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Report of the Testing and Diagnostics Subcommittee to the Tick-Borne Disease Working Group

Note: All subcommittee members actively participated in the development of this report. Members voted to approve submission of the report to the working group and on the wording of each of the possible actions contained in the report. The vote to submit the report indicates agreement with the main suggestion for action to develop improved diagnostic tests for Lyme disease. An individual member's vote to submit the report does not necessarily indicate complete agreement with each and every statement in the full report.

Readers should not consider the report or any part of it to be guidance or instruction regarding the diagnosis, care, or treatment of tick-borne diseases or to supersede in any way existing guidance.

Information and opinions in this report do not necessarily reflect the opinions of the working group, the U.S. Department of Health and Human Services, or any other component of the federal government.

Background

Lyme disease is a growing emerging public health epidemic in the United States. Increasingly prevalent, this tick-borne bacterial infection has risen to become the nation's most commonly reported vector-borne disease. According to CDC estimates, more than 300,000 new cases occur each year. Despite federal, state, and local efforts to prevent and control the spread of the disease, the number of cases has continued to increase over the last few decades. This problem is exacerbated by biologic and technical challenges to the diagnosis of Lyme disease described below, limiting the opportunities for early identification and treatment.

Currently, Lyme disease diagnostic tests have well described limitations (see https://www.cdc.gov/lyme/diagnosistesting/index.html). A diagnosis of Lyme disease relies on either the presence of a bull's-eye rash, known as erythema migrans (EM) or a positive Lyme disease serology in the appropriate clinical scenario. A two-tiered Lyme disease serologic test includes a first-tier enzyme immunoassay (EIA) followed by a supplemental Western immunoblot for those with positive or equivocal first-tier test results.

Patients must rely on health care professionals to suspect possible Lyme disease. More than half of patients may not have the characteristic EM rash and this skin lesion may not be present or may present atypically (i.e., not a bulls-eye), making the diagnosis more challenging. In addition, currently available diagnostic tests may have either false negative or false positive test results. Generally, several weeks may elapse between initial infection and the development of antibodies and, for some patients, antibodies may not reach a detectable level. Previous evaluations of Lyme disease tests have focused on patients with EM lesions and as such, the tests may perform less effectively than expected when applied to some patients without an EM lesion. Furthermore, in populations where Lyme disease is endemic, and prior exposure is more likely, positive two-tier serology can represent previous rather than active infection. Finally, current tests do not indicate when treatments have been effective. Delayed or missed diagnosis of Lyme disease increases the risk of progressive or worsening illness. Objective tests to correctly identify patients with Lyme disease and to ensure efficacy of treatment are critical to curb the negative effects of the Lyme disease epidemic.

This report identifies key issues related to the currently available commercial laboratory tests that health care providers routinely order for Lyme disease diagnosis. Specifically, the subcommittee focused on the following three priority areas:

- Biological and technical limitations of serological tests for Lyme disease: Why new approaches are needed.
- Emerging technologies, and previously developed technologies applied to other diseases that may be adapted for use in Lyme disease.
- Consideration of special populations, such as children and minorities, both in the evaluation of new diagnostic approaches and in provider education.

Increased federal investment in the development and evaluation of novel or improved approaches is critical to helping avert the continuing adverse health effects of Lyme disease.

Methods

The Chair and Vice-Chair of the Working Group selected two members for each subcommittee to serve as co-chairs (see Table 1). One was a public member of the HHS federal working group and the other was selected from among the community members of the subcommittee.

Members for the subcommittees were selected from a total of 218 nominations that were received from people who had volunteered pursuant to either one of the requests that were published in the Federal Register to serve on the Working Group or on a subcommittee. Of these, a total of 50 persons expressed primary interest in the Testing and Diagnostics Subcommittee. Each application was evaluated to determine level of knowledge and experience regarding the work of the subcommittee that included efforts to benefit others.

The co-chairs reviewed all of the nominations and identified persons with at least some experience related to the subcommittee's specific content as well as the experience needed to address the subcommittee's work. In addition, the co-chairs made sure that the perspectives of patients and other key stakeholders identified in the 21st Century Cures Act were identified. Subcommittee members included patient advocates, health care providers, laboratory directors, basic and clinical Lyme disease researchers, as well as federal members from the NIH and FDA (Table 1).

In response to a request from the co-chairs, subcommittee members developed an extensive list of issues and/or questions related to Lyme disease diagnostic testing. Members considered barriers and opportunities from the perspectives of patients, family members, health care providers, health department staff, the federal government, state and local governments, and society as a whole. After extensive discussions over the phone and email, the subcommittee agreed that it should select three topic areas, given the limited time available for production of the initial report.

Subsequently, the co-chairs organized the list of issues/questions into three categories: gaps in diagnostic technologies, need for improved approaches and innovative technology, and inclusion of special populations. These categories were reaffirmed after further discussion. The subcommittee also noted that numerous problems may exist with the testing and diagnosis of the various tick-borne diseases other than Lyme disease, but after discussion with co-chairs, determined that these issues would be covered by the subcommittee focused on other tick-borne diseases. During the prioritization process, subcommittee members were asked to consider the potential impact that addressing an issue would have, along with how easy or difficult it would be to bring about the desired change. All of the subcommittee members agreed on the issues that were most important to address in the first report.

The committee work was conducted in a series of conference calls as well as electronic document sharing. All together the group held 11 conference calls (Table 2) with links to summaries of the working group conversations. The group selected three invited guest speakers based on topic expertise and group consensus (Table 3). The committee approved the initial and final subcommittee report within the time constraints of the subcommittee process (Table 4). There were no dissenting opinions for any of the subcommittee votes.

Given that time constraints precluded an exhaustive review of the literature before submitting this report, a limited list of selected references relevant to each area of focus was included. All subcommittee members actively participated in the development of this report. Members voted to approve submission of the report to the working group and on the content of each of the possible actions contained in the report. An individual member's vote to submit the report indicates general agreement with content of the document, but it does not necessarily indicate complete agreement with each and every statement in the full report.

Table 1: Members of the Testing and Diagnostics Subcommittee

Member	Type	Stakeholder Group	Expertise
(Co-Chair) **Lise E. Nigrovic, MD, MPH** Boston Children's Hospital Boston, Massachusetts	Public	Health Care Provider	Associate Professor Pediatrics and Emergency Medicine, Harvard Medical School. Founder and chair of Pedi Lyme Net, a six-center pediatric clinical research network with an associated Pediatric Lyme Disease Biobank.
(Co-chair) **David Roth**, JD Blackstone New York, New York	Public	Patient; Advocate	Member of steering committee for National Institute of Standards and Technology Lyme Disease Workshop. Founder and co-Chairman of Tick Borne Disease Alliance. Founder of Global Lyme Alliance. Board member and chairman of Executive Committee of Project Lyme. Steering Committee member of The Steven & Alexandra Cohen Foundation's Lyme disease initiative.
Holly Ahern, MS, MT (ASCP) The State University of New York Adirondack Queensbury, New York	Public	Advocate; Microbiologist	Associate Professor of Microbiology, SUNY Adirondack. NYS Senate Task Force Advisory Board member. Co-founder and VP of Lyme Action Network. Scientific Advisor for Focus on Lyme and Project Lyme.
Charles Y. Chiu, MD, PhD University of California, San Francisco (UCSF) San Francisco, California	Public	Health Care Provider	Associate Professor of Laboratory Medicine and Medicine, Division of Infectious Diseases, UCSF. Associate Director, UCSF Clinical Microbiology Laboratory.
Roberta DeBiasi, MD, MS Children's National Medical Center Washington, DC	Public	Health Care Provider	Professor of Pediatrics, Microbiology, Immunology, and Tropical Medicine, George Washington University School of Medicine and Health Sciences. Chief, Division of Pediatric Infectious Diseases.

Member	Type	Stakeholder Group	Expertise
Noel Gerald, PhD U.S. Food and Drug Administration Silver Spring, Maryland	Federal	Public Health	Biologist and Senior Scientific Reviewer, Office of In Vitro Diagnostics and Radiological Health, Center for Devices and Radiological Health.
Deborah Hoadley, MD, MPH New England Institute for Lyme Disease and Tick-Borne Illness Longmeadow, Massachusetts	Public	Health Care Provider	Director, New England Institute for Lyme Disease and Tick-Borne Illness.
Maliha Ilias, PhD National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) Rockville, Maryland	Federal	Public Health	Program Officer, Lyme Disease Research Bacteriology and Mycology Branch
Bobbi Pritt, MD, MSc Mayo Clinic Rochester, Minnesota	Public	Health Care Provider	Professor of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine. Co-Director, Vector-Borne Diseases Laboratory Services.
Steven Schutzer, MD Rutgers, New Jersey Medical School Newark, New Jersey	Public	Health Care Provider	Professor of Medicine, Rutgers New Jersey Medical School. Lyme disease and tick-borne infections researcher.

Stakeholder Types = Patient, Family Member, Advocate (nonprofit), Health Care Provider, Public Health, Other (if other please type in description).

Table 2: Overview of Testing and Diagnostics Subcommittee Meetings, 2018

Meeting No.	Date	Present	Topics Addressed

Meeting No.	Date	Present	Topics Addressed
1	February 12, 2018	John Aucott (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Roberta DiBiasi, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, David Roth, Steven Schutzer	Personal introductions.
2	March 2, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Roberta DiBiasi, Noel Gerald, Deborah Hoadley, Maliha Ilias, Steven Schutzer	Pros and cons of producing a report that lists all shortcomings of currently available tests and diagnostic algorithms; issues relevant to the available tests for Lyme disease; selection of three high-priority areas.
3	March 9, 2018	Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Noel Gerald, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Need for new Lyme disease test technology; need for a Lyme disease lexicon; assignment of priority areas.
4	March 16, 2018	Kristen Honey (Working Group Vice-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Coverage of priority areas by subgroup.
5	March 23, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Messages to include in the subcommittee's report to the Tick-Borne Disease Working Group.
6	March 28, 2018	Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Maliha Ilias, Steven Schutzer	National Institutes of Health-funded research around Lyme disease diagnostics; inviting Tom Slezak, MS, to join the March 30 meeting.

Meeting No.	Date	Present	Topics Addressed
7	March 30, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Roberta DiBiasi, Noel Gerald, Deborah Hoadley, Steven Schutzer	Use of deoxyribonucleic acid (DNA) sequencing for molecular detection of *Borrelia burgdorferi;* updates from Subgroups 1, 2, and 3.
8	April 6, 2018	Kristen Honey (Working Group Vice-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Noel Gerald, Deborah Hoadley, Maliha Ilias, Steven Schutzer	Next steps in the process of finalizing draft priorities.
9	April 13, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Charles Chiu, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Next steps in the process of finalizing draft priorities.
10	April 27, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Charles Chiu, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Next steps in the process of finalizing draft report to the working group.
11	May 4, 2018	Kristen Honey (Working Group Vice-Chair), Lise Nigrovic (Subcommittee Co-Chair), David Roth (Subcommittee Co-Chair), Holly Ahern, Roberta DiBiasi, Noel Gerald, Deborah Hoadley, Maliha Ilias, Bobbi Pritt, Steven Schutzer	Finalization of working group report; Lyme disease diagnostics guest speaker.

Table 3: Presenters to the Testing and Diagnostics Subcommittee

Meeting No.	Presenter	Topics Discussed

Meeting No.	Presenter	Topics Discussed
6	Maliha Ilias, PhD National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)	NIH-funded research around Lyme disease diagnostics
7	Tom Slezak, MS Lawrence Livermore National Laboratory	Use of deoxyribonucleic acid (DNA) sequencing for molecular detection of *Borrelia burgdorferi*
11	Ray Datweiler, MD Professor of Medicine Chief of Clinical Immunology, Allergy & Rheumatology New York Medical College	Lyme disease diagnostics<

Table 4: Votes Taken by the Testing and Diagnostics Subcommittee

Meeting or Date	Motion	Results	Minority Response
2	Approve issues and priorities section of the report for submission to the Working Group	Agreed meeting	No
3	Approve draft subcommittee report for initial review including background	Agreed Meeting # 9	No
4	Approve draft subcommittee full report for HHS review	Electronic vote	No

Potential Actions

Priority 1: Gaps in Diagnostic Technologies

Summary

The development of tests for Lyme disease with improved performance would result in prompt diagnosis, higher likelihood of cure, decreased adverse impact to patients and their families, and reduction of health care costs for the United States. With federal focus, we believe these tests can be developed (or adapted from existing technology used in other diseases) and validated within a few years. The causative microbe (i.e., *Borrelia burgdorferi*) has unusual properties that has made test design difficult. Most of the currently

available tests utilize technology that is more than 30 years old. Federal assistance can enable modern infectious disease testing methods to quickly be adapted to Lyme disease, which would help mitigate the adverse impact Lyme disease has had and continues to have on society.

Issue

Lyme disease is an infectious disease that begins in the skin and may disseminate to other organs (joints, heart, and nervous system) over time. The complexity of the infection process and the resulting variability in symptoms makes it difficult to establish a single simple standard to diagnose all patients.

According to existing medical standards, Lyme disease may be diagnosed without laboratory testing in persons from a highly endemic area with objective clinical findings, specifically a near unique rash called erythema migrans (EM). However, fewer than half of patients may develop this specific rash. Additionally, the rash may be missed or take a form other than the classic bull's-eye rash. Laboratory testing to provide evidence of infection with *Borrelia burgdorferi*, the organism that causes Lyme disease, is recommended for patients who do not show an identifiable EM rash but have non-specific symptoms suggestive of Lyme disease.

Serological assays that detect antibodies against *B. burgdorferi* are currently the only type of laboratory test for Lyme disease cleared by the U.S. Food and Drug Administration (FDA) and recommended by the Centers for Disease Control and Prevention (CDC) for the diagnosis of Lyme disease. The current serologic tests have biological and technical limitations that hinder their clinical performance and application. Problems include a poor ability of serologic tests to correctly identify all patients with the disease, subjective interpretation of test results by lab technicians for the type of serological assay called the Western blot, and confusion by health care providers and patients regarding how to interpret serologic test results.

As a result of these limitations, many health care providers have difficulty appropriately diagnosing and effectively treating patients with Lyme disease symptoms.

Evidence and Findings

Criteria for the interpretation of serologic tests currently recommended by the CDC for the diagnosis of Lyme disease were established in 1994. Published peer-reviewed studies show that serological tests have technical limitations, such as cross-reactivity between tests for Lyme disease and those for other infectious diseases. Serologic tests also have biological limitations related to how the human immune system reacts to infection with *B. burgdorferi*. Antibodies may not be produced by the immune system early enough or in high enough quantities to meet the detection limit of the test. These limitations combine to make it difficult for health care providers to determine whether their patient has Lyme disease or not.

Research focusing specifically on the performance of serologic tests for Lyme disease diagnosis demonstrates that test results can be inconsistent among different laboratories or with different test kits, that serologic assays for *B. burgdorferi* can be negative during the first two to four weeks of infection, and

that serology may not detect all cases of Lyme disease, particularly in persons who do not produce detectable levels of antibodies in response to infection, and in Lyme disease patients who were treated with antibiotics at the beginning of the infection.· In addition, many previous evaluations of Lyme disease tests have focused on patients with EM lesions and as such, the tests may perform less effectively than expected when applied to patients without an EM lesion.

Opportunities

The United States is in the position to markedly change Lyme disease diagnosis for the better. A federal response that includes diagnostic test development and implementation would decrease the number of missed Lyme disease diagnoses and, by extension, decrease the number of people who have short and long-term negative health effects stemming from untreated infections. An improved Lyme disease test would also decrease false positive results and reduce unnecessary treatment. Also, current diagnostic measurements do not reliably change with treatment so there is essentially no “test for cure.” Improved Lyme disease tests could decrease the societal burden of Lyme disease and associated costs to public health care systems. A strong federal response and immediate investment would help enable rapid improvements.

Threats or Challenges

Serologic tests for Lyme disease measure a person’s past or present immune response to infection and as such do not indicate whether the infection is active. Health care providers need to know the status of the infection (that is, active or not) in order to make an informed decision on whether antibiotic treatment should be initiated or continued.

Serology, however, remains the most commonly ordered test for Lyme disease in the United States. The greatest threat of not addressing the shortcomings in laboratory testing for Lyme disease is that a significant proportion of patients in the United States who are newly infected with *B. burgdorferi* will not be diagnosed with Lyme disease and will not receive prompt treatment for a disease with the potential to cause disabling illness and death.

Possible Actions for Working Group to Consider

Congress can increase appropriations to the National Institutes of Health (NIH) and other federal organizations to fund research that will advance the ability of health care providers to accurately diagnose and effectively treat patients with Lyme disease. NIH and other federal organizations may then take advantage of current and existing peer-review processes to evaluate the feasibility and impact of proposed research projects, including projects that will:

1. Support translational research leading to the development of diagnostic tests
2. Rapidly translate new diagnostics into test platforms that can be submitted for evaluation by the FDA for clearance or approval

3. Encourage scientists to repurpose existing technologies available for diagnosis of other diseases such as cancer and non-Lyme infectious diseases.

Ideas to explore include funding to develop new or enhance existing repositories of biological samples for basic research and test validation, use of private-public partnerships, open-source data exchange initiatives and cash-based prizes to validate diagnostic technologies (see Section 2002(A)(i) of the 21st Century Cures Act).

Selected References

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Priority 2: Improved Approaches and Innovative Technology for Diagnosing Lyme Disease

Summary

The limitations of many of the currently available diagnostic tests for Lyme disease highlight the need for improved approaches and innovative technologies to detect early Lyme disease, determine effectiveness of treatment, and distinguish between active infection and previous exposure to the causative organism. Laboratory tests for Lyme disease are based on either directly detecting the pathogen in patient samples or detecting an immune response of the patient to the pathogen. Even though both testing methodologies have their limitations, research and development efforts over the years have led to the identification of various innovative approaches to diagnosing Lyme disease that have benefited from recent advancements in technologies. In addition, efforts have been made to adapt existing technologies that have been used for other infectious diseases for the diagnosis of Lyme disease. Further funding and support will ensure that these advancements help overcome some of the gaps in diagnostic tests for Lyme disease, thus resulting in appropriate diagnosis and effective treatment of patients.

Issue

Biological and technical limitations of many currently available diagnostic tests for Lyme disease impact their clinical performance and interpretation, which highlights the need for improved approaches to detecting Lyme disease. As a result, some patients with Lyme disease fail to get diagnosed and others without Lyme disease get diagnosed incorrectly. This can lead to missed and/or incorrect diagnoses, no treatment or inappropriate treatment, increased health care costs, and poorer clinical outcomes.

Evidence and Findings

Lyme disease is particularly challenging to diagnose because of the low numbers of *Borrelia burgdorferi* (the bacterium that causes Lyme disease in the United States) that can be detected in a patient's blood. Additionally, the bacterium grows very slowly in culture. Thus, traditional methods, such as culture and polymerase chain reaction (PCR), may fail to detect the pathogen in persons with the infection. Indirect serologic tests, which measure a patient's immune response, also have limitations because the body takes approximately two or three weeks to generate antibodies in response to this infection, and in a subpopulation of patients the immune response may never be sufficient to allow for detection of

antibodies. As a result, the clinical performance of these tests is limited and improvements are needed. Also, current diagnostic measurements do not reliably change with treatment so there is essentially no "test for cure."

Opportunities and Challenges

Laboratory testing for Lyme disease is based on either directly observing the pathogen in patient samples (direct testing) or by measuring the patient's immune response (indirect testing). As mentioned earlier, there are challenges with both types of testing. Some of the challenges with regards to both type of testing methodologies appear below.

Challenges of direct testing (based on the presence or absence of a pathogen):

- The low levels of bacteria in most patient samples makes direct pathogen detection challenging
- Culturing the bacteria is difficult as *B. burgdorferi* grows slowly, requires special media and expertise, and thus has limited applicability in clinical settings.
- PCR is a technique that allows the replication of genes across several orders of magnitude and can generate millions of copies of a DNA sequence, but use of PCR has biological limitations when it comes to the detection of *B. burgdorferi* without enhancements.

Challenges of indirect testing (based on host response):

- The development of detectable levels of antibodies to *B. burgdorferi* takes time by conventional methods, which makes it difficult to diagnose infection during the first few weeks.
- Subjective interpretation of the results of the Western blot introduces variability.
- Indirect testing ultimately depends on the ability of the host's immune system to respond to infection as well as the composition of the test itself.

However, research has helped us realize progress in not only improving current testing methodologies but also developing new technologies or repurposing existing technologies to overcome some of the challenges identified above. **Table 5** lists existing and emerging technologies for diagnosing Lyme disease. Many of these new tests for infectious diseases are based on emerging approaches and have the potential to be diagnostically useful for Lyme disease. Improved serologic tests targeting multiple and more specific components from *Borrelia* or simultaneously detecting all tick-borne infections are being developed. Metagenomic sequencing of DNA/RNA and proteomics can be used to identify tick-borne pathogens in clinical samples. Transcriptomics and metabolomics are methods to comprehensively assess a patient's host response during all stages of infection and can be potentially leveraged for use as

a method to stage disease. Emerging technologies and diagnostic platforms in other fields, such as cancer and other infectious diseases, are being repurposed for Lyme disease, including microfluidics, affinity capture technology, cytokine release assays, and nanopore sequencing.

Table 5: Existing and Emerging Technologies to Improve Diagnosis of Tick-Borne Diseases

Category of Existing and Emerging Technology	Direct or Indirect Indicator of Infection in a Patient	Description	Comments
Proteomics	Direct – tests for pathogen proteins<	Proteins from specific pathogens are detected in patient samples.	• Existing and emerging technology supporting discovery of biomarkers useful for developing new tests. • Potential to detect early Lyme disease in the absence of rash. • Potential to monitor response to treatment. • Can be paired with affinity capture technology to enhance harvesting of proteins to detect pathogens. • Potential to detect all known sequenced tick-borne pathogens in one test.
Multiplexed next-generation DNA/RNA sequencing	Direct – tests for pathogen DNA or RNA	DNA or RNA from specific pathogens in patient samples are amplified by multiplexed PCR and identified by DNA/RNA sequencing.	• Existing technology with the potential to detect multiple tick-borne pathogens in one test. • Sensitivity needs to be studied.

Category of Existing and Emerging Technology	Direct or Indirect Indicator of Infection in a Patient	Description	Comments
Metagenomic DNA/RNA sequencing	Direct – tests for pathogen DNA or RNA	All of the DNA or RNA from patient samples is captured and sequenced, followed by computational identification of pathogen-specific “reads.”	• Existing technology with the potential to detect all known sequenced tick-borne pathogens in one test. • Technology will support discovery of new targets useful for developing new tests. • Technically complex. • Sensitivity needs to be studied.
Culture-based methods	Direct – tests for presence of live microorganism	Pathogenic microorganisms are grown in culture and identified.	• Existing and new technology • *Borrelia* are difficult to culture • Results are not rapidly available for immediate clinical decision-making. • Culture-based methods are currently in use to study *Borrelia* organisms in a research laboratory setting and may hold promise in identifying potential treatment regimens for further study in clinical trials.
Nanopore sequencing	Indirect – tests RNA expression	Capture and sequencing of pathogen DNA or human host RNA in patient samples using a pocket-sized device.	• Emerging technology with potential for “point-of-care” applications. • Evolving technology that may lack standardization.

Category of Existing and Emerging Technology	Direct or Indirect Indicator of Infection in a Patient	Description	Comments
Metabolomics	Indirect – tests for biomarkers indicating infection	Indicates disease by identifying sets of metabolic biomarkers produced in response to a specific infection.	• Existing and emerging technology supporting discovery of biosignatures or biomarkers useful for developing new tests. • May be prone to false positives. • Technically complex.
Transcriptomics	Indirect – tests patient RNA expressed during infection	Indicates disease by identifying patterns of RNA expression from activated human genes produced in response to a specific infection.	• Existing and emerging technology supporting discovery of biosignatures or biomarkers useful for developing new tests. • May be prone to false positives. • Technically complex.
Next-generation serologic assays	Indirect – tests for patient antibodies produced in response to an infection by a specific pathogen	Indicates disease by detecting antibodies produced by the patient in response to past or recent exposure to a specific pathogen.	• Existing technology evolving from current serologic tests that will detect an expanded range of antibodies. • False negatives in early disease. • False negatives and false positives in non-acute disease.
Microfluidics	Indirect – tests for patient antibodies produced in response to an infection by a specific pathogen.	Indicates disease by detecting antibodies produced by the patient, using small volumes of fluid samples.	• Existing and emerging technology evolving from current serologic tests. • Potential for “point-of-care” applications.

Category of Existing and Emerging Technology	Direct or Indirect Indicator of Infection in a Patient	Description	Comments
Cytokine Release Assays	Indirect – tests for non-antibody proteins produced by immune cells in response to infection	Indicates disease by exposing the patient's immune cells to Lyme-specific proteins and measuring their production of cytokines such as interferon gamma.	• Existing and emerging technology already in use for TB. • Potential to detect early Lyme disease in the absence of rash. • Can be used to monitor response to treatment. • Technology is adaptable to many infectious organisms.

Possible Actions for Working Group to Consider

Support is urgently needed to further develop and refine technologies, such as those highlighted above, and for clinical studies comparing the performance of new technologies to that of currently available serologic tests.

The Testing and Diagnostics subcommittee suggests the following initiatives to encourage development of novel approaches and innovative technologies for Lyme disease diagnosis:

1. Increased funding for the development of diagnostics for Lyme disease
2. Development of new (or support of existing) bio-sample repositories for the purpose of supporting basic research and test validation
3. Foster public-private partnerships, open source data-sharing and support prize-based competitions for the development of diagnostics for Lyme disease

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Priority 3: Inclusion of Special Populations

Summary

In endemic areas, Lyme disease cases in children may outnumber those in adults. This calls for the need to include children in scientific studies. Equally important is the need to include patients from additional populations previously under-represented in Lyme disease studies, as they may hold clues to special risk factors that could help reduce the number of Lyme disease cases and the resulting burden on the health care system.

Issue

Informed by convergent data from expert presentations, review of peer-reviewed publications, and multiple patient stories shared during public comment, the Testing and Diagnosis Subcommittee identified the need to include special populations (especially children) both in the evaluation of Lyme disease diagnostics and in provider education curricula as a priority area for further focus.

Of the more than 300,000 estimated new cases of Lyme disease occurring each year, more than half occur in children. However, to date, the majority of studies evaluating Lyme disease diagnostics have included few, if any, pediatric patients. Unique challenges in diagnosing Lyme disease in children abound. Those challenges include differences in clinical presentation and a reliance on caregivers to recognize illness and seek care for pediatric patients. Additionally, many health care professionals lack the knowledge that would enable them to suspect possible Lyme disease based on presenting signs and symptoms.

In addition to children, there are other smaller patient populations who have been under-represented in studies evaluating Lyme disease diagnostics. Those populations are listed below, (ranked from highest to lowest number of likely cases):

- Under-represented minorities
- Patients from geographical areas with a low prevalence of Lyme disease
- Immunocompromised patients
- Pregnant women

- Neonates born to women who were infected during pregnancy

Recognition of classic erythema migrans (EM) rash in individuals with dark skin pigmentation may be challenging, resulting in either delays or even failure to diagnose Lyme disease. Clinicians who care for patients residing in geographic areas with a low prevalence of Lyme disease require additional education to appropriately suspect Lyme disease in patients with appropriate signs and symptoms and to be cognizant of potential false positives as disease prevalence decreases. Patients with suppressed immune systems may not mount a reliable antibody response to infection; in such cases, reliance on currently available two-tiered, serology may not be appropriate. Hormonal changes during pregnancy can lead to changes in immune function[4] that may affect the detection of clinical or laboratory findings.

Opportunities

The evidence we reviewed allowed us to identify potential opportunities. Chief among these is the need to include children as well as other important patient subgroups in future studies to evaluate new approaches to Lyme disease diagnosis.

Provider awareness and recognition of the possibility of Lyme disease is an important component of diagnosis. Most providers have received little or no specific training on recognition, appropriate evaluation, and interpretation of testing for Lyme disease. Provider and patient education and training should include consideration of additional diagnostic issues pertinent to the special populations under consideration.

Threats or Challenges

Evaluations of Lyme disease diagnostics rely on well-characterized biospecimens from patients with Lyme disease as well as appropriate control patients. Investigators and test manufacturers often rely on previously collected specimens housed in Lyme disease biobanks, which contain biosamples collected from well characterized patients. However, existing biobanks include few if any children and rarely capture samples from other special populations, such as pregnant women, immunocompromised patients, or under-represented minorities.

Patient enrollment and biosample collection for Lyme disease biobanks is expensive and time-consuming. Efforts need to be made to ensure inclusion of special populations in new and existing biobanks.

Medical school curricula and post-graduate continuing education programs devoted to tick-borne illnesses may be quite limited, and it is rare for them to address issues related to the important subpopulations identified.

Possible Actions for Working Group to Consider

This subcommittee identified three potential actions that the federal government could take to improve testing and diagnosis of Lyme disease and tick-borne diseases for the special populations identified.

1. Encourage inclusion of special populations in future federally-funded Lyme disease research.

2. Provide federal funds for the development of high-quality Lyme disease biobanks that include special populations.

3. Develop and disseminate high-quality on-line provider education modules that address diagnosis of tick-borne illness in general, and special populations more specifically.

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