Yield of Large-Volume Blood Cultures in Patients with Early Lyme Disease

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To improve yield, 6 3-mL plasma cultures (18 mL total) were established for adult patients with early Lyme disease associated with erythema migrans. Borrelia burgdorferi was recovered from the blood of 22 (44.0%) of 50 evaluable patients. The recovery rate per plasma culture and the frequency of positive results for plasma cultures for individual patients were consistent with a level of spirochetemia of ~0.1 cultivable cell/mL of whole blood. Our findings suggest that, if further improvements in the yield of blood cultures are possible, they probably will depend on enhancing the sensitivity of the culture method rather than increasing the volume of material cultured.

Methods

In EDTA-blood collection tubes, 6 3-mL plasma samples were obtained from untreated adult patients with a clinical diagnosis of erythema migrans that had been established at the Westchester Medical Center (Valhalla, NY) in 2000. All patients had agreed to participate in the study and satisfied the Lyme disease surveillance criteria of the Centers for Disease Control and Prevention [10]. In total, 18 mL of plasma was obtained by a single venipuncture from each patient. Plasma was separated by centrifugation at 260 g for 15 min. Within 3 h of the time of collection, each 3-mL aliquot of plasma was inoculated into a 70-mL screw-cap plastic flask containing 60 mL of antibiotic-free BSK medium, which was prepared as described elsewhere [2]. Cultures were incubated at 32°C–33°C for up to 12 weeks. The cultures were examined by fluorescence microscopy at 2 weeks and thereafter at 2–4-week intervals. Sampling for each culture was done as follows. A 10-μL aliquot of culture material was mixed with 10 μL of an acridine orange staining solution (100 μg/mL in PBS [pH 7.4]). Ten microliters of this mixture was placed on a slide overlaid with a coverslip and was examined with a microscope (magnification, ×400). A minimum of 20 high-power fields were viewed for the presence of spirochetes.

Results

Plasma cultures were established for 51 untreated adult patients with erythema migrans. One patient was not considered to be evaluable because of gross contamination of the flasks. Of the 50 evaluable patients, 22 (44.0%) had a positive plasma culture, including 7 (46.7%) of the 15 patients with multiple erythema migrans lesions and 15 (42.9%) of the 35 patients with a solitary skin lesion (P = 1.0, Fisher’s exact test). For the 50 evaluable patients, a single flask became contaminated for 1 patient, and, for another patient, there was insufficient plasma to inoculate 1 of the 6 3-mL plasma cultures. Overall, 81 (27.2%) of 298 plasma cultures had positive results.

Of the 22 patients with positive culture results, 3 (13.6%), 4 (18.2%), 0 (0%), 7 (31.8%), 6 (27.3%), and 2 (9.1%) patients were positive by exactly 1, 2, 3, 4, 5, and 6 of the 3-mL plasma cultures, respectively (figure 1A). Among just the patients with positive culture results, the probability of recovering B. burg-
For the 22 spirochetric patients, the filled bars equal the observed percentage of patients (Y-axis) with exactly 1, 2, 3, 4, 5, or 6 positive 3-mL plasma cultures (X-axis). The observed percentage of culture-positive patients is compared with the predicted percentage (open bars) by assuming that the probability of a culture-positive 3-mL plasma sample is constant at 0.6 (B) or 0.7 (C). Calculations of the predicted percentage were based on the binomial method. The probabilities of a culture-positive 3-mL plasma sample of 0.6 and 0.7 gave a reasonably good fit of observed to predicted, whereas for probabilities <0.5 the differences between observed and predicted were much greater.

This experiment has confirmed that high-volume plasma blood cultures are positive in >40% of untreated adult patients with erythema migrans. These results further suggest that the probability of recovery of *B. burgdorferi* is only ~6 per 3-mL plasma sample. This implies either that the culture method is insensitive or that there are relatively few viable spirochetes per milliliter of blood. Little data exist on the sensitivity of BSK for primary isolation of *B. burgdorferi* from clinical specimens. However, sensitivity of BSK culture can approach 1 cell for laboratory propagated strains of *B. burgdorferi* [11, 12]. Published data on the number of *B. burgdorferi* in the blood of humans are quite limited. Goodman et al. [9], in a study of patients with erythema migrans, were able to detect *B. burgdorferi* DNA by polymerase chain reaction (PCR) in 0.5 mL of plasma of 14 (18.4%) of the 76 patients. Using a semiquantitative PCR method, these investigators estimated that the number of spirochetes in plasma varied from <20/mL to >4000/mL. Whether detection of spirochetal DNA necessarily implies the presence of viable microorganisms is, however, open to question even in untreated patients.

The variable number of culture positive–plasma samples per patient (figure 1A) in our study might be due to inconsistent numbers of cultivable spirochetes in different patients. Alternatively, the level of spirochetemia per patient may be relatively constant, with the variability in the number of positive plasma samples explained by random sampling alone. Figure 1 depicts the frequency of positive 3-mL plasma samples if the probability of a single positive 3-mL plasma culture were exactly 0.6 (figure 1B) or 0.7 (figure 1C; calculated by the binomial method). As can be seen, the patterns have reasonably close similarity to the observed frequency of positive plasma cultures. However, too few patients were cultured to determine definitively whether the observed pattern is significantly different from that which was predicted.

Table 1 compares the percentage of culture-positive plasma samples in the present study with our results from 1997 to 1999, which used 3 3-mL plasma cultures in spirochetric patients [2] (authors’ unpublished data). A remarkable consistency in the culture positivity rate per plasma sample (61.4%–71.4%) is evident, which suggests that 0.6–0.7 is a reasonable estimate for the probability of recovery of *B. burgdorferi* per 3-mL plasma culture.

Probabilities of recovery of 0.6–0.7 for *B. burgdorferi* from
Table 1. Positive 3-mL plasma samples from spirochetemic adult patients with erythema migrans, by year of study.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of patients</th>
<th>No. of samples cultured per patient</th>
<th>No. (%) of cultures positivea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>10</td>
<td>3</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>1998</td>
<td>7</td>
<td>3</td>
<td>15 (71.4)</td>
</tr>
<tr>
<td>1999</td>
<td>20</td>
<td>3</td>
<td>42 (70.0)</td>
</tr>
<tr>
<td>2000</td>
<td>22</td>
<td>6</td>
<td>81 (61.4)</td>
</tr>
</tbody>
</table>

a Percentage represents no. of culture-positive plasma samples divided by the total no. of plasma samples cultured (>100) for spirochetemic patients.

a single 3-mL plasma culture mean that ~0.1 cultivable B. burgdorferi cell is present in 1 mL of whole blood. If this is the correct probability range, using the binomial method, it is possible to estimate the frequency of patients who would be expected to have false-negative blood culture results if 3, rather than 6, 3-mL plasma samples were cultured. Given these assumptions, positive blood culture results would be detected in 3%–6% fewer patients by culturing 9 mL versus 18 mL of plasma. On the basis of the actual observations in our study and by use of an analysis that assumes that B. burgdorferi is randomly distributed into the plasma cultures, ~10% of culture-positive patients would have been missed had we inoculated 3 instead of 6 cultures. (Of the 3 patients with exactly 1 positive 3-mL plasma culture, by chance alone, 50% or 1.5 would not have been detected, and among the 4 patients with exactly 2 positive plasma cultures, 20% or 0.8 would not have been detected, for a total of 2.3 fewer patients.)

In summary, high-volume plasma blood cultures have a yield of ~40% in untreated adult patients with early Lyme disease associated with erythema migrans. Our observations suggest that the level of spirochetemia may be ~0.1 cultivable spirochete per milliliter whole blood. Doubling the volume of plasma cultured from 9 to 18 mL (the upper limit of what is practical for patients) is associated with an incremental gain of only ~10% in identifying spirochetemic patients. Therefore, in those circumstances in which a blood culture is done, ~10 mL of plasma should be sufficient. If further improvements in culture yield are still possible, they probably will depend on enhancing the sensitivity of the culture method rather than by further increasing the volume of material cultured. Changes that may be considered in future studies include omitting or altering the choice of anticoagulant used to collect the blood and/or attempting to enrich the BSK culture medium.

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References