Culture of Borrelia burgdorferi

Marques et al. (2) recently evaluated a new culture medium for the growth of *Borrelia burgdorferi* from human blood based on a report by Phillips et al. (3). The MPM medium described by Phillips et al. was prepared with Detroit tap water. However, Marques et al. noted in their report that the MPM medium they used was prepared with deionized distilled water from the National Institutes of Health. While unlikely, it is possible that micronutrients in tap water could promote growth of *B. burgdorferi*, as the MPM medium evaluated by Marques et al. failed to support such growth.

Accordingly, we prepared, in our laboratory, MPM medium identical to that described by Phillips et al. Unlike Marques et al., we obtained Detroit tap water and used it to make liquid MPM medium. Both serum and whole blood were collected from 25 patients who presented with signs and symptoms of persistent Lyme disease and whose disease was confirmed clinically and by serological methods. All patients were informed of the study and consented to the drawing of an additional tube of whole blood.

One-milliliter quantities of whole blood were added to 10-ml tubes containing 5.0 ml of MPM medium and to 10-ml tubes containing 5.0 ml of BSK-H medium (complete medium; Sigma, St. Louis, Mo.). The MPM tubes were incubated for 4 weeks at 30°C, and the BSK-H tubes were incubated for 4 weeks at 35°C. Aliquots of a control culture of *B. burgdorferi* 2591 were added to replicates containing either MPM or BSK-H medium and incubated similarly. All tubes were periodically sampled, and the slides were stained with acridine orange (AO) stain. A terminal PCR targeting the OspA gene (1) was performed on all patient cultures and controls.

None of the patient samples showed growth of *B. burgdorferi*. The control culture in BSK-H grew luxuriantly, as evidenced by the appearance of spirochetes in the AO stain and a positive PCR result. The control organism failed to grow in MPM medium.

We agree with the report of Marques et al., who concluded that (i) MPM medium does not enhance the growth of *B. burgdorferi* in patient samples, (ii) the reference strain of *B. burgdorferi* will not grow in MPM medium, (iii) BSK-H medium remains the best medium for cultivation of *B. burgdorferi*,

and (iv) blood culture of patients suspected to have Lyme disease is a low-yield test.

We conclude that the use of Detroit tap water for preparation of the MPM medium as described by Phillips et al. has no effect on the growth of *B. burgdorferi* from either patient samples or controls.

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Authors' Reply

We thank Dr. Tilton and colleagues for sharing their experience with the MPM medium, which not only confirms our findings that this medium is not useful for the culture of *B. burgdorferi*, but also demonstrates that the provenience of the water used in its preparation does not affect the outcome.

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