



## Detection of Only Viable Bacterial Spores Using a Live/Dead Indicator in Mixed Populations

**This technology can be used by the food and pharmaceutical industries to validate sterility and quality.**

*NASA's Jet Propulsion Laboratory, Pasadena, California*

This method uses a photoaffinity label that recognizes DNA and can be used to distinguish populations of bacterial cells from bacterial spores without the use of heat shocking during conventional culture, and live from dead bacterial spores using molecular-based methods.

Biological validation of commercial sterility using traditional and alternative technologies remains challenging. Recovery of viable spores is cumbersome, as the process requires substantial incubation time, and the extended time to results limits the ability to quickly evaluate the efficacy of existing technologies. Nucleic acid amplification approaches such as PCR (polymerase chain reaction) have shown promise for improving time to detection for a wide range of applications. Recent real-time PCR methods are particularly promising, as these methods can be made at least semi-quantitative by correspondence to a standard curve.

Nonetheless, PCR-based methods are rarely used for process validation, largely because the DNA from dead bacterial cells is highly stable and hence, DNA-based amplification methods fail to discriminate between live and inactivated microorganisms.

Currently, no published method has been shown to effectively distinguish between live and dead bacterial spores. This technology uses a DNA binding photoaffinity label that can be used to distinguish between live and dead bacterial spores with detection limits ranging from  $10^9$  to  $10^2$  spores/mL.

An environmental sample suspected of containing a mixture of live and dead vegetative cells and bacterial endospores is treated with a photoaffinity label. This step will eliminate any vegetative cells (live or dead) and dead endospores present in the sample. To further determine the bacterial spore viability, DNA is extracted from the

spores and total population is quantified by real-time PCR.

The current NASA standard assay takes 72 hours for results. Part of this procedure requires a heat shock step at 80 °C for 15 minutes before the sample can be plated. Using a photoaffinity label would remove this step from the current assay as the label readily penetrates both live and dead bacterial cells. Secondly, the photoaffinity label can only penetrate dead bacterial spores, leaving behind the viable spore population. This would allow for rapid bacterial spore detection in a matter of hours compared to the several days that it takes for the NASA standard assay.

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## Intravenous Fluid Generation System

**This system can be used in remote medical facilities where limitations such as lack of refrigeration may limit the type and volume of medical fluids being stored or transported.**

*John H. Glenn Research Center, Cleveland, Ohio*

The ability to stabilize and treat patients on exploration missions will depend on access to needed consumables. Intravenous (IV) fluids have been identified as required consumables. A review of the Space Medicine Exploration Medical Condition List (SMEML) lists over 400 medical conditions that could present and require treatment during ISS missions.

The Intravenous Fluid Generation System (IVGEN) technology provides the scalable capability to generate IV fluids from indigenous water supplies. It meets USP (U.S. Pharmacopeia) standards. This capability was performed using potable water from the

ISS; water from more extreme environments would need preconditioning. The key advantage is the ability to filter mass and volume, providing the equivalent amount of IV fluid: this is critical for remote operations or resource-poor environments. The IVGEN technology purifies drinking water, mixes it with salt, and transfers it to a suitable bag to deliver a sterile normal saline solution.

Operational constraints such as mass limitations and lack of refrigeration may limit the type and volume of such fluids that can be carried onboard the spacecraft. In addition, most medical fluids have a shelf life that is shorter

than some mission durations. Consequently, the objective of the IVGEN experiment was to develop, design, and validate the necessary methodology to purify spacecraft potable water into a normal saline solution, thus reducing the amount of IV fluids that are included in the launch manifest.

As currently conceived, an IVGEN system for a space exploration mission would consist of an accumulator, a purifier, a mixing assembly, a salt bag, and a sterile bag. The accumulator is used to transfer a measured amount of drinking water from the spacecraft to the purifier. The purifier uses filters to separate any air bubbles that may have gotten

trapped during the drinking water transfer from flowing through a high-quality deionizing cartridge that removes the impurities in the water before entering the salt bag and mixing with the salt to create a normal saline solution.

*This work was done by John McQuillen and Terri McKay of Glenn Research Center, and Daniel Brown and John Zoldak of ZIN Technologies, Inc. Further information is contained in a TSP (see page 1). Inquiries concerning rights for the commercial use of this*

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