Combining Spore Germination and Heat Inactivation to Decontaminate Materials Contaminated with *Bacillus anthracis* Spores

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Aims: To add a spore germination step in order to reduce decontamination temperature and time requirements compared to the current hot, humid air decontamination parameters, which are 75-80°C, ≥72 h, 70-90% RH, down to ≤60°C and ≤24 h total decontamination time.

Methods and Results: Spore germination with L-alanine, inosine and calcium dipicolinate was quantified at 0-40°C, several time points, and spore concentrations of 5-9 $\log_{10}$ mL^{-1}. A single germinant application followed by 60°C, 1-h treatment consistently inactivated >2 $\log_{10}$ (>99%) of spores. However, a repeat application of germinant was needed to achieve the objective of ≥6 $\log_{10}$ spore inactivation out of a 7 $\log_{10}$ challenge (≥99.9999%) for ≤24 h total decontamination time for nylon and Aircraft Performance Coating. Characterization of alanine racemase and inosine hydrolase mutants will assess the impact of those signaling molecules on heat sensitivity.

Conclusions: High efficiency germination (>99% of a spore population) and a post-germination, treatment of 60°C, 1 h, no RH control, followed by a second application of germinant was able to trigger germination of persister spores to achieve ≥6 $\log_{10}$ inactivation over a wide germination temperature range (0-40°C).

Significance and Impact of the Study: Germination expands the scope of spore decontamination to include any materials from any industry sector that can be sprayed with an aqueous germinant solution. The decontamination time and efficacy requirements will determine if a heating step is required.