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Comparison of Bacillus Anthracis to the Surrogate Bacillus Atrophaeus for Spore Inactivation on a Novel Antimicrobial Fabric

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Comparison of *Bacillus anthracis* to the Surrogate *Bacillus atrophaeus* for Spore Inactivation on a Novel Antimicrobial Fabric

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BACKGROUND: Military fabric amended with an antimicrobial compound may reduce the viability of biological threat agents that could be encountered in contaminated environments. *Bacillus atrophaeus* (formerly *Bacillus subtilis* var. *niger*) is typically employed in the evaluation of antimicrobial compounds and has been reported to be less susceptible to disinfection than *Bacillus anthracis*, and thus is commonly used as a surrogate whenever direct evaluation with *B. anthracis* may not be feasible. In this study, a direct comparison of the sporicidal activity of a novel antimicrobial fabric was evaluated for *B. anthracis* and the surrogate *B. atrophaeus*. **METHODS**: Fabric amended with a chlorine-based compound and fabric minus this compound were inoculated with a liquid suspension of dormant spores of either *B. anthracis* or *B. atrophaeus* and incubated at 30°C for one hour at relative humilities ranging from 60 to greater than 90 percent relative humidity (RH). Spores were eluted from their respective fabrics and enumerated by direct microscopic count. The number of viable spores was determined by cultivation on Nutrient Agar and the percent of cultivable spores were calculated as the ratio of cultivable spores to total spores as a function of exposure time. **RESULTS**: Cultivability of *B. anthracis* spores on fabric amended with the antimicrobial compound decreased significantly with an increase in the percent of relative humidity ($R^2 = 0.97$) with approximately five logarithms (5.5 ± 0.4) in reduction at 90 percent RH. *Bacillus atrophaeus* spores were not correlated with the percent RH ($R^2 = 0.31$) and only experienced about a one logarithm reduction (0.9 ± 0.3) in cultivability at 90 percent RH. Additionally, spores eluted from control fabric with no antimicrobial maintained cultivability under the same exposure conditions. **CONCLUSIONS**: The antimicrobial-treated fabric was capable of inactivating dormant spores of *B. anthracis* and to a lesser extent those of *B. atrophaeus* ($P < 0.001$). Thus, military protective gear amended with this antimicrobial compound could provide protection from possible exposure to a biological agent like *B. anthracis*, which in turn, can be conservatively evaluated by the more tolerant and non-pathogenic surrogate *B. atrophaeus*.

FIGURE 1.

MATERIALS AND METHODS:

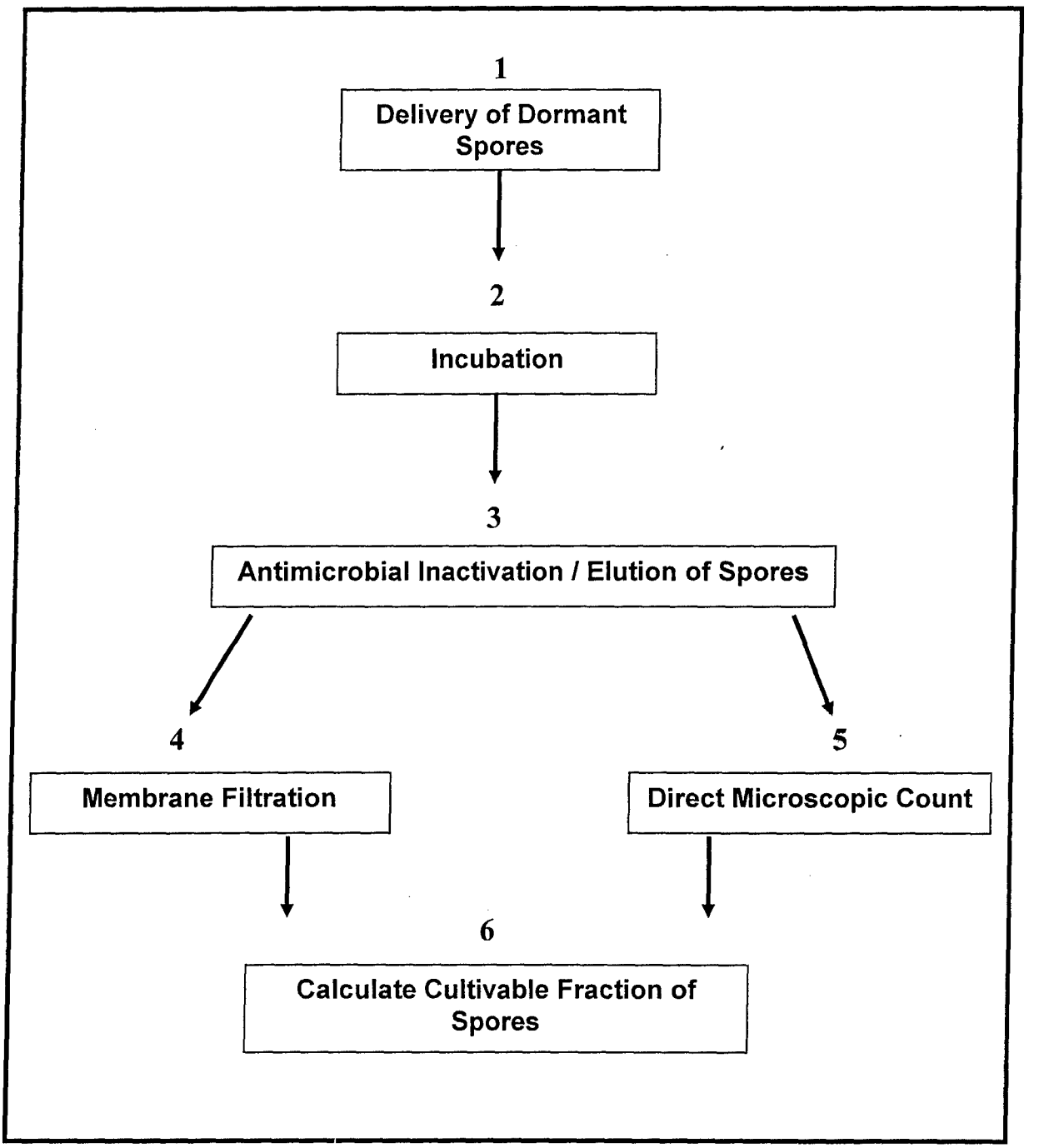

FIGURE 1. Schematic diagram representing the dormant spore surface assay for determining the efficacy of fabric amended with an antimicrobial compound.

1. Purified dormant *B. anthracis* (Sterne) and *B. atrophaeus* (NRRL-B-4418) spores suspended in distilled water and Tween 20 (0.001%) were delivered individually to fabric samples (25 mm diameter) amended with an antimicrobial compound and control fabric with no antimicrobial compound.

2. Samples were placed in a controlled environmental chamber with equilibrated temperature and relative humidity and incubated for one hour.

3. Samples were then submerged in 5.0 ml elution buffer containing sodium thiosulfate (0.1%) to inactive the antimicrobial compound, vortexed, and sonicated in a water bath to suspend the spores in the buffer.

4. Suspended spores were evaluated by membrane filtration plate count on nutrient agar for cultivable fraction of eluted spores.

5. Suspended spores were examined by phase-contrast microscopy and directly enumerated for total individual spore count with a Petroff-Hausser Counting Chamber.

6. The percent of viable spores for each sample was determined as the ratio of cultivable spores to total spores. Antimicrobial efficacy was calculated as the logarithm of the ratio of cultivable spores as compared to control spores.

RESULTS:

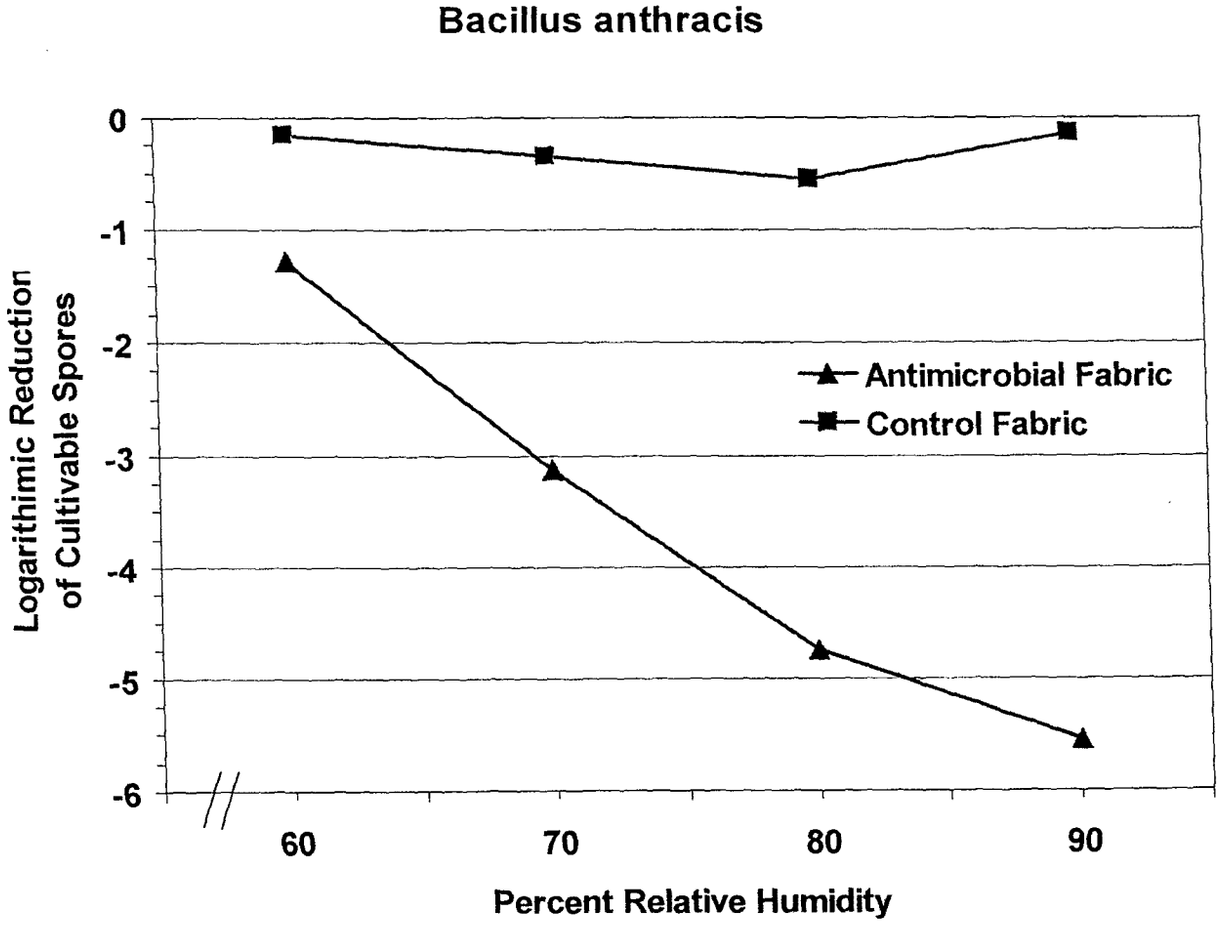

FIGURE 2. The amount of spore inactivation for *Bacillus anthracis* (Sterne) represented as logarithmic reduction of the ratio of cultivable spores as compared to control spores over increasing levels of percent relative humidity.

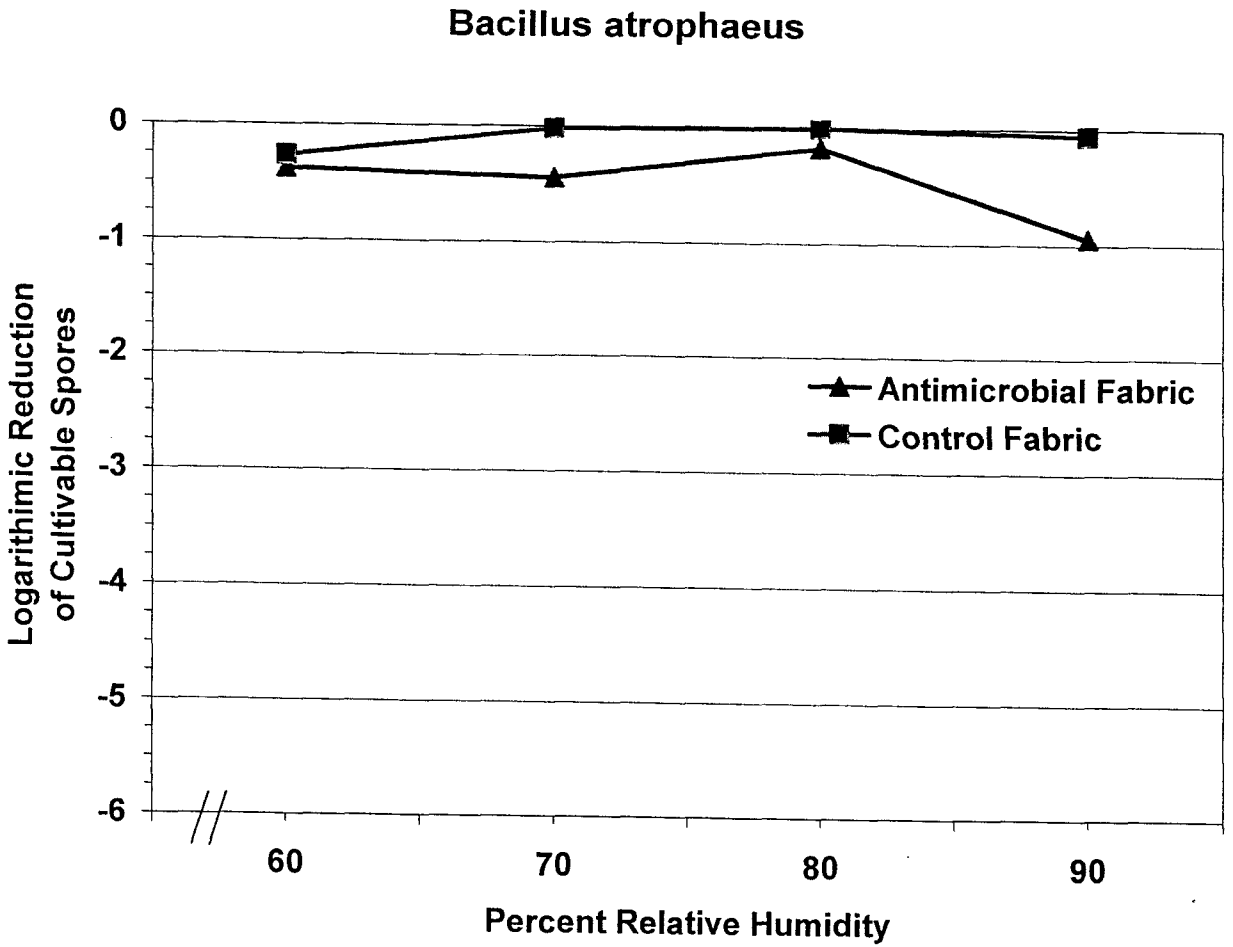

FIGURE 3. The amount of spore inactivation for *Bacillus anthracis* (Sterne) represented as logarithmic reduction of the ratio of cultivable spores as compared to control spores over increasing levels of percent relative humidity.