Sensitivity and Applicability of a Rapid Micro Detection System in the Enumeration of Bacterial Spores

Fei Chen, Gregory Kuhlman, Larry Kirshner, Asahi Matsuyama, Andrew Sage, Molly Pickett, Kasthuri Venkateswaran and Roger Kern.

NASA monitors spacecraft surfaces to assure very low levels of bacterial spores are landed on the surface of Mars. In order to meet rigid schedules of spacecraft assembly, a more rapid, sensitive spore detection assay is being considered as an alternate method for the current three-day NASA standard culture based assay. The Millipore Microstar™ Rapid Micro Detection System (RMDS) has been used successfully for rapid bioburden enumeration in a wide range of applications and sample types. It combines membrane filtration, adenosine triphosphate (ATP) bioluminescence chemistry, and image analysis based on photon detection with a CCD camera. The RMDS is rapid, simple, shows high sensitivity (1 cfu/sample), and an excellent correlation with traditional culture based methods. In addition, by utilizing the Milliflex™ filtration system, the RMDS is ideal for sampling both large sample volumes and those samples containing inhibitory substances. In this study, we have evaluated the use of the RMDS as a rapid spore detection tool for NASA applications. This is accomplished by preceding the RMDS incubation protocol with a heat shock step, 15 minutes at 80°C, as a direct selection of spores. Different luciferases were tested in order to reduce the typical 18-24 hour incubation time required by the RMDS to ~5 hours. Of reagents evaluated, a formulation of highly sensitive bioluminescence reagent was found to be more sensitive than the present commercially available reagents. Assay times of ~5 hours were repeatedly demonstrated along with low image background noise. In order to evaluate the applicability of this method, 7 species of *Bacillus* totaling 9 strains, which have been repeatedly found in clean room environments, were assayed. All strains were detected in ~5 hours. The improved RMDS-based spore detection is now under field trials and is expected to achieve the goal of "same shift" measurement of spore bioburden during spacecraft assembly.

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