Validation of a rapid bacteria endospore enumeration system for use with spacecraft assembly

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NASA planetary protection policy sets forth strict limits on the number of bacterial endospores that can be present on a spacecraft at launch. Currently, the only approved method for counting the spores is a culture based assay that requires three days to produce results, a timeframe that can be at odds with the rapid pace and rigorous deadlines of spacecraft assembly. A possible alternative to the traditional culture based approach is the Millipore Rapid Microbiology Detection System (RMDS) which has previously been used for process and contamination control in the pharmaceutical and food industries. The RMDS is rapid and simple, shows high sensitivity (1 colony forming unit [CFU]/sample), and correlates well with traditional culture-based methods. It combines membrane filtration, adenosine triphosphate (ATP) bioluminescence chemistry, and image analysis based on photon detection with a Charge Coupled Device (CCD) camera.

In this study, we have optimized the assay condition and evaluated the use of the RMDS as a rapid spore detection tool for NASA applications. Seven species of *Bacillus* (nine strains) that have been repeatedly isolated from clean room environments were assayed. In order to select for spores, the samples were subjected to a heat shock step before proceeding with the RMDS incubation protocol. All strains were detected by the RMDS in $\sim$5 hours and these assay times were repeatedly demonstrated along with low image background noise. The RMDS-based spore detection method is undergoing the final stages of validation and is expected to achieve the goal of "same shift" measurement of spore bioburden during spacecraft assembly.