New Lyme Culture Test Failed CDC Analysis

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Eighty percent of the patient samples used to demonstrate a novel method of culturing Lyme disease spirochetes from serum contained gene sequences identical to those found in laboratory strains used to develop the test and were likely false positives, Centers for Disease Control and Prevention (CDC) researchers report in an article published online August 14 in the *Journal of Clinical Microbiology*.

"Taken together, our data and those of Sapi et al. indicate that laboratory contamination was the probable source of the borrelial DNA found in the patient samples. The vast majority of patient *pyrG* sequences (41/51) are indistinguishable from laboratory strains used by the investigators. The clinical relevance of the other *pyrG* sequences (10/51) is unclear; these findings also may be consistent with laboratory contamination," the authors write.

The CDC researchers warned that independent verification is critical for novel findings that contradict a large body of previous work and for tests that might lead to unnecessary antibiotic treatment.

"We caution clinicians and patients to wait for independent verification by scientifically sound methods before using this culture service for diagnostic purposes," they write.

The CDC research team, led by Barbara J.B. Johnson, PhD, from the Division of Vector-Borne Disease in Fort Collins, Colorado, were trying to understand why the majority of the spirochetes described in an article published earlier this year in the *International Journal of Medical Sciences* were related by *pyrG* gene sequences to species of Borrelia that had not previously been detected in North American patients other than those with a history of travel to Europe or Asia. The *pyrG* gene encodes CTP synthase, which interconverts UTP and CTP in pyrimidine biosynthesis.

The authors of the previous article had used 2 *B burgdorferi* reference strains (B31 and 297) and 2 Eurasian reference strains (*Borrelia afzelii* and *Borrelia garinii*) for method development and testing of culture medium. To rule out the possibility that the sequence similarities were a result of laboratory contamination, the CDC researchers compared the *pyrG* sequences reported by Sapi et al. for 51 patient isolates to sequences for *B burgdorferi* B31 and 297 for *B afzelii*, and for *B garinii*, using the same primers used in the original study. Previously, *pyrG* gene sequence had been reported only for *B burgdorferi* strain B31, so Dr. Johnson's team sequenced the other 3 laboratory strains and deposited them in GenBank.

The analysis showed that 53% (27/51) of the patient-related sequences reported in the previous article were from samples infected by *B garinii* and 20 the 27 clones were identical to the *B garinii* reference strain. The other 7 *B garinii* sequences had either a single nucleotide polymorphism (n = 5), 2 differences (n = 1), or 3 differences (n = 1) from the laboratory strain.

Twenty-one (41%) of the 51 patients had nucleotide sequences related to *B burgdorferi*, and in 20 of these patients, the sequences matched the laboratory strain B31 exactly.

Two of the 51 patients had sequences closely related to *B afzelii*, which is not found in the United States.

"Eighty percent (41/51) of the reported patient-derived pyrG sequences are identical to one of the laboratory strains and an additional 12% (6/51) differ by only a single nucleotide across a 603bp region of the pyrG gene. Thus, false positivity due to laboratory contamination of patient samples cannot be ruled out and further validation of the proposed novel culture method is required," the authors conclude.

They also note that the patient cultures reported by Sapi et al. had been subjected to nested polymerase chain reaction, "a contamination-prone method that is unnecessary when bacteria are numerous enough to be seen by microscopy." The authors also point out that control samples from healthy blood donors had not been tested by polymerase chain reaction but had been classified as negative based only on dark field microscopy and antibody staining.

The authors have disclosed no relevant financial relationships.

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