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Evaluation of a New Culture Medium for *Borrelia burgdorferi*

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We evaluated the new MPM medium for the growth of *Borrelia burgdorferi*. All 18 blood samples from 17 patients with Lyme disease were negative. Growth studies showed that by day 4, most organisms in MPM were not viable. Our results reinforce the use of BSK medium as the primary choice for growing *B. burgdorferi*.

Lyme disease is a complex multisystem infection caused by *Borrelia burgdorferi* and is the most common vector-borne disease in the United States (5). Chronic Lyme disease and post-treatment Lyme syndrome (CLD/PLDS) are names used to describe the clinical picture of patients who suffer from chronic symptoms after what is thought to be adequate antibiotic therapy. The underlying mechanism of these symptoms is unknown, and the management of these patients is controversial. Evidence of a persistent infection in these patients, as assessed by the available direct methods, is generally lacking. Unfortunately, these methods have low sensitivity in some disease manifestations that are clearly associated with persistent *B. burgdorferi* infection. Therefore, negative results may not exclude the possibility of persistent infection. For example, *B. burgdorferi* can be cultivated in vitro by using an enriched artificial medium, named Barbour-Stoenner-Kelly medium BSK), supplemented with serum (2). However, culture of the spirochete from clinical specimens has very low sensitivity outside of samples taken from patients with erythema migrans, where the reported sensitivity varies from 20 to 90% (7, 9, 13, 14, 16). The yield of this microorganism in cultures of whole, untreated blood from patients with acute disease has usually been 5% or less (6), but the use of plasma, serum, and larger quantities of blood has increased the yield to about 25% to 50% in blood samples from patients with early disease who have not received antibiotics (17, 18). Culture of cerebrospinal fluid yields bacteria in less than 10% of samples (8), and the spirochete has never been reliably cultured from joint fluid. PCR results have been variable and usually are similar to the culture results (6, 11, 13–15). The exception is for joint fluid from patients with Lyme arthritis, where PCR sensitivity is up to 85% (10).

In 1998, Phillips et al. described a new medium and methods for culturing *B. burgdorferi*, called MPM. Using this new medium, they reported being able to culture *B. burgdorferi* from the blood of 43 of 47 patients with CLD/PLDS, all of whom had relapsed after long-term oral and intravenous antibiotics (12). Such an improvement in culture sensitivity would be a major advance in the laboratory diagnosis of Lyme disease. In a prospective evaluation of MPM and BSK for blood cultures from CLD/PLDS patients, we were unable to duplicate the findings of Phillips et al. We tested 18 blood samples from 17 patients evaluated at the National Institutes of Health (NIH) Clinical Center between July and September 1999. The patients included 10 patients referred to the NIH with suspected CLD/PLDS, 5 patients with Lyme arthritis, 1 patient who had recovered from Lyme disease, and 1 patient with early Lyme disease. The patients were from Maryland ($n = 12$), New Jersey ($n = 2$), Massachusetts ($n = 1$), Wisconsin ($n = 1$), and Florida ($n = 1$). The patients with suspected CLD/PLDS had a history of Lyme disease according to the Centers for Disease Control and Prevention (CDC) clinical definition (six had a history of erythema migrans), positive serologic analysis confirmed by immunoglobulin G Western blot analysis using the CDC interpretation criteria (3, 4), and persistent or intermittent symptoms for at least 6 months after appropriate antibiotic therapy. The usual symptoms included widespread musculoskeletal pain and fatigue, memory and/or concentration impairment, radicular pain, and paresthesias or dysesthesias. The onset of symptoms was coincident with or within 6 months of initial *B. burgdorferi* infection, the symptoms were significant enough to interfere with daily activities, and other causes were excluded. PCR of cerebrospinal fluid and blood, using the outer surface protein A gene target or the 16S RNA gene target, was negative in all CLD/PLDS patients. Patients with Lyme arthritis had mono- or oligoarticular arthritis, primarily of large joints, exposure to a known area of endemic infection, exclusion of other causes, and positive Lyme disease serologic test results. The patient who recovered from Lyme disease was asymptomatic 1 year after receiving therapy for early neuroborreliosis (disseminated erythema migrans, lymphocytic meningitis, and bilateral facial nerve palsy). The patient with early Lyme disease had localized erythema migrans and fever. Two 5-ml blood samples were collected in EDTA tubes from each patient. From these, one MPM culture and one BSK culture (described below) were processed from each of 17 participants, while 1 participant had 2 sets of cultures done. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all patients signed informed-consent forms.

The MPM medium was prepared as described by Phillips et al., except that NIH (Bethesda, Md.) distilled water rather than Detroit tap water was used. Briefly, the MPM formula contained the following per liter of water: 20 g of proteose peptone, infusion from 1,000 g of beef, 10 g of dextrose, 10 g of NaCl, 4 g of dipotassium phosphate, 1 g of sodium thioglycolate, 1 g of purified agar, 0.004 g of Bacto Methylene Blue, 100 g of sucrose, and 5 g of soluble starch. The medium was autoclaved for 15 min at 120°C and refrigerated overnight before the final tube and slide cultures were prepared. To prepare the tube cultures, 10 ml of medium was boiled to melt the agar, and 1 ml of a sterile 10% solution of autoclaved yeast extract and 1 ml of a sterile solution of $NaHCO_3$ were added. The slide cultures were prepared similarly, except that 3 ml of the yeast extract solution and 10 ml of the $NaHCO_3$ solution were added to 30 ml of the basal medium and the mixture was

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TABLE 1. Comparative growth of *B. burgdorferi* in two culture media

Inoculum dilution[a]	Growth[b] after:									
	2 days		4 days		7 days		14 days		21 days	
	BSK	MPM	BSK	MPM	BSK	MPM	BSK	MPM	BSK	MPM
1/10	+++	+++	+++	−−−	+++	−−	+	−	±	0
1/100	++	+±	+++	−	+++	−	+	−	±	0
1/1,000	+	+	+++	0	+++	0	+±	0	±	0
1/10,000	0	0	+	0	+++	0	++	0	±	0
1/100,000	0	0	0	0	+++	0	+++	0	0	0
1/1,000,000	0	0	0	0	+	0	+	0	0	0

[a] Tenfold dilution series of a suspension of *B. burgdorferi* HB19.
[b] Cultures were observed by AO staining. +, the organism stains orange, ±, a mixture of orange and green organisms; −, only green-staining organisms; 0, no organisms were seen. Where the same symbol is listed multiple times, it refers to the estimate of the number of organisms per slide.

then poured into a sterile Coplin jar. To make the blood agar plates, the agar content was adjusted to 16 g/liter and 60 ml of sheep blood was added directly after autoclaving the medium to prepare a chocolatized agar. Sterile yeast extract was also added to a final concentration of 1%. Tube, slide, and plate cultures were performed using blood (0.1 ml/tube, 0.1 ml/slide, and 0.5 ml/plate) as specified in the procedures of Phillips et al. (12). The cultures were incubated at 30°C and held for 4 weeks. Samples were taken from the tube cultures at 2, 4, 7, 14, and 21 days for staining with acridine orange (AO). AO stain (Becton Dickinson Microbiology Systems, Sparks, Md.) contains 0.1 g of acridine orange in 1 liter of 0.5 M acetate buffer (pH 4.0). The slides were fixed in methanol for 2 min, stained with AO for 2 min, and read with a 40× objective. All 18 cultures were negative by tube, slide, and plate cultures.

Simultaneously, blood cultures in BSK broth were prepared by our standard *Borrelia* culture method. Approximately 2 to 4 ml of plasma from each patient was inoculated into 100 ml of BSK broth containing rabbit serum (Sigma, St. Louis, Mo.). These cultures were incubated at 35°C, examined visually for growth once a week, and stained with AO if growth appeared to be occurring or at the end of the 4-week incubation period. All 18 BSK blood cultures were negative.

To further document the comparative growth characteristics of MPM and BSK, we used *B. burgdorferi* strain HB19 to perform a series of growth studies. High-passage *B. burgdorferi* sensu stricto HB19 was obtained from the Rocky Mountain Laboratories. HB19 was originally isolated from the blood of a patient from Connecticut (1). Table 1 describes the results of cultivation of a series of 10-fold dilutions of a suspension (10^6 organisms/ml) of the organism. A 1-ml volume of each dilution was inoculated into tubes of MPM broth and of BSK broth. After incubation, each broth culture was examined for growth by AO staining, which is more sensitive than the Gram stain for the detection of slender organisms. AO stains bacteria by binding to bacterial DNA, and when the staining is done at low pH, it results in bright orange fluorescence of viable bacteria while background debris are generally yellow to green. Estimates of growth were determined by determining the number of organisms per high-power field if organisms were numerous or per slide if organisms were very few. Although staining the organism could be demonstrated equivalently in both media on day 2 by AO staining, the majority of organisms in MPM were green rather than orange by day 4, suggesting decreasing viability. Growth was readily apparent in all except the highest dilution of the BSK, reaching an optimum at around 7 days. However, no significant growth occurred at any dilution of the MPM series. Subcultures from each of the BSK and MPM tubes into new tubes of BSK at 3 weeks confirmed that the organisms from the BSK tubes were still viable while those from the MPM tubes were nonviable and did not initiate growth when transferred to fresh medium (either MPM or BSK).

This study demonstrates that the use of MPM does not enhance the detection of *B. burgdorferi* from cultures of blood samples from CLD/PLDS patients. We were unable to recover the spirochete from the blood of patients with CLD/PLDS by using either this new medium or BSK. Using a reference strain of *B. burgdorferi*, we have also presented evidence that culturing in BSK remains the best method for growing and maintaining *B. burgdorferi* in medium.

REFERENCES

1. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41:**795–804.
2. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
3. **Centers for Disease Control and Prevention.** 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morb. Mortal. Wkly. Rep. **44:**590–591.
4. **Centers for Disease Control and Prevention.** 1997. Case definitions for infectious conditions under public health surveillance. Morb. Mortal. Wkly. Rep. **46:**1–55.
5. **Centers for Disease Control and Prevention.** 1997. Lyme disease—United States, 1996. Morb. Mortal. Wkly. Rep. **46:**531–535.
6. **Goodman, J. L., J. F. Bradley, A. E. Ross, P. Goellner, A. Lagus, B. Vitale, B. W. Berger, S. Luger, and R. C. Johnson.** 1995. Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using the polymerase chain reaction. Am. J. Med. **99:**6–12.
7. **Jurca, T., E. Ruzic-Sabljic, S. Lotric-Furlan, V. Maraspin, J. Cimperman, R. N. Picken, and F. Strle.** 1998. Comparison of peripheral and central biopsy sites for the isolation of *Borrelia burgdorferi* sensu lato from erythema migrans skin lesions. Clin. Infect. Dis. **27:**636–638.
8. **Karlsson, M., K. Hovind-Hougen, B. Svenungsson, and G. Stiernstedt.** 1990. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis. J. Clin. Microbiol. **28:**473–479.
9. **Nadelman, R. B., J. Nowakowski, G. Forseter, S. Bittker, D. Cooper, N. Goldberg, D. McKenna, and G. P. Wormser.** 1993. Failure to isolate *Borrelia burgdorferi* after antimicrobial therapy in culture-documented Lyme borreliosis associated with erythema migrans: report of a prospective study. Am. J. Med. **94:**583–588.
10. **Nocton, J. J., F. Dressler, B. J. Rutledge, P. N. Rys, D. H. Persing, and A. C. Steere.** 1994. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N. Engl. J. Med. **330:**229–234.
11. **Nocton, J. J., B. J. Bloom, B. J. Rutledge, D. H. Persing, E. L. Logigian, C. H. Schmid, and A. C. Steere.** 1996. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. J. Infect. Dis. **174:**623–627.
12. **Phillips, S. E., L. H. Mattman, D. Hulinska, and H. Moayad.** 1998. A proposal for the reliable culture of *Borrelia burgdorferi* from patients with chronic Lyme disease, even from those previously aggressively treated. Infection **26:**364–367.
13. **Picken, M. M., R. N. Picken, D. Han, Y. Cheng, E. Ruzic-Sabljic, J. Cimperman, and F. Strle.** 1997. A two year prospective study to compare culture

and polymerase chain reaction amplification for the detection and diagnosis of Lyme borreliosis. Mol. Pathol. **50:**186–193.

14. **Schwartz, I., G. P. Wormser, J. J. Schwartz, D. Cooper, P. Weissensee, A. Gazumyan, E. Zimmermann, N. S. Goldberg, S. Bittker, G. L. Campbell, et al.** 1992. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans lesions. J. Clin. Microbiol. **30:**3082–3088.
15. **von Stedingk, L. V., I. Olsson, H. S. Hanson, E. Asbrink, and A. Hovmark.** 1995. Polymerase chain reaction for detection of *Borrelia burgdorferi* DNA in skin lesions of early and late Lyme borreliosis. Eur. J. Clin. Microbiol. Infect. Dis. **14:**1–5.
16. **Wormser, G. P., G. Forseter, D. Cooper, J. Nowakowski, R. B. Nadelman, H. Horowitz, I. Schwartz, S. L. Bowen, G. L. Campbell, and N. S. Goldberg.** 1992. Use of a novel technique of cutaneous lavage for diagnosis of Lyme disease associated with erythema migrans. JAMA **268:**1311–1313.
17. **Wormser, G. P., J. Nowakowski, R. B. Nadelman, S. Bittker, D. Cooper, and C. Pavia.** 1998. Improving the yield of blood cultures for patients with early Lyme disease. J. Clin. Microbiol. **36:**296–298.
18. **Wormser, G. P., S. Bittker, D. Cooper, J. Nowakowski, R. B. Nadelman, and C. Pavia.** 2000. Comparison of the yields of blood cultures using serum or plasma from patients with early Lyme disease. J. Clin. Microbiol. **38:**1648–1650.