



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).

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Simplified Microarray Technique for Identifying mRNA in Rare Samples

This method can be implemented by use of portable equipment.

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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.