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Reply to "No Evidence for Contamination of *Borrelia* Blood Cultures: a Review of Facts"

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A basic obligation of a commercial clinical laboratory is to validate a test before offering it for sale to diagnose patients. Advanced Laboratory Services offers a culture method to diagnose patients with a *Borrelia* species infection (1), but we do not find the evidence provided in support of this method by Sapi et al. adequate to establish that the new culture test has the clinical sensitivity (94%) and specificity (100%) claimed (2). We disagree, therefore, with the opinion expressed by Dr. MacDonald in his letter to the editor (3) stating that the analytical methods of Advanced Laboratory Services are sound.

Sapi et al. sequenced a portion of a single gene after nested-PCR amplification and concluded that they had ruled out laboratory contamination. Our analysis demonstrated that the vast majority of the patient-related DNA sequences were identical to those of the laboratory strains used to develop this culture method. These identities can readily be seen in GenBank using BLASTn and support our claims (2). (One way among many to see these relationships is to enter the accession number of each patient-associated *pyrG* sequence into the search box of the BLASTn program.) The published data are insufficient to determine the source(s) of the DNA used by Sapi et al. to produce patient-related gene sequences. Possibilities include borrelial cells, DNA, and/or PCR amplicons. Because 80% of the patient-associated sequences matched the controls over the region sequenced, the possibility of contamination cannot be excluded. To show that purported *Borrelia* isolates from patients are different from control strains, more-robust analyses, such as multilocus sequence typing, would be required.

We also noted inconsistencies between the DNA sequences and the immunofluorescence results from patient-related material. According to Sapi et al., an internal validation study demonstrated that monoclonal antibody MA1-7006 recognized *Borrelia burgdorferi* strains B31 and 297 but did not recognize the *B. garinii* or *B. afzelii* reference strains. All patient-related cultures were reported to be positive for MA1-7006 staining, indicating the presence of *B. burgdorferi* cells. However, patient-related DNA sequences matched all three *Borrelia* species used as reference strains.

The majority of patient-related sequences matched the *pyrG* gene of the Japanese control strain of *Borrelia garinii* (Fuji P1). If well validated, these data would constitute the first evidence of human infection by *B. garinii* acquired in the Western Hemisphere. However, it is unwarranted to conclude based on the work of Sapi et al. (1) that *B. garinii* is endemic in the United States. The assertions made by Dr. MacDonald (3) are not supported by the literature, and none of the references cited established *B. garinii* as the infecting agent in the patients studied.

To properly validate a Lyme disease diagnostic test, investigators must examine blindly by the same procedures samples from well-characterized patients, healthy controls, and patients with unrelated conditions that have similar symptoms. Sapi et al. did not do this in their report. We are not aware of any published evaluation that appropriately characterizes the sensitivity and specificity of this culture method.

Finally, we recall the reason that culture is considered the "gold standard" for laboratory diagnosis in microbiology. Cultures can be archived and shared. Advanced Laboratory Services can send their isolates of *Borrelia* from clinical material to independent laboratories for analysis. However, despite commercial use for more than 2 years, this test has not been corroborated in an independent report. For all of these reasons, we stand by our critique and strongly recommend that patients and clinicians wait for independent verification of these findings before relying on results of this culture method to diagnose and treat patients.

REFERENCES

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