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Improving the Yield of Blood Cultures for Patients with Early Lyme Disease

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This study was designed to improve the recovery of *Borrelia burgdorferi* from blood. With the techniques used, *B. burgdorferi* could be recovered from the blood of approximately 25% of patients with early Lyme disease associated with erythema migrans. Serum was a better source of culture material than whole blood. The volume of blood cultured correlated directly with yield, particularly for patients with a single erythema migrans lesion.

The majority of patients with early Lyme disease present with a single, expanding, annular skin lesion, called erythema migrans. While this lesion may at times be almost unmistakable in appearance, diagnostic confusion can occur with insect bites, plant dermatitis, or other cutaneous erythemas (11). In addition, Lyme disease serology is often negative at this stage of illness because of insufficient time for the development of antibodies to the etiologic agent, *Borrelia burgdorferi* (1).

Although *B. burgdorferi* can be cultivated in vitro by using an enriched artificial medium (Barbour-Stoenner-Kelly [BSK] medium), the yield of this microorganism in cultures of blood from patients with erythema migrans has usually been 5% or less (3, 5, 10, 12–15). This low yield may be surprising in view of the presumed hematogenous route of dissemination of the spirochete.

One factor which influences the yield of blood cultures for other bacterial infections is the volume of blood cultured (8, 16, 17). This is due to the small number of microorganisms in blood. For example, for 50% of bloodstream infections in adult patients, the concentration of microorganisms per milliliter of whole blood is less than or equal to 1 CFU; in nearly 20% of cases, it is less than or equal to 0.1 CFU (4, 7). Consequently, 20 to 30 ml is the volume of blood recommended for culture (17). Despite this experience with other bacterial infections, the amount of blood cultured in most studies of Lyme borreliosis has been less than 1 ml (3, 5, 9, 10, 12–15).

The purpose of this study was to determine the effect on the culture positivity rate of culturing different volumes of blood and of culturing whole blood or serum from patients with early Lyme disease associated with erythema migrans.

Experiment 1. In 1995, three 3-ml samples of whole blood were collected in heparinized tubes from untreated adult patients with a clinical diagnosis of erythema migrans that had been established at the Westchester County Medical Center. In addition, three 3-ml samples of serum were collected in sterile tubes without anticoagulant; serum was separated by centrifugation at 1,100 × *g* for 10 min. All blood samples were obtained by a single venipuncture. Within 3 h of the time of collection, each 3-ml aliquot of either whole blood or serum was inoculated into a 70-ml screw-cap plastic flask containing 60 ml of BSK medium prepared as previously described (9), except that antimicrobial agents were omitted.

Experiment 2. In 1996, six 3-ml samples of serum were obtained for culture by a single venipuncture from untreated adult patients with erythema migrans. Each sample was inoculated into BSK medium as described above.

For both experiments, the cultures were incubated at 32 to 33°C for up to 12 weeks. The cultures were examined by fluorescence microscopy at 2 weeks and thereafter at 2- to 4-week intervals (9). Sampling was done as follows. Ten-microliter aliquots of culture material were mixed with 10 μl of an acridine orange staining solution (100 μg/ml of phosphate-buffered saline [pH 7.4]). These mixtures were examined microscopically (magnification, ×400) on a slide overlaid with a coverslip. A minimum of 20 high-power fields were viewed for the presence of motile spirochetes. Confirmation that the visualized spirochete was *B. burgdorferi* was done by PCR with a sample of the culture medium as previously reported (18).

To selectively remove nonborrelial microorganisms, contaminated cultures were filtered twice, first through a 0.45-μm-pore-size filter (Nalgene; Nalge Co., Rochester, N.Y.) and then through a 0.2-μm-pore-size filter (Nalgene) (6).

Statistics. Fisher's exact test, two-tailed, was used for comparisons of proportions. Continuous variables were compared by Student's *t* test (two-tailed).

In experiment 1, each of three 3-ml aliquots of either whole blood or serum was cultured in modified BSK medium for 31 patients with erythema migrans. Because of the limited amounts of available blood or serum, only two 3-ml aliquots of blood were cultured for three patients, one 3-ml aliquot of blood was cultured for one patient, and no whole blood was cultured for one patient. For one additional patient, only two 3-ml aliquots of serum were available for culture. Contaminated cultures occurred in 6 (7.1%) of the 85 aliquots of whole blood compared to 4 (4.3%) of the 92 serum samples (P = 0.52). After filtration, one of the four contaminated serum cultures grew *B. burgdorferi*.

Eight (25.8%) of the 31 patients had a positive whole-blood or serum culture, including 3 (50%) of the 6 patients with multiple erythema migrans lesions and 5 (20%) of the remaining 25 patients with solitary skin lesions (P = 0.16). Whole blood was culture positive for 3 (10%) of the 30 evaluable patients (1 patient did not submit any whole-blood sample), whereas serum cultures yielded *B. burgdorferi* for 6 (19.4%) of 31 patients (P = 0.47). Three (3.5%) of 85 whole-blood samples were culture positive for *B. burgdorferi*, compared to 10

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(10.9%) of 92 serum samples ($P = 0.08$). Three (9.7%) of 31 patients had two or more samples of serum that were culture positive, while none (0%) of the patients had two or more positive whole-blood cultures ($P = 0.24$). In addition, the time to first detection of spirochetes in culture was significantly shorter for serum cultures than for blood cultures (2.7 ± 1.8 weeks [mean ± standard deviation] versus 7.7 ± 3.3 weeks, $P < 0.01$). Whole blood, however, was the only culture-positive sample for 2 (6.7%) of the 30 evaluable patients.

Because of the higher yield of positive cultures with serum than with whole blood in experiment 1, six 3-ml aliquots of serum (and no whole blood) were cultured for 26 untreated patients (total, 156 samples cultured) in experiment 2. Seven patients (26.9%) were culture positive for *B. burgdorferi*, including 2 (28.5%) of the 7 with multiple erythema migrans lesions and 5 (26.3%) of the 19 with a single skin lesion ($P = 1.0$). Contamination occurred in 13 of the 156 (8.3%) samples, but after filtration, two of these cultures grew *B. burgdorferi*. The overall culture positivity rate for the serum samples cultured was 22 of 156 (14.1%). Two patients were culture positive for six of six serum aliquots, one was culture positive for five of six, one was culture positive for two of six aliquots, and three were culture positive for only one of the six aliquots.

This series of experiments has shown that 3-ml serum aliquots from untreated adult patients with erythema migrans are at least twice as likely to yield *B. burgdorferi* on culture as 3-ml samples of whole blood (10.9 versus 3.5%, $P = 0.08$). The relatively small number of samples tested may explain why this difference did not reach the level of significance of $P = 0.05$. Indeed, in experiment 2, in which a larger number of serum samples was cultured, the yield per sample was even greater (14%, 22 of 156). Since *B. burgdorferi* is an extracellular pathogen, the most straightforward explanation is that the volume of serum cultured is the critical determinant. Three milliliters of whole blood contains only approximately one-half that volume of serum. Alternatively, substances released from the hemolysis of whole blood or the breakdown of leukocytes or the heparin anticoagulant may be detrimental to the growth of *B. burgdorferi* (3).

Although it was observed previously that *B. burgdorferi* could be cultured from serum specimens (9), a systematic study of serum cultures had been done until now for only a small number of patients (15). Goodman and colleagues (5) investigated the yield from cultures of 0.1-ml aliquots of whole blood and various blood components. Although serum cultures were not done in this study of Goodman et al., cultures of plasma were at least twice as likely to yield *B. burgdorferi* as those of whole blood. However, in their study of 76 patients with erythema migrans, only 4 patients (5.2%) in total were culture positive based on any blood-derived culture.

The results of our second experiment, in which six separate 3-ml aliquots of serum were cultured for 26 untreated adult patients with erythema migrans, confirmed the finding in experiment 1 that *B. burgdorferi* can be recovered from peripheral blood in approximately 25% of patients, provided that a sample of sufficient volume is cultured. It should be noted that three of the seven culture-positive patients in experiment 2 had a positive culture for only one of the six aliquots of serum cultured, implying that there might have been a still-greater yield had an even larger volume of serum been cultured. However, since 18 ml of serum represents more than 30 ml of whole blood, this volume is probably the upper limit of what may be considered acceptable to patients. Whether the yield might be further improved by culturing plasma instead of serum deserves further study since plasma appears to be the blood component most likely to be associated with a positive PCR signal for *B. burgdorferi* (5).

It is also interesting to note that all of the patients in experiment 2 for whom culture positivity was limited to a single sample of serum, or, at most, two samples, had a solitary erythema migrans lesion. In contrast, both of the culture-positive patients with multiple erythema migrans lesions had at least five positive serum cultures. When both experiments are considered together, the mean (± standard deviation) number of negative serum cultures per patient for the five culture-positive patients with multiple erythema migrans lesions was 0.8 ± 0.84, compared to 3.0 ± 1.76 for the 10 culture-positive patients with a solitary erythema migrans lesion ($P = 0.02$). This difference suggests that patients with multiple erythema migrans lesions are more likely to have a higher grade of spirochetemia.

In summary, our results demonstrate that the yield of blood-derived cultures in early Lyme disease is approximately 25%, a rate comparable to those of other common infectious diseases such as pneumococcal pneumonia (2). Serum is preferable to whole blood as a source of culture material. Analogous to the results with other bacterial infections (8, 16, 17), the volume of blood cultured correlates directly with yield, particularly for patients with a solitary erythema migrans lesion.

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