



Surface Bacterial-Spore Assay Using Tb^{3+} /DPA Luminescence

A total spore count could be obtained in minutes.

NASA's Jet Propulsion Laboratory, Pasadena, California

Equipment and a method for rapidly assaying solid surfaces for contamination by bacterial spores are undergoing development. The method would yield a total (non-viable plus viable) spore count of a surface within minutes and a viable-spore count in about one hour. In this method, spores would be collected from a surface by use of a transparent polymeric tape coated on one side with a polymeric adhesive that would be permeated with one or more reagent(s) for detection of spores by use of visible luminescence. The sticky side of the tape would be pressed against a surface to be assayed, then the tape with captured spores would be placed in a reader that illuminates the sample with ultraviolet light and counts the green luminescence spots under a microscope to quantify the number of bacterial spores per unit area. The visible luminescence spots seen through the microscope would be counted to determine the concentration of spores on the surface.

This method is based on the chemical and physical principles of methods described in several prior *NASA Tech Briefs* articles, including "Live/Dead Spore Assay Using DPA-Triggered Tb Luminescence" (NPO-30444), Vol. 27, No. 3 (March 2003), page 7a. To recapitulate: The basic idea is to exploit the observations that (1) dipicolinic

acid (DPA) is present naturally only in bacterial spores; and (2) when bound to Tb^{3+} ions, DPA triggers intense green luminescence of the ions under ultraviolet excitation; (3) DPA can be released from the viable spores by using L-alanine to make them germinate; and (4) by autoclaving, microwaving, or sonicating the sample, one can cause all the spores (non-viable as well as viable) to release their DPA.

One candidate material for use as the adhesive in the present method is polydimethylsiloxane (PDMS). In one variant of the method — for obtaining counts of all (viable and nonviable) spores — the PDMS would be doped with $TbCl_3$. After collection of a sample, the spores immobilized on the sticky tape surface would be lysed by heating or microwaving to release their DPA. Tb^{3+} ions from the $TbCl_3$ would become bound to the released DPA. The tape would then be irradiated with ultraviolet and examined as described above. In another variant of the method — for obtaining counts of viable spores only — the PDMS would be doped with L-alanine in addition to $TbCl_3$.

As now envisioned, a fully developed apparatus for implementing this method would include a pulsed source of ultraviolet light and a time-gated electronic camera to record the images seen

through the microscope during a prescribed exposure interval at a prescribed short time after an ultraviolet pulse. As in the method of the second-mentioned prior article, the pulsing and time-gating would be used to discriminate between the longer-lived Tb^{3+} /DPA luminescence and the shorter-lived background luminescence in the same wavelength range. In a time-gated image, the bright luminescence from bacterial spores could easily be seen against a dark background.

This work was done by Adrian Ponce of Caltech for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1).

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
(818) 354-2240*

E-mail: iaoffice@jpl.nasa.gov

Refer to NPO-40646, volume and number of this NASA Tech Briefs issue, and the page number.

Simplified Microarray Technique for Identifying mRNA in Rare Samples

This method can be implemented by use of portable equipment.

Ames Research Center, Moffett Field, California

Two simplified methods of identifying messenger ribonucleic acid (mRNA), and compact, low-power apparatuses to implement the methods, are at the proof-of-concept stage of development. These methods are related to traditional methods based on hybridization of nucleic acid, but whereas the traditional methods must be practiced in laboratory settings, these methods could be practiced in field settings.

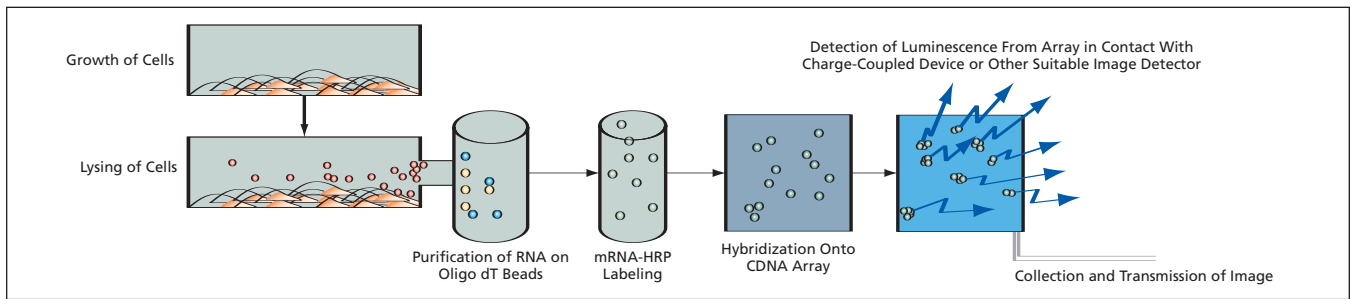
Hybridization of nucleic acid is a

powerful technique for detection of specific complementary nucleic acid sequences, and is increasingly being used for detection of changes in gene expression in microarrays containing thousands of gene probes. A traditional microarray study entails at least the following six steps:

1. Purification of cellular RNA,
2. Amplification of complementary deoxyribonucleic acid [cDNA] by poly-

- merase chain reaction (PCR),
3. Labeling of cDNA with fluorophores of Cy3 (a green cyanine dye) and Cy5 (a red cyanine dye),
4. Hybridization to a microarray chip,
5. Fluorescence scanning the array(s) with dual excitation wavelengths, and
6. Analysis of the resulting images.

This six-step procedure must be performed in a laboratory because it requires bulky equipment.



mRNA Is Labeled with a reporter enzyme (HRP), then hybridized onto a cDNA array. The array is then treated to induce chemiluminescence, which is detected by contact digital imaging.

The present developmental methods require fewer steps and are not restricted to laboratory settings because they do not require bulky equipment. In principle, they could be implemented by means of low-power, portable, lightweight units having sizes of the order of a cubic foot ($\approx 0.03\text{m}^3$). These methods could be used to perform field studies as precursors to full laboratory gene-expression analyses and can be used for detecting rare and little-expressed mRNA samples. This present method does not require the PCR-amplification, fluorescent-labeling, and scanning steps (steps 2, 3, and 5 listed above).

The steps involved in the method are depicted schematically in the figure. In this method, the initial mRNA from cell or tissue lysates is purified in one step, using oligo dT beads, and is then directly labeled by cross-linking to a reporter enzyme [horseradish peroxidase (HRP)]. The HRP-linked mRNA is then hybridized to a cDNA gene array printed on a nylon membrane, the membrane is incubated with a chemiluminescence substrate, and the resulting chemiluminescence from the affected area of the membrane is detected by contact digital imaging. The whole procedure takes less than five hours. This method is useful for identifying rare genes without much

processing, and for diagnostic genomic screening for biomarkers. The apparatus for implementing this method can be miniaturized for rapid screening for stem-cell research or analyzing rare cell samples from tissues.

This work was done by Eduardo Almeida of Ames Research Center and Geeta Kadambi of National Space Grant Foundation. Further information is contained in a TSP (see page 1).

This invention is owned by NASA and a patent application has been filed. Inquiries concerning rights for the commercial use of this invention should be addressed to the Ames Technology Partnerships Division at (650) 604-2954. Refer to ARC-15177-1.