dimensional, tissuelike assemblies have been successful.

Anchorage-dependent cells, including primary cells, transformed cells, genetically engineered cells and cell lines, and suspension hybridoma cells were successfully cultured either as mono- or co-cultures in the HFB for times as long as 2 weeks. Cultures were initiated by inoculating cells, variously with or without attachment materials, into the vessel through the sampling port, filling the vessel completely with a culture medium, and setting rotation rates to maintain suspension. Large three-dimensional, tissuelike assemblies were obtained from HFB cultures of anchorage-dependent cells (see Figure 2). In addition, critical stages in three-dimensional, tissuelike growth (cell attachment, three-dimensional aggregation, and formation of an extracellular matrix) were observed with all anchorage-dependent cells cultured in the HFB.

This work was done by Steve R. Gonda and Glenn F. Spaulding of **Johnson Space Center**, *Yow-Min D. Tsao, Scott Flechsig and Leslie Jones of Wyle Laboratories, and Holly Soehnge of Universities Space Research Associates.*

Title to this invention, covered by U.S. Patent No. 6,001,642 has been waived under the provisions of the National Aeronautics and Space Act {42 U.S.C. 2457 (f)}. Inquiries concerning licenses for its commercial development should be addressed to

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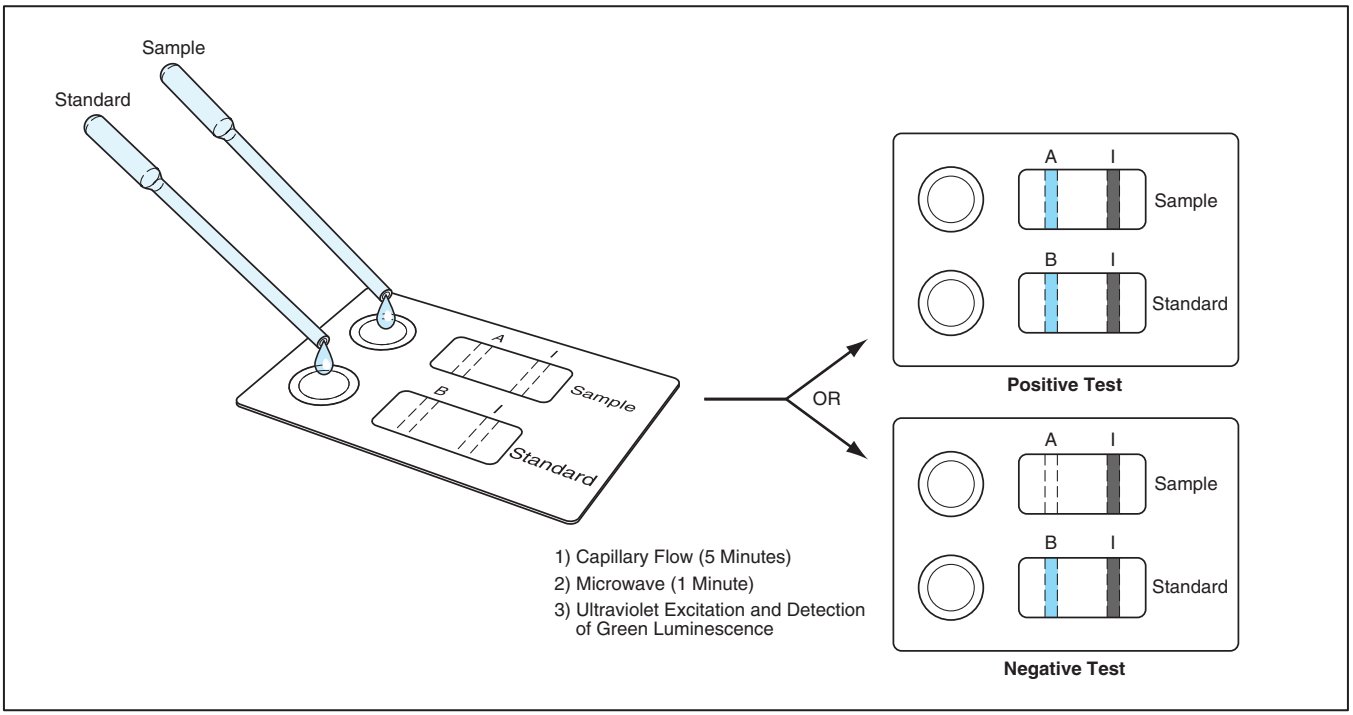
Refer to MSC-22538, volume and number of this NASA Tech Briefs *issue, and the page number.*

Species Specific Bacterial Spore Detection Using Lateral-Flow Immunoassay With DPA-Triggered Tb Luminescence

A highly-sensitive assay can be performed in minutes.

NASA's Jet Propulsion Laboratory, Pasadena, California



An **Assay Is Performed** on a sample suspected of containing bacterial spores of a species of interest, in parallel with an assay of a standard containing a known concentration of *Bacillus subtilis*. The intensity of luminescence in region A under ultraviolet illumination is proportional to the number density of the spores.

A method of detecting bacterial spores incorporates

- A method of lateral-flow immunoassay in combination with
- A method based on the luminescence of Tb^{3+} ions to which molecules of dipicolinic acid (DPA) released from the spores have become bound.

The second-mentioned component method, denoted the method of DPA-triggered Tb luminescence, was described in somewhat more detail as a precursor of a related development reported in "Improved Technique for Detecting Endospores via Luminescence" (NPO-21240) *NASA Tech Briefs*, Vol. 26, No. 7 (July 2002), page 56.

The present combination of lateral-flow immunoassay and DPA-triggered Tb luminescence was developed as a superior alternative to a prior lateral-flow immunoassay method in which detection involves the visual observation and/or measurement of red light scattered from colloidal gold nanoparticles. The advantage of the present combination method is that it affords both

- High selectivity for spores of the species of bacteria that one seeks to detect (a characteristic of lateral-flow immunoassay in general) and
- Detection sensitivity much greater (by virtue of the use of DPA-triggered Tb luminescence instead of gold nanoparticles) than

that of the prior lateral-flow immunoassay method. The sensitivity afforded by the present method is so much greater that whereas the previously reported detection limit of lateral-flow immunoassay was 10^5 spores/mL, the estimated detection limit of the present method is 100 spores/mL.

DPA in a 1:1 complex with Ca^{2+} ions is present in high concentration in bacterial spores, and has not been observed in any lifeforms other than bacterial spores. Hence, DPA is an indicator molecule for the presence of bacterial spores. Fortuitously, DPA is also a classic inorganic-chemistry ligand that binds metal ions with high affinity. When bound to Tb^{3+} ions, DPA triggers intense green luminescence of the ions under ultraviolet excitation. The intensity of the luminescence can be correlated with the number density of bacterial spores per milliliter. Moreover, the concentrations of compounds that could potentially give rise to spurious luminescence are typically much smaller than the concentration of DPA, and the strengths with which they bind to Tb^{3+} are of the order of a millionth of that of DPA, so that the desired luminescence signal appears against a dark background.

The figure summarizes the steps of the present method. A sample suspected of containing bacterial spores is prepared by suspending raw sample material in an aqueous solution that contains Tb^{3+} ions. A volume of ≈100 μL of the sample is placed on a test strip — a nitrocellulose membrane on which species-specific antibodies are bound in an area denoted the sample region (area A in the figure). Capillary action moves the spores along the strips. In the sample region, specific binding of membrane-bound antibodies captures and immobilizes the bacterial spores. Next, the strip is exposed to microwave power to release DPA from the spores. The released DPA binds to the Tb^{3+} ions in the solution. Hence, when the strip is exposed to ultraviolet light, the Tb^{3+} ions luminesce green, signaling the presence of the bacterial spores from which the DPA was released.

At the same time, a volume of ≈100 μL of a similar solution containing a known concentration of *Bacillus subtilis*, to be used as a standard, is placed on a similarly prepared, parallel membrane denoted the standard strip, which includes an antibody-coated area designated the standard region (area B in the figure). The standard strip is subjected to the same process as is the test strip. A combination of green luminescence from the region B and a change in color in regions I of both strips indicates that the assay has worked properly. In that case, the ratio of between the intensity of luminescence in region A and that in region B is proportional to the number density of bacterial spores in the sample. The entire assay can be performed in 10 minutes or less.

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

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Refer to NPO-30469, volume and number of this NASA Tech Briefs *issue, and the page number.*

Live/Dead Bacterial Spore Assay Using DPA-Triggered Tb Luminescence

A relatively simple procedure can be executed in about 20 minutes.

NASA's Jet Propulsion Laboratory, Pasadena, California

A method of measuring the fraction of bacterial spores in a sample that remain viable exploits DPA-triggered luminescence of Tb^{3+} and is based partly on the same principles as those described in the immediately preceding article. Unlike prior methods for performing such live/dead assays of bacterial spores, this method does not involve counting colonies formed by cultivation (which can take days), or counting of spores under a microscope, and works whether or not bacterial spores are attached to other small particles (i.e., dust), and can be implemented on a time scale of about 20 minutes.

Like the method of the preceding article, this method exploits the facts that (1) DPA is present naturally only in bacterial spores; (2) when bound to Tb^{3+} ions, DPA triggers intense green luminescence of the ions under ultraviolet excitation; and (3) the intensity of the luminescence can be correlated with the concentration of DPA released from spores and, thus, with the number density of the spores. It has been found that in the case of a sample comprising bacterial spores suspended in a solution, the DPA can be released from the viable spores into the solution by using L-alanine to make them germinate. It has also been found that by autoclaving, microwaving, or sonicating the sample, one can cause all the spores (non-viable as well as viable) to release their DPA into the solution. When the released DPA binds Tb^{3+} ions in the solution and the sample is exposed to ultraviolet light, the solution luminesces, as described in the preceding article.

Therefore, in this method, one divides a sample into two parts. For the first part, germination is used to release the DPA from the viable spores; for the second part, one of the three other techniques is used to release DPA from all the spores. The intensities of the DPA-triggered luminescence of both parts of the sample are measured. Then the fraction of viable spores is calculated as the ratio between the measured luminescence intensities of the first and second parts of the sample.

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

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