

Evaluation of an ATP-Based Bioluminescence Assay for Determination of the Microbial Burden of Spacecraft

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NASA has an ongoing research effort to introduce new technologies to evaluate trace biological contaminants on spacecraft outbound from Earth to other planetary bodies. Viable organisms are of particular concern for spacecraft cleanliness, but conventional culture-based methodologies detect only a small fraction. In general, NASA evaluates the bioburden of spacecraft surfaces by the determination of aerobic spore-forming microbes. We have recently reported the application of the ATP-based bioluminescence assay for the evaluation of microbial burden of spacecraft assembly facilities. In order to implement this technique for verifying spacecraft cleanliness, we conducted an extensive QA/QC validation approach on this ATP-assay by determining the method detection limits, accuracy, reproducibility, and hold times for the assay. Based on calibration curves of selected microbes isolated from spacecraft surfaces and known strains, the ATP assay detects a minimum of 100 cells in a given sample. This method accurately measures ATP to 10^{-14} moles per sample with a confidence level of 99% and is also reproducible with a coefficient of variation <5%. When this ATP-assay was tested in the field ~500 Mars Exploration Rover (MER) surface samples were collected. The results indicated that 27% of the samples were contaminated with $>10^{-11}$ moles of ATP. However, only 9% of the total samples contained spore-forming microorganisms. This might be due to the detection of ATP originating from both live and dead microbes. Although ATP and the NASA standard assay detect different biosignatures, both methods indicate that the MER surfaces are not significantly contaminated. Since spacecraft achieve very low bioburden levels after rigorous cleaning processes, a simple and rapid method such as the ATP assay is useful to validate “biological cleanliness” and might also help to meet rigid schedules of the spacecraft assembly processes. Although the ATP assay does not correlate with the spore counts, this 10 minute rapid assay can be used as an indicator of the total microbial burden of the spacecraft.