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Effects of Bovine Serum Albumin on the Ability of Barbour-Stoenner-Kelly Medium To Detect *Borrelia burgdorferi*

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The ability of decreasing inocula of *Borrelia burgdorferi* to grow in otherwise identical Barbour-Stoenner-Kelly (BSK) media containing different lots of bovine serum albumin (fraction V) was determined. These media differed significantly in ability to detect *B. burgdorferi*. Some BSK media required inocula of 2×10^5 organisms per ml for detection, while other media could stimulate growth after inoculation with <2 organisms per ml. In addition, organisms from the less sensitive BSK media were thinner, longer, and less tightly coiled. The endpoint dilutions of indirect fluorescent-antibody titers, especially immunoglobulin M, exhibited up to 16-fold decreases, and both immunoglobulin G and M titers were more difficult to interpret with diagnostic slides prepared from some longer, thinner *B. burgdorferi*. These results demonstrate that, when performing laboratory investigations which rely on *B. burgdorferi*, it is essential that the quality of the BSK medium be determined.

Lyme disease is a multisystem disorder caused by the spirochete *Borrelia burgdorferi*. The microorganism is transmitted by infected ticks, primarily *Ixodes* sp. (6, 8), to humans (15–17) and to wild and domestic animals (1, 4, 6, 11, 12). The increased recognition and rapid dispersal of infected ticks have made Lyme disease the most common tickborne illness in the United States (7).

The significant increase in the number of animal and human Lyme disease cases has augmented scientific investigations. The recovery of *B. burgdorferi* from tissue is an important requirement for many of these studies. For example, isolation of *B. burgdorferi* from infected animals and ticks has been effective for defining endemic Lyme disease areas (1, 2, 5, 6), determining a response to infection (10), or evaluating experimental therapy (9).

Various modifications of Barbour-Stoenner-Kelly (BSK) medium (3) are routinely used to grow *B. burgdorferi* from infected tissues. We observed that the morphologic characteristics of *B. burgdorferi* varied with different BSK batches even though the medium was prepared by the same procedure. After investigating several different variables, we report that bovine serum albumin (fraction V) is a significant factor affecting the ability of BSK to grow *B. burgdorferi*.

We prepared modified BSK medium as described previously (6). Briefly, 3 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (Sigma Chemical Co., St. Louis, Mo.), 2.5 g of neopeptone (Difco Laboratories, Detroit, Mich.), 0.35 g of sodium citrate, 2.5 g of glucose, 1.1 g of sodium bicarbonate, 1.27 g of TC yeastolate (Difco), 0.4 g of sodium pyruvate (Sigma), and 0.2 g of *N*-acetyl-D-glucosamine (Sigma) from the same lots were added to six separate 1-liter flasks which contained 450 ml of distilled water. A 25-g amount of six separate lots of bovine serum albumin (fraction V) from three different manufacturers (Sigma; GIBCO Laboratories, Grand Island, N.Y.; and Armour Pharmaceutical Co., Kankakee, Ill.) was added.

The suspensions in each flask were slowly stirred (speed set at 1) for 5 h at room temperature on a hot plate (model PC 351; Corning Glass Works, Corning, N.Y.). After stirring, the media were adjusted to pH 7.5 with 1 N NaOH and sterilized by filtration through a 0.22-μm filter (Millipore Corp., Bedford, Mass.). After sterilization, 50 ml of sterile CMRL 1066 liquid medium (10×) without glutamine (GIBCO), 7 g of gelatin (Difco) dissolved in 100 ml of sterile water, and 32 ml of heat-inactivated (56°C, 45 min) sterile rabbit serum (Pelfreez Biologicals, Inc., Rogers, Ariz.), previously shown to support growth of *B. burgdorferi*, were added to each flask. After preparation, 6.0-ml aliquots of each BSK medium were dispensed into plastic tissue culture tubes (13 by 100 mm; Becton Dickinson and Co., Lincoln Park, N.J.).

A Wisconsin strain of *B. burgdorferi* (S-1-10), isolated from a white-footed mouse (*Peromyscus leucopus*), was incubated in BSK medium at 32°C until the number of organisms was 10^7/ml. The number of *B. burgdorferi* organisms per milliliter was determined with a Petroff-Hausser counting chamber. Duplicate tubes containing 6-ml aliquots of the six separate BSK media were then inoculated with 10^6 *B. burgdorferi* to yield a final concentration of 2×10^5 organisms per ml. After inoculation, the tubes were serially diluted 10-fold, using nine additional tubes containing the appropriate BSK medium. The culture tubes were incubated in the dark at 32°C for 21 days.

The presence of *B. burgdorferi* organisms in each tube was determined by examining 25 fields (25×) of a 10-μl sample by dark-field microscopy. The number of *B. burgdorferi* organisms per milliliter in each culture was calculated. The last tube that supported the growth of the diluted *B. burgdorferi* was also determined.

Although each BSK medium supported the growth of *B. burgdorferi* after inoculation with 2×10^5 organisms per ml,

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TABLE 1. Inoculum of *B. burgdorferi* needed for growth from separate BSK media prepared with six different lots of fraction V bovine serum albumin

BSK medium (company, lot no.)	Inoculum (organisms per ml) needed for detection ($n = 2$)	No. of *B. burgdorferi* per ml in last dilution (10^6)[a]	*B. burgdorferi* morphology
A (Armour, 45206)	<2	37 ± 13	Motile, 9–14 μm long, uniformly refractile
B (Sigma, 37F-0641)	<2	26 ± 24	Motile, 9–14 μm long, uniformly refractile
C (GIBCO, 24377)	<2	42 ± 1	Less refractile, granulated, 23–30 μm long, less motile
D (GIBCO, 16218)	<2	2 ± 0	Less refractile, granulated, 23–30 μm long, less motile
E (Sigma, 67F-0799)	≥200	2 ± 0	Less refractile, granulated, 23–30 μm long, less motile
F (Armour, 97105)	≥200,000	4 ± 2	Less refractile, granulated, 23–30 μm long, less motile

[a] Incubated for 21 days at 32°C; $n = 2$; ± standard error.

the media differed in ability to grow smaller inocula of *B. burgdorferi* (Table 1). BSK media A, B, C, and D could successfully grow *B. burgdorferi* with an inoculum of <2 organisms per ml. BSK media E and F required a inocula of 2×10^2 and 2×10^5 organisms per ml, respectively. These results demonstrate that different lots of fraction V bovine serum albumin can affect the ability of BSK to detect small numbers of *B. burgdorferi*.

Dark-field and transmission electron microscopy, using negative staining (13) with minor modifications, revealed that the morphology of *B. burgdorferi* was also affected by the media. BSK media C, D, E, and F yielded organisms which were 23 to 30 μm ($n = 10$) long and were coiled 8 to 10 times, while the organisms from BSK media A and B were 9 to 14 μm ($n = 10$) long with 3 to 5 coils (Table 1). The longer *B. burgdorferi* were less refractile and less motile. These organisms also had a granulated appearance.

Finally, we determined that *B. burgdorferi* grown in different media affected the quality of the Lyme disease indirect fluorescent-antibody test. Slides were prepared as described previously (14), using *B. burgdorferi* organisms cultured in each separate BSK medium. These diagnostic slides were then reacted with five Lyme disease case sera with immunoglobulin G (IgG) or IgM antibody titers of 128 to >2,048 (reciprocal of dilution). After reaction with the case sera, the diagnostic slides were read as blind tests by three experienced serologists. The individual IgG and IgM antibody titers were determined after standardization with slides of each antigen preparation to a known serum endpoint. Generally, each batch of *B. burgdorferi* antigen yielded comparable IgG and IgM serum antibody levels for each case serum, although some fourfold differences between serum endpoints by individual readers were reported. However, there was considerable variation in the quality of the indirect fluorescent-antibody slides. The diagnostic slides prepared from *B. burgdorferi* cultured in BSK media C, D, E, and F contained excessive background debris, even after three washings, and the organisms were often clumped together. Generally, the serum endpoint antibody titers were more difficult to interpret when slides prepared with these longer thinner *B. burgdorferi* were used.

Since the diagnostic slides were initially compared after standardization with each antigen preparation and a fourfold titer difference could be due to reader variability, we investigated whether more variability would be observed after standardization with our current control *B. burgdorferi* antigen. In this instance, the IgG antibody titers were similar to our previous observations. However, up to 16-fold decreases of IgM titers were observed when slides prepared from BSK media E and F were used. It seems probable that at least some of this variance was due to differences among the *B. burgdorferi* organisms from each BSK medium. We could not attribute these differences to varying protein concentrations since sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis and densitometer tracings, prepared by using identical total protein concentrations of *B. burgdorferi* organisms cultured in each BSK medium, revealed no significant differences among *B. burgdorferi* surface proteins.

We have demonstrated that the fraction V bovine serum albumin used in modified BSK medium can affect the recovery of low numbers of *B. burgdorferi*. The ability to recover small numbers of *B. burgdorferi* from human and animal tissues may become increasingly important for diagnosis and treatment of Lyme disease. Before investigations are undertaken to recover *B. burgdorferi*, BSK medium should be prepared with different fraction V bovine serum albumin lots so that the optimum efficacy of the BSK medium will be assured.

In addition, we showed that the serum albumin preparation can affect the indirect fluorescent-antibody test. It is possible that the differences in the quality of *B. burgdorferi* antigen from different BSK media may affect the results of other diagnostic Lyme disease tests. When performing laboratory investigations which rely on *B. burgdorferi*, it is essential that the quality of the BSK medium be determined.

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