June 20, 2025

Susan Monarez, PhD
Director of the Centers for Disease Control and Prevention (CDC)
susan.monarez@nih.hhs.gov

Re: Molecular diagnosis of Lyme disease spirochetemia by Sanger sequencing

Dear Dr. Monarez:

I am writing to inform the CDC that according to a published peer-reviewed study titled "Sanger Sequencing of *Borrelia burgdorferi flaB* Paralogs Detected Spirochetemia at the Early Localized Stage of Lyme Disease" https://www.imrpress.com/journal/FBS/17/2/10.31083/FBS31280/htm :

- The *flaB* gene of the *Borrelia burgdorferi* bacteria invading the blood stream of the Lyme disease patients at the early localized stage of infection is found to have duplicate paralogs.
- *The flaB* gene with its paralogs is a more sensitive chromosomal target than the *16S rRNA* gene in PCR-based molecular testing for detecting *B. burgdorferi* spirochetemia.
- For sensitive and specific molecular diagnosis of *B. burgdorferi* spirochetemia with very low bacterial densities, the platelet-rich plasma needs to be separated from the blood cells by differential centrifugation immediately after venous blood collection. The entire DNA content extracted from the platelet pellet is used for *flaB* gene nested PCR amplification followed by Sanger sequencing of the PCR products.

The serology testing recommended by the CDC for laboratory diagnosis of Lyme disease is deeply flawed because it is known to generate both false-negative and false-positive results. Lyme disease is a systemic bacterial infectious disease. As we all know, in clinical microbiology, laboratory diagnoses of infectious diseases are based on culturing pathogens or nucleic acid amplification methods, which include techniques like PCR and subsequent DNA sequencing.

It is generally believed that at the early stage of infection Lyme disease can be treated successfully with a proper course of antibiotics, but the spirochete bacteria may disseminate from the site of the tick bite to other regions of the body if not timely and properly treated.

Since *Borrelia burgdorferi* does not have lipopolysaccharides, the endotoxin found in Gram-negative bacteria, patients at early stage of Lyme disease infection even with spirochetemia may have little symptoms. A sensitive and specific direct detection method is needed for early diagnosis. Antibodies against the bacteria are not detectable until a few weeks after infection at the convalescent stage. By then, the bacteria may have infected the tendons around the joints or the nervous system, causing neuroborreliosis. Patients with chronic Lyme disease or post-treatment Lyme Disease Syndrome may also have spirochetemia, confirmed by nested PCR and Sanger sequencing: https://pubmed.ncbi.nlm.nih.gov/24968274/

The total cumulative number of patients with chronic Lyme disease or post-treatment Lyme Disease Syndrome in the United States is very large, but unknown due to a lack of diagnostic

tools. The pathogenesis of chronic Lyme disease or post-treatment Lyme Disease Syndrome is highly debatable. Persistent bacterial infection of the deep tissues and autoimmune reaction (https://autoimmune.org/lyme-disease-and-the-autoimmune-connection/) have been proposed as the underlying pathology. But it seems to be clear that if Lyme disease can be detected early and treated timely and properly, the causative agent inducing persistent infection or autoimmune reaction can be eliminated. In recent medical history rheumatic fever and rheumatic heart disease were very common disabling diseases in the 1950s and 1960s, but we hardly hear about them nowadays because the pediatricians in the U.S. started to treat children with group A beta-hemolytic streptococcal pharyngitis with penicillin in the 1950s. By virtue of the same rationale the solution for the Lyme disease crisis in the U.S. is to set up routine PCR/sequencing testing for direct detection of *Borrelia burgdorferi* spirochetemia in community hospital laboratories located in Lyme disease-endemic areas for early diagnosis and early treatment.

I would like to point out that some of the CDC managers have used “lack of FDA approval” as a pretext to suppress implementation of Sanger sequencing of nested PCR amplicon of *Borrelia burgdorferi* chromosomal DNA as a sensitive and specific tool for the diagnosis of Lyme disease spirochetemia to deprive the current and future Lyme disease patients from having an accurate diagnostic test for timely and proper management of their infections.

However, most hospital laboratories are still using laboratory-developed tests regulated by the Centers for Medicare & Medicaid Services (CMS) under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) and not approved by the FDA to diagnose bacteremias.

The CDC scientists were also quite comfortable to use Sanger sequencing of nested RT-PCR products of the SARS-CoV-2 RNA genome for the diagnosis of the first COVID-19 case in the United States [1] with the full knowledge that the nested RT-PCR/Sanger sequencing test was not approved by the FDA. In fact, to this day the CDC does not recommend serologic tests for the diagnosis of SARS-CoV-2 infections as it still does so for the diagnosis of *Borrelia burgdorferi* infections. This double standard leading to misdiagnoses of Lyme disease infections has created many ill-defined illness cases in the U.S.

In view of the low density of spirochetes in the circulating blood specimens of most Lyme disease patients, nested PCR amplification of the target chromosomal DNA of *Borrelia burgdorferi* is needed to increase the sensitivity of detection while automated Sanger sequencing is needed to verify the authenticity of the nested PCR product to avoid PCR false-positive results. This is a high-complexity technology, which involves selection of a high-fidelity DNA polymerase to reduce *Taq* errors, which tend to be associated with nested PCRs, and involves proprietary sequencing reagents and instruments. It is unlikely for any commercial entity to find all the needed ingredients to manufacture such a complex test kit for FDA clearance in the near future. The CDC should assume a leading role to implement this revolutionary project.

References:

1. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, Queen K, Tao Y, Paden CR, Zhang J, Li Y, Uehara A, Wang H, Goldsmith C, Bullock HA, Wang L, Whitaker B, Lynch B, Gautam R, Schindewolf C, Lokugamage KG, Scharton D, Plante JA, Mirchandani D, Widen SG, Narayanan K, Makino S, Ksiazek TG, Plante KS, Weaver SC, Lindstrom S, Tong S, Menachery VD, Thornburg NJ. Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. Emerg Infect Dis. 2020 Jun;26(6):1266-1273. doi: 10.3201/eid2606.200516. Epub 2020 Jun 17. PMID: 32160149; PMCID: PMC7258473.

Thank you for your consideration. I will be glad to answer any questions about this suggestion.

Respectfully yours,

Sin Hang Lee, MD

Director
Milford Molecular Diagnostics Laboratory
2044 Bridgeport Avenue
Milford, CT 06460
Email: shlee01@snet.net

Copy of this letter is also sent via USPS to:

The Honorable Robert F. Kennedy, Jr.
Secretary
U.S. Department of Health and Human Services
200 Independence Avenue, S.W.
Washington, D.C. 20201