ASSOCIATION FOR MOLECULAR PATHOLOGY
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Delivered electronically to: LymeInnovation@hhs.gov

Subject: RFI RESPONSE - Accelerating Innovation in Diagnostic Testing for Lyme Disease

Dr. Honey:

On behalf of the Association for Molecular Pathology (AMP), thank you for the opportunity to submit comments in response to the Department for Health and Human Services' Request for Information (RFI) on Accelerating Innovation in Diagnostic Testing for Lyme Disease. AMP is an international medical and professional association representing approximately 2,500 physicians, doctoral scientists, and medical technologists who perform or are involved with laboratory testing based on knowledge derived from molecular biology, genetics, and genomics. Membership includes professionals from the government, academic medicine, private and hospital-based clinical laboratories, and the in vitro diagnostics industry. A subset of our members has a professional interest in infectious diseases, so we are pleased to engage in this conversation on how to advance laboratory testing in such a way that improves the care of patients with Lyme disease.

As the RFI details, there are numerous challenges associated with Lyme disease diagnostic testing as it currently stands. We outline factors that should be considered as we work to advance this area of medicine in below.

What challenges/barriers exist for the development and validation of innovative diagnostic tests for Lyme disease?

As you know, the standard for Lyme disease testing is a two-step serodiagnostic approach, which is only able to aid in the diagnosis of Lyme disease after a patient develops antibodies to *Borrelia burgdorferi*, well after the optimal window for patient care, which introduces the potential for unnecessary and ineffective treatment and the delay of care specific to the etiology of the disease. Unfortunately, the sensitivity of polymerase chain reaction (PCR) testing using a whole blood sample to detect an active infection is low as a result of the low concentration and transient nature of *B. burgdorferi* in the bloodstream following infection. Emphasis has been put on increasing the sensitivity of the PCR test; however, we also recommend that test developers explore approaches to concentrate the number of *B. burgdorferi* spirochetes in the sample used. For instance, antibodies to *B. burgdorferi* could be used as part of a pull-down assay to collect the pathogen to be deposited into more concentrated form that can then be used for PCR-based detection.

The ability to develop and assess the performance of tests directed at detection of *B. burgdorferi* is also limited by the lack of access to gold standard specimens for validation purposes. As a result of the serology testing being

the principal means of laboratory diagnosis, patient specimens are often collected sometime after a person has been infected. Because *B. burgdorferi* eventually leaves the bloodstream to invade and colonize other tissues, there is a limited window for the direct detection of the pathogen in peripheral blood samples that are collected at the time of symptom onset. This results in limited access to positive samples to aid in test development and validation. We note that the Centers for Disease Control and Prevention in partnership with the National Institutes of Health have developed a comprehensive panel of sera from patients with various stages of Lyme disease and other conditions, as well as healthy persons[1], and we applaud the agencies for advancing this important work. We believe that test developers would greatly benefit from building on the current effort to increase the size of the biorepository of well characterized samples to specifically include additional specimens collected early in the infection (i.e., specimens more likely to contain the organism at a greater concentration). Additionally, the effort should be expanded to include negative-control disease categories such as specimens from individuals infected with other tick-borne diseases to account for the diversity of organisms with overlapping clinical features that are observed in the clinical setting.

What types of diagnostic technologies are being developed (or could be developed or adapted) to detect Lyme disease, including technologies and breakthroughs adapted from COVID-19 diagnostics with potential applications for Lyme disease (e.g., highly sensitive nucleic acid amplification testing [NAAT])?

As mentioned above, opportunities to take pre-analytical steps to concentrate the number of spirochetes in the sample for DNA detection will aid in the improved ability to directly detect *B. burgdorferi*. A number of innovative approaches could be limited by the number of pathogens in whole blood and serum, so a method to concentrate organism or free nucleic acid in blood or serum could improve the success of many technologies. As examples, concentrated samples could synergistically contribute to the advancements that have been made to increase the sensitivity of nucleic acid amplification testing and to overcome the challenges, described by others[2], to employ sequencing-based detection. Additionally, we are encouraged by the development of testing using cell-free DNA (cfDNA) in plasma to improve early diagnosis of Lyme disease.[3]

What emerging technologies (e.g., epigenetic mapping, inflammatory markers, gene arrays, NAAT, or others) might be developed or adapted to characterize different stages of Lyme disease, including Post-Treatment Lyme Disease Syndrome (PTLDS), etc.?

Our comments focus on the improvement of early and direct detection of *B. burgdorferi*, but we applaud you for also inviting comment on this other important topic. It has been reported that the incidence of Post-Treatment Lyme Disease Syndrome is higher among those who are not treated early for Lyme disease[4], so there may be significant benefit that can be gained from early detection alone. We applaud LymeX Innovator Accelerator for exploring all ways that care for patients with Lyme disease can be improved through testing.

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[1] Molins CR, Sexton C, Young JW, et al. Collection and characterization of samples for establishment of a serum repository for Lyme disease diagnostic test development and evaluation. J Clin Microbiol 2014;52:3755–62. https://doi.org/10.1128/JCM.01409-14

[2] Schutzer SE, Body BA, Boyle J, et al. Direct Diagnostic Tests for Lyme Disease. Clin Infect Dis. 2019;68(6):1052-1057. doi:10.1093/cid/ciy614

[3] Branda JA, Lemieux JE, Blair L, et al. Detection of Borrelia burgdorferi Cell-free DNA in Human Plasma Samples for Improved Diagnosis of Early Lyme Borreliosis. Clin Infect Dis. June 2020. doi:10.1093/cid/ciaa858

[4] Asch ES, Bujak DI, Weiss M, et al. Lyme disease: an infectious and postinfectious syndrome. J. Rheumatol. 1994; 21(3):454-61.

Additionally, we also feel strongly that any novel diagnostics should also consider other tick-borne pathogens. An array of other *Borrelia* species, as well as *Anaplasma*, *Ehrlichia*, and *Babesia*, are transmitted by the same tick species as *B. burgdorferi*, or have overlapping geographic range. Further, ticks may carry, and transmit, these organisms together which may lead to co-infections. Given the similar clinical presentation and exposure risk of these other tickborne pathogens, the ability to detect them in a person presenting with symptoms of "Lyme disease" can greatly aid in the diagnosis and management of these patients. Fortunately, these organisms are comparatively easy to detect using PCR-based tests because the concentration in the bloodstream is significantly higher than that of *B. burgdorferi* and as such may be relatively easy to incorporate into novel diagnostic tests for *B. burgdorferi*.

What is the optimal sample type (e.g., whole blood, plasma) for the detection of a test analyte in patients with Lyme disease? The optimal sample type can be generally defined as the one where the analyte can be best detected.

Currently, whole blood has limited utility because the recovery of *B. burgdorferi* from this sample type is low. However, we believe that whole blood can potentially be an optimal sample type because 1) the pathogen is present in the bloodstream in the days and weeks following the onset of infection, and 2) whole blood can be obtained through minimally invasive procedures. Moreover, if other limitations can be overcome, it can be used for a range of testing approaches including PCR-based testing, sequencing, cfDNA testing, point-of-care testing, and more.

We recognize that synovial fluid of the joint is another specimen type that can be used for direct diagnostic testing, however, it requires an invasive procedure in order to be collected and is therefore not ideal. Further, invasion of tissues or joints is typically a later presentation of *B. burgdorferi* infection.

An alternative specimen that had demonstrated some utility for diagnosing acute or active infection is urine. A major advantage of urine is that it can be collected non-invasively. Although much more research needs to be done, *B. burgdorferi* surface proteins have been detected in the urine of patient with acute and active infection. This type of assay has been successfully used for other bacterial and fungal pathogens including *S. pneumioniae*, *L. monocytogenes*, and *Blastomyces* which provides a solid precedent for this approach. A further benefit is that the use of urine as a specimen type could help to enable at-home testing, which would greatly expand the accessibility of testing. The COVID-19 pandemic has renewed energy for engaging patients in their healthcare at home especially for those who are unable to travel to seek care, which will be particularly impactful for those in rural areas.

What challenges exist in the implementation and use of Lyme disease diagnostic testing in clinical practice?

The early diagnosis of Lyme disease while a person is still actively infected to ensure prompt, targeted therapy is the preferred situation. A laboratory-based testing service using whole blood is likely to be the most sensitive and specific approach to ensure that an appropriate treatment plan is selected. We note that emphasis has been made on the use of FDA cleared or approved tests. In vitro diagnostic test kits for the detection of *B. burgdorferi* are an important and central component of the array of test types that will be needed to meet the needs of laboratory professionals in the United States and other countries especially because of the test development and validation challenges noted above. However, laboratory developed testing procedures (LDPs) fill gaps in testing and are often the foundation for advances in molecular pathology. We hope that the LymeX Innovation Accelerator program will consider LDPs and the molecular professionals that perform them as valuable contributors to patient care.

It is also important to note that patients are in a range of situations and an alternative approach may be best suited to meet an individual's needs. Some patients may benefit from having a test result in minutes while sitting in their healthcare provider's office, as is made possible by current[5], and hopefully future, CLIA waived point of care tests. Alternatively, some patients may be unable or reluctant to travel to a provider's office and are much more likely to take a test if it is from the comfort of their own home. With the rapid expansion of telehealth and other technologies that have improved our ability to access remote healthcare, providers can continue to be involved in ensuring that patients are receiving the best care possible.

Lastly, we would like to note that innovation in Lyme disease diagnostic testing would help to improve patient care for other tick-borne infectious diseases. For instance, *B. burgdorferi* and *Borrelia miyamotoi* present in ticks in the same geographical locations, and unfortunately, *B. miyamotoi* leads to cross-reactive antibodies to the C6 peptide when performing an enzyme immunoassay (EIA) for *B. burgdorferi*.[6] Additionally, it has been reported that when broad multiplex tests to detect tick-borne pathogens were performed for patients presenting with Lyme-like symptoms, nine percent of the positive results were *B. miyamotoi*[7]. Therefore, we strongly suggest test developers and clinical guideline setting bodies consider the benefits of sequential or panel pathogen testing to ensure that patients with another tick-borne disease are treated in a timely manner; however, we would like to emphasize that while ability to multiplex should be considered, it should not be a requirement for a better Lyme-specific test.

Thank you again for the opportunity to provide these comments in response to the RFI. We are hopeful that this "moonshot" approach will accelerate achievements in Lyme disease testing. We hope that you will consider the Association for Molecular Pathology and our members as a resource as you take the next steps in this effort. If you have any questions, please do not hesitate to contact Sarah Thibault-Sennett, Senior Manager of Public Policy and Advocacy, at sthibaultsennett@amp.org.

Sincerely,

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President, Association for Molecular Pathology

[5] https://www.quidel.com/immunoassays/rapid-lyme-tests/sofia-2-lyme-fia

[6] Koetsveld J, Platonov AE, Kuleshov K, et al. Borrelia miyamotoi infection leads to cross-reactive antibodies to the C6 peptide in mice and men. Clin Microbiol Infect. 2019;26(4). doi:10.1016/j.cmi.2019.07.026

[7] Buchan B, Jobe D, Mashock M, et al. Evaluation of a Novel Multiplex High-Definition PCR Assay for Detection of Tick-Borne Pathogens in Whole-Blood Specimens. J Clin Microbiol. 2019;57(11). doi: 10.1128/JCM.00513-19.