Sensitive, Rapid Detection of Bacterial Spores

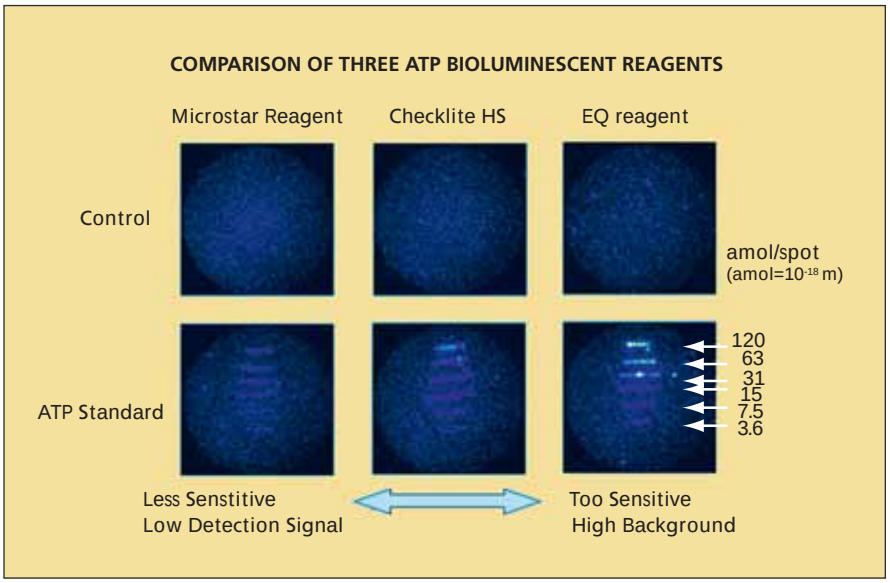
This capability is beneficial for medicine, public health, and biowarfare defense.

NASA's Jet Propulsion Laboratory, Pasadena, California

A method of sensitive detection of bacterial spores within delays of no more than a few hours has been developed to provide an alternative to a prior three-day NASA standard culture-based assay. A capability for relatively rapid detection of bacterial spores would be beneficial for many endeavors, a few examples being agriculture, medicine, public health, defense against biowarfare, water supply, sanitation, hygiene, and the food-packaging and medical-equipment industries.

The method involves the use of a commercial rapid microbial detection system (RMDS) that utilizes a combination of membrane filtration, adenosine triphosphate (ATP) bioluminescence chemistry, and analysis of luminescence images detected by a charge-coupled-device camera. This RMDS has been demonstrated to be highly sensitive in enumerating microbes (it can detect as little as one colony-forming unit per sample) and has been found to yield data in excellent correlation with those of culture-based methods. What makes the present method necessary is that the specific RMDS and the original protocols for its use are not designed for discriminating between bacterial spores and other microbes.

In this method, a heat-shock procedure is added prior to an incubation procedure that is specified in the original RMDS protocols. In this heat-shock procedure (which was also described in a prior *NASA Tech Briefs* article on enumerating spore-forming bacteria), a sample is exposed to a temperature of 80 °C for 15 minutes. Spores can survive the heat shock, but non-spore-forming bacteria and spore-forming bacteria that are not in spore form cannot survive. Therefore, any colonies that grow during incubation after the heat shock are deemed to have originated as spores.



These **Luminescence Images** were obtained in tests of three bioluminescence reagents with successively diluted samples of an ATP solution. The tests led to the selection of one of the reagents (Checklite HS) as offering the best compromise between requirements for high sensitivity and low background.

This method also provides for reduction of the incubation time from the typical range (18 to 24 hours) required by the original RMDS protocols. This reduction was effected by evaluation of three commercial bioluminescence reagents (see figure), leading to the selection of one of them that makes it possible to effect detection after an incubation time of only ≈5 hours. The sensitivity and rapidity afforded by this method were demonstrated in tests in which seven species of *Bacillus* that had been repeatedly isolated from clean rooms were detected after incubation times of about 5 hours.

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Adenosine Monophosphate-Based Detection of Bacterial Spores

AMP is released by means of heat shock, then detected via bioluminescence.

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A method of rapid detection of bacterial spores is based on the discovery that a heat shock consisting of exposure to a temperature of 100 °C for 10 minutes causes the complete release of adenosine monophosphate (AMP) from the spores. This method could be an alternative to the method described in the immediately preceding article. Unlike that method and related prior methods, the present method does not involve germination and cultivation; this feature is an important advantage because in cases in which the spores are those of pathogens, delays involved in germina-