisms of this spirochete in its varied environments and possible approaches to better understand and improve human diagnostics and minimize disease.

With the varied tissues and organs potentially involved in human disease and the limitations of culture for laboratory confirmation of infection, alternative methods of diagnosis have been explored since the early 1980s. These include microscopy, immunohistochemistry, nucleic acid amplification, and immune response detection. This article provides an overview of the state of the diagnostic art for Lyme borreliosis, namely, serology, for the past 30 years. Most serology-specific understanding presented will focus on findings in North American patients infected with *B burgdorferi* sensu stricto (ss). Finally, recent advances and likely future directions for Lyme disease serodiagnostics are presented.

INFECTION AND IMMUNE RESPONSE

Natural Lyme borreliosis infection of humans and other mammals occurs over a period of hours and is initiated 1 to several days after the infected tick attaches to the host's skin and starts imbibing its blood. At this point in tick feeding, spirochetes residing in its midgut lumen are stimulated to replicate and migrate through the circulatory hemolymph to the salivary glands and finally into the skin of the host. Changes in spirochete gene expression and antigen profile occur during this migration as the spirochete prepares for its drastically altered environments and the defensive assault, both passive and active, awaiting in the mammalian host.[8,9] Through these physical, physiologic, and defensive processes, it is estimated that spirochetes from as few as 5% of infected ticks feeding on humans are transmitted successfully.[10] When successful, hundreds to thousands of bacteria may be inoculated over a multi-hour period before the tick terminates feeding. The likelihood of a continued infection and dissemination from the tick bite site is further reduced by both innate and active immunity. Despite these odds, without early and appropriate treatment, roughly 60% of *B burgdorferi*–infected patients in the United States will manifest disseminated infections with multiple EMs and other noncutaneous systems involvement (available: http://www.cdc.gov/lyme/signs_symptoms/index.html).

After infected tick feeding, the first indication of transmission and early infection in most North American patients is the development of an EM rash at the site of the previous tick bite. A rash developing within the first hours of tick attachment is not an EM or indicative of infection. EM development is a product of the innate immune response and occurs between 3 and 30 days after infection with an average of 7 days. The innate immune system is preprimed to recognize and quickly respond to a limited number of foreign patterns including those displayed by the outer surface proteins (Osp) of *B burgdorferi*.[11] Spirochete numbers (inoculated dose and subsequent replication) as well as their migration from the bite site factor into this early immune response and the subsequent EM appearance and behavior. Prospective and carefully attended patient studies indicate that EM occurs in about 85% or more of newly infected US Lyme patients, but is in practice less frequently observed and reported in nonstudy patients.[12,13] Reasons for discrepancies in EM detection include location (hidden or not frequently viewed anatomic sites), atypical or unrecognized