Process to Selectively Distinguish Viable From Non-Viable Bacterial Cells

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The combination of ethidium monoazide (EMA) and post-fragmentation, randomly primed DNA amplification technologies will enhance the analytical capability to discern viable from non-viable bacterial cells in spacecraft-related samples. Intercalating agents have been widely used since the inception of molecular biology to stain and visualize nucleic acids. Only recently, intercalating agents such as EMA have been exploited to selectively distinguish viable from dead bacterial cells. Intercalating dyes can only penetrate the membranes of dead cells. Once through the membrane and actually inside the cell, they intercalate DNA and, upon photolysis with visible light, produce stable DNA monoadducts. Once the DNA is crosslinked, it becomes insoluble and unable to be fragmented for post-fragmentation, randomly primed DNA library formation. Viable organisms’ DNA remains unaffected by the intercalating agents, allowing for amplification via post-fragmentation, randomly primed technologies. This results in the ability to carry out downstream nucleic acid-based analyses on viable microbes to the exclusion of all non-viable cells.

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