

ples suggest that by first treating a sample with PMA and covalently modifying the DNA originating from dead cells, downstream FISH-based microscopy should then enable the direct, specific visualization and enumeration of only living, viable microorganisms. An effective and efficient coupling of PMA-based chemistry with downstream FISH-microscopic methods would significantly empower the current ability to discern viable from dead microbes by direct visualization.

The basic principle of this method is that PMA penetrates only the dead cells and/or spores, due to their compromised membrane structures. Once inside the cell, PMA strongly intercalates with DNA. PMA has a photoactive azide group that allows covalent cross-linkage to DNA upon exposure to bright white light. This photoactivation results in the formation of PMA-DNA complex that renders DNA inaccessible for hybridization reaction during FISH assay. To avoid the difficulties and problems associated with current methods for determining the actual numbers of living versus dead cellular entities examined, and biases associated therewith, a novel mo-

lecular-biological protocol was developed for selective detection and enumeration of viable microbial cells. After having been subjected to the procedures described herein, the viability (live vs. dead) of bacterial cells and spores could be discerned. Following treatment with PMA, living, viable cells and spores were shown to be receptive to fluorescently labeled oligonucleotide probes, as hybridization and FISH-based microscopy was successful. Dead cells and spores, however, were not detected, as the pretreatment with PMA rendered their DNA unavailable to hybridization with the FISH-probes.

The true novelty of the technology is the coupling of a downstream, highly specific means of visualizing microbial cells and spores with a chemical pretreatment that precludes the portion of the microbial consortium that is not living (non-viable) from being detected. This results in the ability to selectively visualize and enumerate only the living cells and spores present in a given sample, in a molecular biological fashion, without the need for heavily biased cultivation-based methodologies. This novel study demonstrates that PMA penetrates

only the heat-killed spores, which precludes downstream hybridization reactions in the FISH assay. This novel PMA-FISH method is an attractive tool to detect viable endospores in spacecraft-associated environments, which is of crucial importance and benefit to planetary protection practices aimed at reducing the abundance of spacecraft-borne microbial contaminants.

This work was done by Myron T. La Duc and Kasthuri Venkateswaran of Caltech, and Bidyut Mohapatra of the University of South Alabama for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

*Innovative Technology Assets Management
JPL*

Mail Stop 202-233

4800 Oak Grove Drive

Pasadena, CA 91109-8099

E-mail: iaoffice@jpl.nasa.gov

Refer to NPO-48040, volume and number of this NASA Tech Briefs issue, and the page number.

Portable Intravenous Fluid Production Device for Ground Use

This small, portable device with high output produces medical injection-grade sterile water from potable water sources.

John F. Kennedy Space Center, Florida

There are several medical conditions that require intravenous (IV) fluids. Limitations of mass, volume, storage space, shelf-life, transportation, and local resources can restrict the availability of such important fluids. These limitations are expected in long-duration space exploration missions and in remote or austere environments on Earth. Current IV fluid production requires large factory-based processes. Easy, portable, on-site production of IV fluids can eliminate these limitations. Based on experience gained in developing a device for spaceflight, a ground-use device was developed.

This design uses regular drinking water that is pumped through two filters to produce, in minutes, sterile, ultrapure water that meets the stringent quality standards of the United States Pharmacopeia for Water for Injection (Total Bacteria, Conductivity, Endo-

toxins, Total Organic Carbon). The device weighs 2.2 lb (1 kg) and is 10 in. long, 5 in. wide, and 3 in. high (\approx 25, 13, and 7.5 cm, respectively) in its storage configuration. This handheld device produces one liter of medical-grade water in 21 minutes. Total production capacity for this innovation is expected to be in the hundreds of liters.

The device contains one battery powered electric mini-pump. Alternatively, a manually powered pump can be attached and used. Drinking water enters the device from a source water bag, flows through two filters, and final sterile production water exits into a sealed, medical-grade collection bag. The collection bag contains pre-placed crystalline salts to mix with product water to form isotonic intravenous medical solutions. Alternatively, a hypertonic salt solution can be injected into a filled bag. The filled collection bag is de-

tached from the device and is ready for use or storage. This device currently contains one collection bag, but a manifold of several pre-attached bags or replacement of single collection bags under sterile needle technique is possible for the production of multiple liters. The entire system will be flushed, sealed, and radiation-sterilized.

Operation of the device is easy and requires minimal training. Drinking water is placed into the collection bag. Inline stopcock flow valves at the source and collection bags are opened, and the mini-pump is turned on by a switch to begin fluid flow. When the collection bag is completely filled with the medical-grade water, the pump can be turned off. The pump is designed so it cannot pump air, and overfilling of the collection bag with fluid is avoided by placing an equal amount of water in the source bag. Backflow is avoided by in-

line check valves. The filled collection bag is disconnected from its tubing and is ready for use. The source bag can be refilled for production of multiple liters, or the source bag can be replaced with an input tube that can be placed in a larger potable water source if the device is attended. The device functions in all orientations independent of any gravity fields.

In addition to creating IV fluids, the device produces medical-grade water, which can be used for mixing with medications for injection, reconstituting freeze-dried blood products for injection, or for wound hydration or irrigation.

Potential worldwide use is expected with medical activities in environments that have limited resources, storage, or resupply such as in military field opera-

tions, humanitarian relief efforts, submarines, commercial cruise ships, etc.

This work was done by Philip J. Scarpa of Kennedy Space Center and Wolfgang K. Scheuer of Tiger Purification Systems, Inc. For more information, contact Dr. Philip Scarpa at (321) 867-6386 or Philip.J.Scarpa@nasa.gov. KSC-13598

Adaptation of a Filter Assembly to Assess Microbial Bioburden of Pressurant Within a Propulsion System

NASA's Jet Propulsion Laboratory, Pasadena, California

A report describes an adaptation of a filter assembly to enable it to be used to filter out microorganisms from a propulsion system. The filter assembly has previously been used for particulates $>2\ \mu\text{m}$. Projects that utilize large volumes of nonmetallic materials of planetary protection concern pose a challenge to their bioburden budget, as a conservative specification value of $30\ \text{spores}/\text{cm}^3$ is typically used.

Helium was collected utilizing an adapted filtration approach employing

an existing Millipore filter assembly apparatus used by the propulsion team for particulate analysis. The filter holder on the assembly has a 47-mm diameter, and typically a $1.2\text{-}5\ \mu\text{m}$ pore-size filter is used for particulate analysis making it compatible with commercially available sterilization filters ($0.22\ \mu\text{m}$) that are necessary for biological sampling.

This adaptation to an existing technology provides a proof-of-concept and a demonstration of successful use in a ground equipment system. This adapta-

tion has demonstrated that the Millipore filter assembly can be utilized to filter out microorganisms from a propulsion system, whereas in previous uses the filter assembly was utilized for particulates $>2\ \mu\text{m}$.

This work was done by James N. Benardini, Robert C. Koukol, Wayne W. Schubert, Fabian Morales, and Martin F. Klatte of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1). NPO-48304