

KILL-TIME STUDIES
Antimicrobial Activity of Advanced Cellular Silver (ACS) 200
Using *Borrelia burgdorferi*
Test Solution: ACS 200
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PREPARED FOR:

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I. PURPOSE.

The purpose of this study was to estimate the antimicrobial activity of ACS 200 test formulation on *Borrelia burgdorferi*. Since there is no current protocol for disinfectant testing with *Borrelia burgdorferi*, and since this organism does not form colonies on agar, loss of motility observed through dark-field microscopy was used to determine the extent of kill.

II. MATERIALS AND METHODS.

A. Test organism.

The test suspension was prepared by growing *Borrelia burgdorferi* (ATCC 35210; Type strain) in 10ml of BSK-H Medium Complete (Sigma Aldrich) for approximately six days at 37 °C without shaking.

B. Kill-Time Procedure.

1. A 0.9ml aliquot of the test solution was added to a 2-ml cryovial. Then, 100 µl of the *B. burgdorferi* culture was added at time zero.
2. The solution was vortexed and 30 µl of the suspension was placed on a slide with a cover slip.
3. The slide was immediately placed on the microscope for observation under dark-field microscopy.
4. A control slide using PSS was prepared in an identical manner.
5. The test and control slides were observed for loss of motility every 2 minutes. Motility counts from representative fields were recorded until there was no more movement observed in the test solution.

D. Controls.

1. A baseline count was established by counting several fields of motile organisms on a control slide and estimating an average number of motile organisms per field.

III. RESULTS.

***Borrelia burgdorferi* baseline count:**

Mean number of motile organisms: 130/field

The estimated volume of 1 field is approximately 3.4×10^{-6} ml. Therefore, the starting concentration of the *B. burgdorferi* culture was approximately 3.8×10^7 organisms/ml.

ACS 200:

(Received 04/29/09)

<u>Time</u>	<u>No. motile organisms</u>
1.5 min	17
4 min	1
6 min	1
8 min	0

PSS:

(Control)

<u>Time</u>	<u>No. motile organisms</u>
1 min	11
4 min	10
6 min	3
8 min	10

Since a 1:10 dilution of the culture was performed in both the test and PSS control suspensions, the number of live organisms observed after 8 minutes in the PSS control was about 77% of that expected.

IV. CONCLUSION.

From these preliminary studies, it appeared that most of the *B. burgdorferi* motility ceased after about 4 minutes of exposure to the ACS 200 solution. No motility was observed after 8 minutes of exposure.

Date of test: September 15, 2009

Performed By:

Emily Moore
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Supervised by:

A handwritten signature in black ink, appearing to read "Richard A. Robison", written over a horizontal line.

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