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Re: Comments on Accuracy of Diagnostic Tests for Lyme Disease

Dear Ms. Fabel:

At your request, I have reviewed the article titled “The Accuracy of Diagnostic Tests for Lyme Disease in Humans, A Systematic Review and Meta-Analysis of North American Research” by Lisa A. Waddell and colleagues [1]. My comments are as follows.

1. Lyme disease, or Lyme borreliosis, in North America is a group of systemic infections which may be caused by *Borrelia burgdorferi* sensu lato (including *B. mayonii*) [2], *B. miyamotoi* [3-5], and other unnamed tick-borne borrelia strains [3, 6]. Currently the diagnosis of emerging or re-emerging infectious diseases largely depends on finding evidence of the causative agents, including borrelia, in the host by nucleic acid-based tests [7]. The accuracy of any diagnostic tests must be measured against this standard of microbiological diagnosis. Using a serologic test kit developed for the detection of antibodies against the epitopes of *B. burgdorferi* sensu stricto strain B31 will fail to diagnose most Lyme borreliosis patients in the first two weeks of acute infection and probably all clinical Lyme borreliosis cases caused by a strain of borrelia other than *B. burgdorferi* sensu stricto B31 at any stages of the disease. The inherent inaccuracy of serologic tests for Lyme disease can be compared with that of the Widal test for the diagnosis of typhoid or paratyphoid fever (Salmonella infections). A comment extracted from a Centers for Disease Control and Prevention (CDC) document is copied as follows [8].

*“The Widal test is unreliable but is widely used in developing countries because of its low cost. It is a serologic assay for IgM and IgG to the O and H antigens of Salmonella Typhi, but is not specific and false positives may occur. Acute- and convalescent-phase titers are more sensitive than a single serum sample. Newer serologic assays for Salmonella Typhi infection are occasionally used in outbreak situations, and are somewhat more sensitive and specific than the Widal test, but are not an adequate substitute for blood, stool, or bone marrow culture.”*

The term “accuracy” refers to the closeness of a measured value to a reference standard. Since Waddell and colleagues did not define a scientifically acceptable reference standard for “Lyme disease”, it is meaningless to compare all the serologic tests to one another, FDA-approved or not.

The authors seemed to rely on using “clinical diagnosis” and “clinical reference standard” as the yardstick to measure accuracy of the serologic tests for Lyme disease, but did not acknowledge the facts that clinical manifestations of early Lyme disease which are fever, headache and

fatigue with or without a skin rash significantly overlap those of many other disorders. Clinical diagnosis is not a reliable constant that can be used to validate any laboratory diagnostic tests. By the same token, arthritis is a late clinical manifestation, and cannot be used to evaluate the accuracy of laboratory assays.

2. Systematic reviews typically involve a detailed and comprehensive plan and search strategy derived *a priori*, with the goal of reducing bias by identifying, appraising, and synthesizing all relevant studies on a particular topic [9]. The review by Waddell et al. did not identify, appraise and synthesize **all relevant** studies on the topic of Diagnostic Tests for Lyme Disease in Humans. In particular, nucleic acid tests based on polymerase chain reaction (PCR) [10-12] and DNA sequencing [3, 4, 6, 13, 14] for the detection and validation of borrelial 16S ribosomal RNA gene (16S rDNA) are conspicuously absent in the review. It is hard to believe that the authors were not aware of the classic microbiological diagnostic assays based on “genetic sequence using 16S ribosomal RNA sequencing, which is a well-established method to compare and identify bacteria” [15]. At best the Waddell et al. review [1] can only be a “systemic review of serologic tests for human Lyme disease in North America”, namely a review of certain tests which were cherry-picked to the exclusion of others by the authors.

3. In their meta-analysis, Waddell and colleagues stated “Across the direct detection studies sensitivity was low and in most cases lower than the two-tier test regime, assays or immunoblots reported for early LD.” The authors could make this biased statement because they chose to exclude inconvenient literature using 16S rDNA assays, such as the report by Santino et al. [12] and those by others [3, 4, 6, 10-14]. Santino et al. reported that *Borrelia burgdorferi* 16S rDNA was detected by PCR in all serum samples from seropositive patients (100%) when patients presenting clinical manifestations of Lyme borreliosis, including early manifestations (erythema migrans, fever, malaise, fatigue, skin rash, arthralgia, myalgia) were tested [12]. In one summer, the emergency room of a small hospital in Connecticut saw 7 DNA sequencing-proven *B. burgdorferi* spirochetemic patients. Only one of the seven (1/7) had a positive two-tier serologic Lyme test on their split blood samples [14]. Therefore, the statement “the direct detection studies sensitivity was low and in most cases lower than the two-tier test regime, assays or immunoblots reported for early LD” is not accurate. Lyme arthritis was first described in the mid-1970’s before nucleic acid tests were discovered. If Lyme borreliosis were described today as an emerging infectious disease, it would probably be tested by an accurate DNA test, like those for Ebola and Zika virus infections. The 1970 diagnostic dogma takes time to be replaced.

4. The statement in the Review “Due to the above limitations, bacterial isolation and PCR are not routinely used as diagnostic tools in clinical practise, although bacterial isolation is considered the gold standard to confirm diagnosis.” is scientifically questionable because bacterial isolation *per se* is not the gold standard. The gold standard in molecular identification of bacteria is bacterial genome (DNA) sequencing. Bacterial isolation is only the preliminary step to “isolate” a spirochete which may or may not belong to a species of borrelia causing

Lyme disease from a patient. PCR is a DNA replicating process like using a Xerox copier to exponentially generate large numbers of copies of a certain questionable paragraph selected with a few specific words at the beginning and at the end from a page in a book. DNA sequencing is a technology designed to read (decipher) the order (sequence) of the alphabets (nucleotide bases) in the copied paragraph in question. Only when the alphabets in the paragraph which have been replicated are shown to be arranged in the right order (sequence), the products of the copier (PCR) can be confirmed to be the paragraph anticipated (diagnostic). All PCR diagnoses without DNA sequencing to confirm the PCR products can be wrong. Waddell and colleagues avoided mentioning DNA sequencing-based assays for Lyme disease to justify their one-sided endorsement of serologic tests.

5. The statement of Waddell *et al.* "Currently, only serology tests have been licensed for use by the FDA and the Health Canada Medical Devices Branch (HC) for LD testing. Other direct detection tests such as PCR may be commercially available, but they have not been licensed for use by a governing body." is not accurate. The Centers for Medicare & Medicaid Services (CMS) regulates all laboratory testing performed on humans in the U.S. through the Clinical Laboratory Improvement Amendments (CLIA). CMS is a U.S. government agency, a governing body. All CLIA-certified clinical laboratories performing PCR are licensed by the State Department of Public Health and CMS. The FDA regulates the introduction or delivery for introduction into interstate commerce of any food, drug, device, or cosmetic, by law, not the tests *per se*. There is no guarantee that any FDA-approved test kit is more accurate than laboratory-developed tests, especially nucleic acid tests based on Sanger DNA sequencing which are usually not FDA-approved for a variety of reasons.

6. Waddell and colleagues concluded "The performance of the commercially available Immunitics1C6 *B. burgdorferi* ELISA™ shows the most promise as a possible standalone test or as part of a two-tiered test protocol; however it did not overcome the low sensitivity of LD diagnostic tests in patients with early LD. Addressing this shortcoming is a significant challenge to improving LD diagnostics."

To overcome the low sensitivity of LD diagnostic tests in patients with early LD at the spirochetemic stage, we must first acknowledge a need to develop direct detection tests for *Borrelia burgdorferi* and related borrelia species known to cause Lyme borreliosis in North America. To survey the existent useful direct detection tests which may not have been published due to global editorial censorship by the mainstream medical journals, it is recommended that blind-coded simulated blood samples spiked with various species of known borreliae or blank be distributed by government regulatory agencies to all clinical laboratories performing Lyme disease testing for a bacteriology proficiency survey, as routinely conducted by the College of American Pathologists for *Neisseria gonorrhoeae*. The laboratories which return the correct answers would be invited to further develop a generally accepted diagnostic protocol to be used by hospital laboratories located in Lyme disease-endemic areas. To be of use for timely patient care, the results must be generated within 5 working days, preferably in

48 hours. **I believe this technology is already available. The first step to the Lyme disease solution is to cut out the tribalism among the scientists whose careers were built on Lyme disease research.**

I would be happy to answer any questions that you and your colleagues may have concerning my comments.

Sincerely,

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## References

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