

Sensitivity and Applicability of a Rapid Micro-Detection System for the Enumeration of Bacterial Endospores

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*Abstract*¹—NASA continuously monitors spacecraft surfaces to assure a very low presence of bacterial endospores upon landing on the surface of Mars. In order to meet the rigid schedules of spacecraft assembly, a more rapid, sensitive spore detection assay is being considered as an alternate method to the current three-day NASA standard culture-based assay. The Millipore MicroStar Rapid System (RMDS) has been used successfully for rapid bioburden enumeration in a wide range of applications. It combines membrane filtration, adenosine triphosphate (ATP) bioluminescence chemistry, and image analysis based on photon detection with a CCD camera. The RMDS is rapid, simple, shows high sensitivity (1 CFU/sample), and an excellent correlation with traditional culture-based methods. In addition, by utilizing the Milliflex filtration system, the RMDS is ideal for sampling both large sample volumes and those samples containing inhibitory substances. In this study, we have evaluated the use of the RMDS as a rapid spore detection tool for NASA applications. This is accomplished by preceding the RMDS incubation protocol with a heat shock step, 15 minutes at 80°C, as a direct selection of spores. Different luciferase enzymes were tested in order to reduce the typical 18–24 hour incubation time required by the RMDS to ~5 hours. Of the reagents evaluated, a formulation of highly sensitive bioluminescence reagent was found to be more sensitive than the present commercially available reagents. Assay times of ~5 hours were repeatedly demonstrated along with low image background noise. In order to evaluate the applicability of this method, seven species of *Bacillus* (nine strains) that have been repeatedly isolated from clean room environments were assayed. All strains were detected in ~5 hours. The improved RMDS-based spore detection is under validation and is expected to achieve the goal of “same shift” measurement of spore bioburden during spacecraft assembly.

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1. INTRODUCTION

The international scientific community has been pressed from early on to reduce the number of organisms that could hitchhike on a spacecraft to celestial bodies as low a level as possible. This policy has been followed with the intent of preserving the pristine nature of the planetary environment as well as to protect the science by preventing interference with scientific life-detection investigations [1]. National Aeronautics and Space Administration (NASA) has been responsible for the implementation of planetary protection policies that are developed in the international community and within the Committee on Space Research (COSPAR) [1]. Policies on planetary protection requirements have evolved over time as new information about Mars and other bodies have been acquired [1]. At the direction of NASA, the Jet Propulsion Laboratory (JPL) has been advancing methods for assessment of biological contamination levels on spacecraft and their assembly facilities [2]. Currently, the long-standing method to assess microbial contamination on spacecraft hardware during assembly has been the “NASA standard assay” (NHB 5340.1), in which the number of cultivable aerobic bacterial endospores in surface samples swabbed from a defined surface area (25.8 cm²) of a spacecraft is measured [2]. According to current Planetary Protection specifications, Mars lander spacecraft not carrying life-detection instrumentation (category IVa) have a bioburden limit [3] not to exceed 3x10⁵ cultivable bacterial spores (NPR 8020.12C) on surfaces open to Mars. For missions carrying life-detection instrumentation (category IVb) or missions to special regions where terrestrial organisms have a likelihood of propagating or where there is a high potential for the existence of extant life forms (category IVc) [1], the requirements are more stringent: 30 cultivable bacterial spores on the total of all surfaces, mated joins, and in the bulk of non-metallic material.

It has been recognized that the development of a more rapid spore assay would be an important contribution to the field of planetary protection. It would reduce risk associated with meeting demanding spacecraft assembly schedules, and could be considered as an alternative to the current three-day NASA standard culture-based assay. The Millipore MicroStar Rapid Micro Detection system (RMDS) offers a way to quantify microbial contamination more rapidly than

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traditional microbial contamination detection methods, such as membrane filtration and pour plates. The RMDS is a unique system that detects and counts viable microorganisms on a membrane (Figure 1). It combines membrane filtration, Adenosine Triphosphate (ATP) bioluminescence chemistry, and image analysis based on photon detection with a CCD camera. The system consists of a detection tower, image intensifier controller, image processor, computer and MicroStar image analysis software. It also includes unique membranes, ATP Bioluminescence reagent kits, and an improved AutoSpray station. In addition, by utilizing the Milliflex PLUS Vacuum pump for filtration, the RMDS system can be used ideally for sampling both large volumes and those samples containing inhibitory substances in a much more time efficient manner. The RMDS system allows detection of viable microorganisms within hours of sample filtration. It can count as low as one colony forming unit (CFU) per membrane sample [4]. However, it has not been used for the detection of bacterial endospores to date. This paper reports the integration and modification of the RMDS for the specific detection of bacterial endospores.

Detection Tower Components

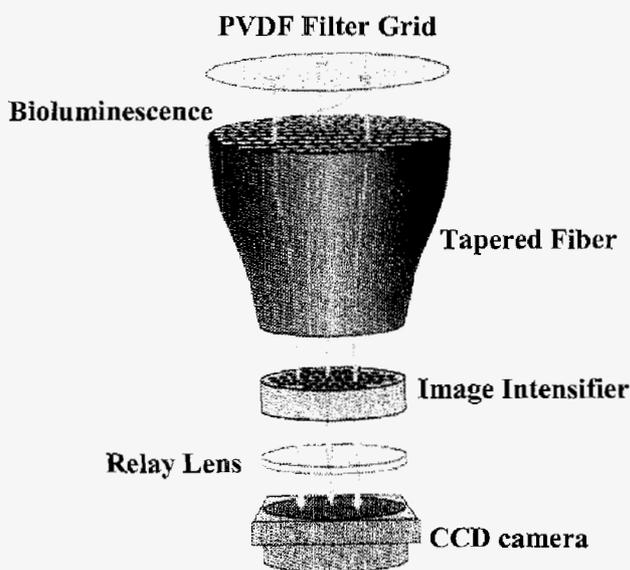


Figure 1. - MicroStar Rapid Microbial Detection System.

The RMDS system uses proven technologies that are recognized as microbial detection methods both nationally and internationally in both the pharmaceutical and food processing industries. The filtration system is highly reliable, automatic, has a sanitary design with autoclavable pump heads, and contains internal calibration programs to ensure accuracy. In view of the fact that concentrated ATP is found only in living cells, it is an indicator of cell viability and happens to be its primary source of energy. The concentration of ATP required for detection is about 20 attomoles. This is equivalent to one yeast or mold cell, or

approximately 50 to 100 bacterial cells. The detection of ATP as a reliable indicator of general microbial metabolic activity is a technology extensively developed for commercial application by the Kikkoman Corporation in Japan. It is widely used in the food industry to determine gross bacterial contamination in a liquid assay format. The standard Kikkoman assay uses a bioluminescent reagent containing luciferin and the firefly enzyme, luciferase. ATP transfers its energy to the luciferin molecule, which forms luciferyl adenylate, which is then oxidized by the enzyme luciferase in the presence of magnesium ions and molecular oxygen, to form oxyluciferin, carbon dioxide, and to emit bioluminescence (Figure 2).

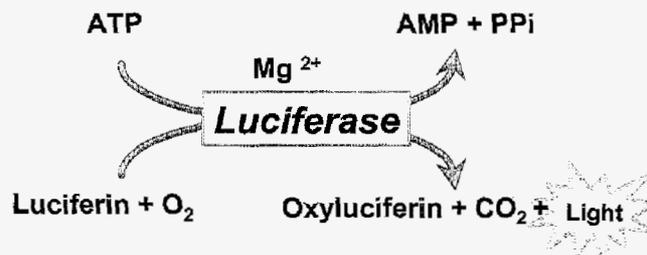


Figure 2. - Firefly luciferase bioluminescence reaction. Luciferase catalyzes the reaction of luciferin with ATP to form an intermediate luciferyl adenylate, whose subsequent oxidation results in light emission with a quantum yield of about 90% (Seliger, 1989; Wilson and Hastings, 1998).

In this study however, we use a solid membrane format for the visualization of microcolonies developed by Millipore Corporation. When using the AutoSpray Station, a uniform, repetitive application of two reagents takes place for the detection of microorganisms on the sample membrane. These reagents initiate the release of ATP by making the cell walls of the microorganism permeable. This is followed by spraying the bioluminescent reagent onto the microorganisms present on the surface of the membrane, which react with the released ATP and emits light (photons). The photons are then converted into electrons and multiplied by about one million by a microchannel plate to produce a cascade of electrons. The electrons are then converted back to photons by a phosphorous screen. The image intensifier controller supplies the high voltage power needed for this process. The photons then hit an array of silicone chips found inside the CCD camera. The chips have the same function as film inside an ordinary camera. During this process, the photons register as a signal when they contact each chip or pixel. The camera scans the pixel every 1/30th second and develops an image of the membrane's light spots. The image processor removes the background and transfers the image to the PC monitor. The RMDS software verifies the size and intensity of each light spot as compared to the background using preset parameters. At that time, each light point is counted then stored for downloading. The location of each photon spot corresponds exactly to the location of each CFU. Total photon/light detection time is approximately two minutes per sample.

The study presented here evaluates the RMDS system to see whether it can be used as a rapid spore detection tool for NASA applications and if it will consequently meet the criteria referred to earlier.

2. METHODS

Sample collection, isolation, and identification

Environmental samples were obtained from the Spacecraft Assembly Facility at JPL as well as from surfaces of spacecraft following swab-rinse procedures described in NPR: 5340.1 [3]. Upon collection of a surface sample, each swab was placed individually into 10 mL sterile water. Samples were sonicated for 2 minutes and heat shocked at 80°C for 15 minutes. Appropriate aliquots were added into Petri dishes and aerobic spores were cultured by pour-plate method using tryptic soy agar (TSA, Difco) as the growth medium. The plates were incubated at 32°C for three days. Isolates were selected, purified, and stored in glycerol at -80°C. Identification of purified strains was accomplished by 16S rDNA sequencing.

Sporulation and spore purification

A selected small set of environmental isolates was sporulated and purified. *Bacilli* spore cultures were inoculated onto 20–30 NSM agar plates at 32°C and allowed to grow for two days [4]. The plates were left at room temperature until >90% had sporulated and were confirmed by phase contrast microscopy. The spore lawn was then scraped off and placed into sterile, deionized, cold water in a centrifuge tube. After agitation to wash the slurry, the tube was centrifuged for twenty minutes at 10,000g, 4°C. This was repeated three times, and then the pellets were brought up to volume with ice-cold, sterile deionized water and placed into an 11°C shaker and shaken at 100 rpm overnight, on ice. The next day, the cultures were removed from the incubator, centrifuged at 10,000 g, and resuspended with ice-cold, sterile, deionized water. Bottles were shaken to loosen the pellets, then placed back into 11°C shaker, and shaken at 100 rpm overnight, on ice. The samples were removed from the shaker on the next day, centrifuged at 10,000g again, and resuspended with ice-cold, sterile, deionized water, and transferred to sterile, glass screw-cap tubes. The spore suspensions were stored at 4°C for later use.

NASA standard assay

NASA has developed specific methodologies based on microbial culturing procedures for the sampling and collection of microorganisms from spacecraft assembly facilities, as well as spacecraft themselves. Generally, samples are obtained by surface swabbing and/or by surface wiping (NHB: 5340.1).

Microbiological Sampling of Environmental Surfaces Swab-Rinse Method—Remove a sterile cotton swab aseptically from its container and moisten the head of the swab in

10.0 mL of sterile distilled water. Moist swabs are then used to sample an area of up to 25.8 cm². Return the head of the swab to the original tube of water, breaking off the head of the swab below any portion of the handle that was touched by the sampler. Allow the swab head to drop into the water and replace the screw cap. Repeat these steps for any other required samples. Transport the samples to the laboratory and process within 24 hours.

Prior to sonication, place each tube containing the water and the swab on a vortex mixer and agitate at maximum power for 5–6 seconds. Suspend the samples in the middle of an ultrasonic water bath filled to normal capacity. Not more than 12 tubes should be placed into the bath at one time. Make sure that the bath fluid is above the liquid level in the tubes and then sonicate for 2 minutes ± 5 seconds.

Heat shock procedure—Heat shock each sample by placing the tube with the vortexed and sonicated solution into a water bath heated to 80 ± 2°C. The temperature within the water bath may be monitored with a pilot tube containing an equal amount of water and a thermometer. Begin the 15-minute exposure as soon as the temperature in the pilot tube reads 80 ± 2°C. Place the heat shocked sample tubes in an ice bath immediately afterwards. The sample tubes should reach 30 to 35°C within 2 minutes at most. Process the samples as soon as possible.

Spore isolation for cultivable isolates—As soon as the heat shock step has been completed (within 45 minutes), aseptically pipette 2.0 mL aliquots into four Petri dishes. Add approximately 20 mL sterile, molten (48 to 50°C) Trypticase Soy Agar (TSA) to each plate and mix the contents by gentle swirling. Allow the mixture to solidify. Aerobically incubate the solidified agar plates at 32°C in an inverted position. Scan the plates for three consecutive days to identify growing colonies. *Bacilli* species can be identified by this preliminary cultivation method, and then confirmed by 16S ribosomal (r) DNA sequence analysis [5]. These procedures may be applied to both swab-rinse and wipe-rinse samples for spore isolation and identification.

Environmental Surface sample collection using Wipes

In a laminar flow hood, wearing sterile gloves, aseptically remove a sterile wipe from its wrapper and place the individual wipe into a clean transport jar or sterile Petri dish. Moisten the wipe with approximately 15 mL of sterile, distilled water. Place the wipe flat on the sample surface and rub over the entire surface using a firm, steady pressure. Refold the wipe by reversing the direction of the open fold so the “contaminated” surface is interior to the new configuration. Rub the wipe over the sample area three times, rotating the direction of motion 90 degrees after each complete coverage of the sample area. Transfer the wipe into a dry, sterile, glass jar with appropriate capacity to accommodate the solution volume. Rinse hands with filtered, sterilized 70% isopropyl alcohol between each sample,

Add 200 mL of sterile distilled water to each sample and reseal the jar. Place the sample jars (not to exceed four) in the ultrasonic bath. Make sure that the liquid level in the bath is above the liquid level in the sample jars. Sonicate for 2 minutes, \pm 5 seconds. The sample is aseptically transferred to a new sterile jar for further processing.

RMDS spore assay

The RMDS system was evaluated for rapid spore detection with some modifications based on the NASA standard assay and the RMDS procedure itself.

Bacterial spore samples were heat shocked for 15 minutes at 80°C as described above. Sample filtration was carried out using the Milliflex PLUS Pump. Milliflex funnel was washed twice with 25 mL ADase treated sterile, distilled water. 25 mL of ADase treated water was added to the funnel after the second wash. Consequently, the heat-shocked spore suspension was added to the funnel and swirled with a sterile pipette, then filtered. This was followed by two wash cycles, with final filtration of the membrane. All the samples were processed in this manner (Figure 3).

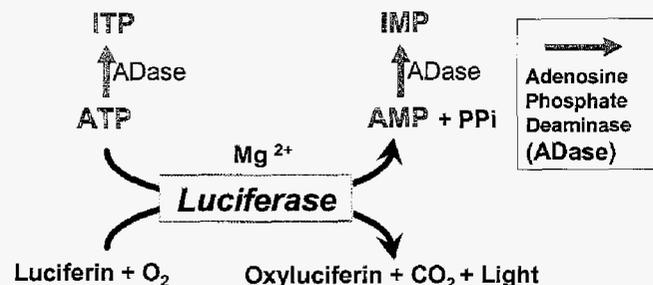


Figure 3. - Background reduction. Adenosine phosphate deaminase (ADase) was used to eliminate background noise levels in TSA medium and rinse water.

Following filtration, the sample funnel was removed from the pump and placed onto a compatible Millipore Petri dish containing TSA, which is used for the incubation of Millipore membranes. It was possible to separate the filter membrane from the filter unit by applying even pressure and breaking the two sections apart. Therefore, the filter membrane came in direct contact with TSA and could be left to incubate for 5 hours at 35°C.

After incubation, the filter membrane and its cover were separated from the nutrient source and placed in a laminar flow hood, and then dried to completion. The dried membrane was exposed to the ATP releasing reagent as well as the bioluminescence reagent. The ATP releasing reagent was purchased from the manufacturer ready to use. However, the bioluminescent reagent needed to be resuspended with the reconstitution buffer provided in the reagent kit. The resuspended bioluminescent reagent has a shelf life of one week at 4°C. The bioluminescent reagent

was resuspended under aseptic conditions, and swirled slightly to obtain a uniform solution. Afterwards, the reagents were loaded into their appropriate positions in the AutoSpray Station, and primed. After priming, the sample membrane was placed onto the membrane holder inside the unit and sprayed with the ATP releasing agent first, underwent a drying cycle, and then finally sprayed with the bioluminescence reagent. Immediately afterwards, the membrane was removed from the unit, and placed in the detection tower face down, and begin the detection and enumeration process.

Optimizing Results—Certain improvements in the procedure were made for obtaining optimum results. It was intended to develop a modification to the system whereby one could process and obtain results within five hours of sample acquisition and filtration— instead of the 18–24 hour incubation interval required— and in the meantime, reduce the background luminescence of the sample just by decreasing the exposure period.

A comparison was carried out to determine the highest sensitivity between three different reagents. A dilute solution of ATP standard was prepared and inoculated in replicates onto Milliflex membranes, following a gradient of decreasing concentration, and allowed to dry. Three different ATP bioluminescent reagents were used to enumerate the spots.

The same ATP bioluminescent reagents were also compared to demonstrate an optimum interval of exposure to TSA cassettes for incubation of *Bacillus subtilis* spores after filtration onto Milliflex membranes.

Growth controls were carried out simultaneously with the analysis to verify the spore concentration of the standard spore stock. Hence, they served as the positive controls. These were finally enumerated three days after plating, reflecting NASA's procedures.

Background reduction

In order to reduce the background noise, it was necessary to expose all rinse solutions (water) and growth media (TSA) to Adenosine phosphate deaminase (ADase). ADase is an enzyme purified from the marine red macroalga *Gloiopeltis furcata* [6]. It is used to catalyze the irreversible nonspecific deamination of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) from all solutions used for the study [7], which could cause higher interference.

A stock solution of ADase was prepared, filter-sterilized at a concentration of 500 units/mL, and then stored at -20°C until use. ADase was added aseptically to any rinse solution or growth media at a final concentration of 0.05 U/mL, and then allowed to incubate for two hours at room temperature (for water) or 55°C (for TSA) before use.

3. RESULTS/DISCUSSION

Spore enumeration using RMDS system

In order to evaluate the applicability and sensitivity of the RMDS system for spore enumeration, conditions had to be optimized before the evaluation was carried out efficiently and thoroughly. These included reduction of background, selection of bioluminescent reagent, and determination of exposure interval to growth medium for optimum results.

Reduction of background using ADase—For NASA planetary protection application, it is important to eliminate any background noise, because most samples are clean with spore counts ranging from zero to fewer than five. Two methods have been evaluated to minimize background noise levels: vapor H_2O_2 and Adenosine phosphate deaminase (ADase). Initially, when filter units were exposed to vapor H_2O_2 , and ATP was spotted onto the membranes, a marked decrease in the ATP signal was observed when compared to negative controls. This indicated that there remained a residual amount of H_2O_2 on the membranes that interfered with the detection chemistry. Additionally, it was determined that if the rinse solution and the growth medium were treated with ADase, there was a noticeable reduction in background noise levels. Consequently, it facilitated spore detection and enumeration. All rinse solutions (sterile, ultra pure water at room temperature) and growth media (Trypticase Soy Agar, molten and at $55^\circ C$) were treated with Adenosine phosphate deaminase (ADase) at a

concentration of $0.05 U/mL$ and incubated for a minimum of two hours with occasional stirring or mixing. This resulted in more constant and amplified bioluminescence observed during detection. The effect of ADase on spore germination and growth was also evaluated. A known amount of spores suspensions were filtered onto the membrane. The membranes were either washed with untreated or ADase treated sterile, distilled water. The membranes were placed on TSA and incubated at $35^\circ C$. The colony forming units on each membrane were compared. No significant different was observed (data not shown) (Figure 4).

Selection of bioluminescent reagent—three different bioluminescent reagents were evaluated: Millipore's Microstar Reagent (R), Millipore's Highly Sensitive Reagent (EQ) and Kikkoman's Checklite Luciferin-Luciferase HS reagent. These reagents were evaluated with both ATP solution and spore suspension. $0.5 \mu L$ ATP solution of increments ranging from $120-3.5 \text{ amol}$ were spotted onto the membranes, dried, and then detected with the three reagents. As shown in Figure 5, Millipore's reagent EQ is the most sensitive one, with the detection limit of 3.6 amol ATP . However, this reagent has high background which can not be eliminated by ADase treatment. Millipore's Microstar reagent is much less sensitive compared to the other two reagents. Kikkoman's Luciferin-Luciferase HS reagent gave the best signal. It is sensitive and the background signal can be eliminated by ADase treatment.

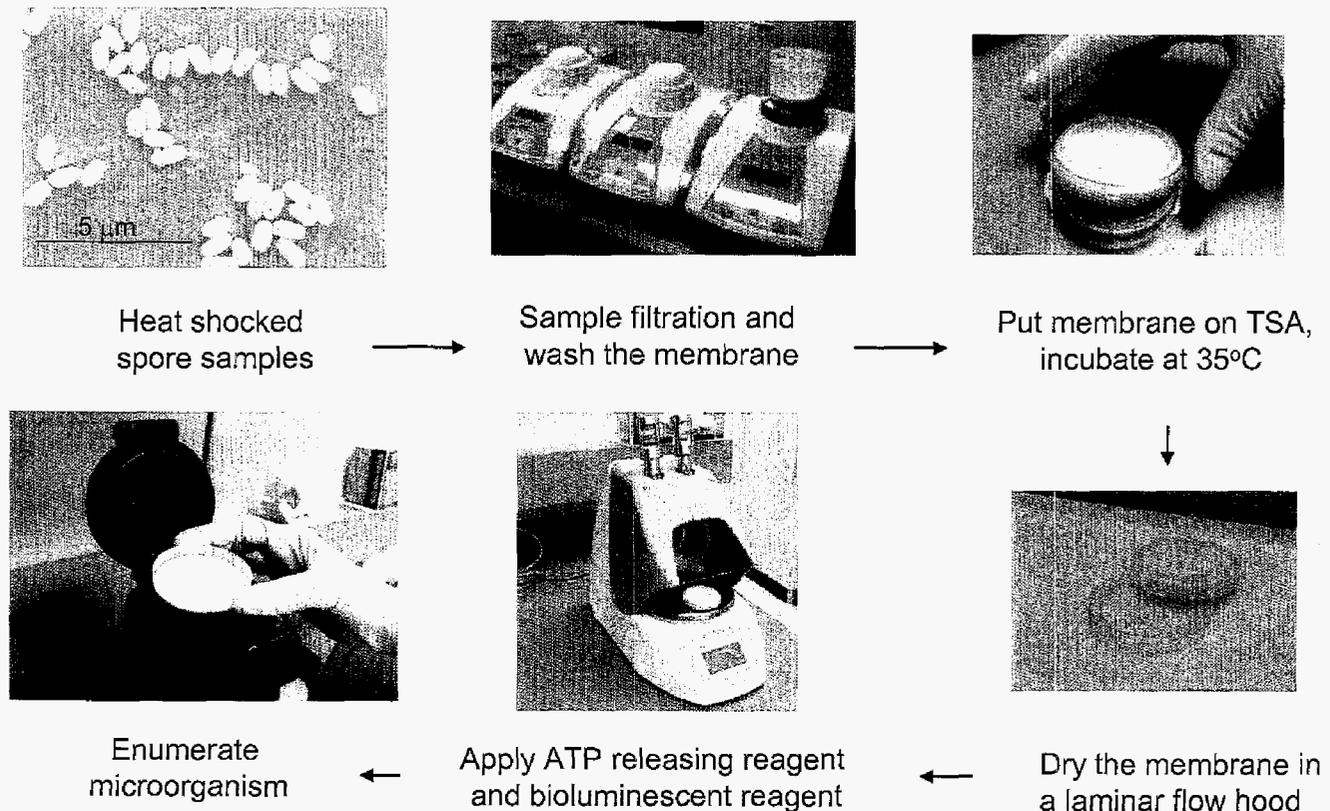


Figure 4. - Procedure for rapid spore enumeration.

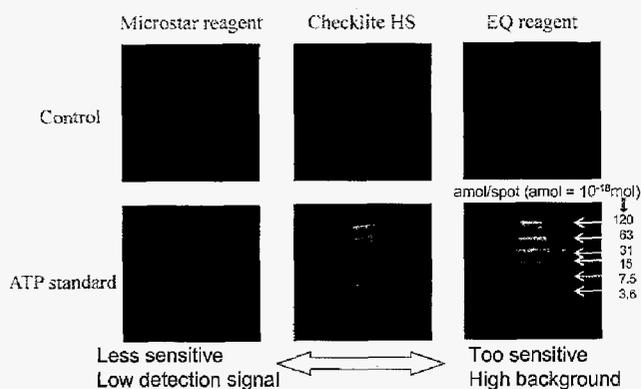


Figure 5. - Comparison of three ATP bioluminescence reagents using ATP solution. A serial dilution of ATP solution was spotted on membrane, and enumerated with three different reagents. Checklite HS has higher sensitivity yet low background.

The same reagents were used in the detection of spores to determine the optimum reagent to be used. *B. subtilis* (ATCC 9372) spores were filtered onto membranes, incubated on TSA, and then enumerated with three different bioluminescent reagents. Kikkoman's Luciferin-Luciferase HS reagent repeatedly demonstrated the optimum spore detection, with high sensitivity and low image background noise (Figure 6). Millipore's reagents gave poorer results in terms of both number of spores and signal background. They were either too dark and spores were almost invisible to detect at the five hour point, or they were too bright and sometimes merged into one detectable colony forming unit if they were close to each other. A high background noise level was also observed which resulted in interference and false-positives during interpretation of the images. Therefore, they were unsatisfactory in comparison to the other independent (Kikkoman) reagent. The HS reagent was selected for this spore assay.

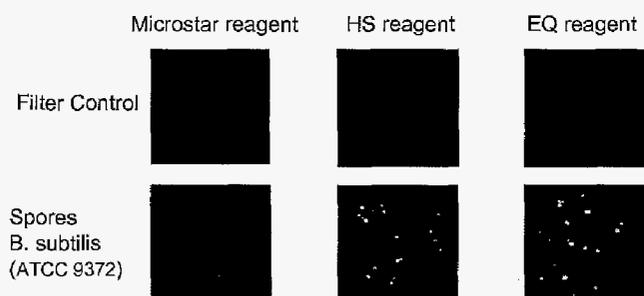


Figure 6. - Evaluated three ATP bioluminescent reagents using *Bacillus* spore (ATCC 9372). Checklite HS demonstrated optimum spore detection after only 5 hours of incubation on TSA.

Determine the optimal minimal incubation time—An optimum TSA incubation period was determined for the

best reproducible spore detection. *B. subtilis* (ATCC 9372) spores were filtered in sets onto membranes, then allowed to incubate on ADase treated TSA at 35°C for four or five hours. After incubation, the membranes were separated from the TSA, and then dried in the laminar flow hood. The dried membranes were processed with one of the three different bioluminescent reagents listed above. Average spore counts were determined from the membranes exposed to TSA for four or five hours, respectively. As a growth control, one set was allowed to incubate on TSA at 35°C for three days, and the spore counts were determined by colony forming units (CFU). The average spore count of the growth control was used as standard (100%). The average spore count at four or five hours with different reagents are listed in Table 1. Four hour incubation is not enough to give a comparable result as growth control even with the most sensitive reagent. Five hour incubation is sufficient to give comparable and reproducible spore count using HS reagent. Although EQ results are similar as the HS results, however, EQ reagent gives higher background, and can not be used for single spore detection.

Table 1. Spore counts on Microstar images at 4 hours and 5 hours with three different ATP reagents.

Reagent	Time	Spore Counts on Image			Average	SD	%
		1	2	3			
R	4h	1	1	3	1.67	1.15	6.49%
HS	4h	NA	13	13	13	0.00	50.84%
EQ	4h	19	17	14	16.67	2.82	64.93%
R	5h	9	11	10	10.00	1.00	38.96%
HS	5h	21	21	29	23.67	4.62	92.20%
EQ	5h	25	21	23	23.00	2.00	89.60%
TSA Growth Control	72h	20	26	31	25.67	5.51	100%

Evaluate the applicability of RMDS spore assay in the detection of environmental spore contamination

Evaluate the detection sensitivity for various strains of spores—Spores of seven species of *Bacilli* totaling nine strains (that have been repeatedly found in clean room environments) were used to evaluate the RMDS spore assay. The list of the strains is shown in Table 2. All strains were detected in five hours using HS reagent. The spore counts are reproducible and comparable with the growth control. Figure 7 shows the RMDS images of three representative strains.

Evaluate the background interference of environmental samples—two surface wipe samples were collected from clean room environment. Wipe sample #1 (W-1) was collected from a lab bench surface; while wipe sample #2 (W-2) was collected from a stainless steel surface. The procedures for sample collection and the release of microbes from the wipe are described above. Each sample was split in half. One half was autoclaved to kill all microbes before filtered onto the membrane.

Table 2. Bacillus species from clean rooms.

Species	Strains	Remarks
<i>B. subtilis</i>	168	Genome sequenced
<i>B. subtilis</i>	42HS1	Spacecraft isolate
<i>B. cereus</i>	ATCC11778	Type strain
<i>B. pumilus</i>	FO36b	Clean-room isolate
<i>B. odysseyi</i>	34HS1	Spacecraft isolate
<i>B. megaterium</i>	ATCC14581	Type Strain
<i>B. mojavensis</i>	KL154	Clean-room isolate
<i>B. megaterium</i>	KL197	Clean-room isolate

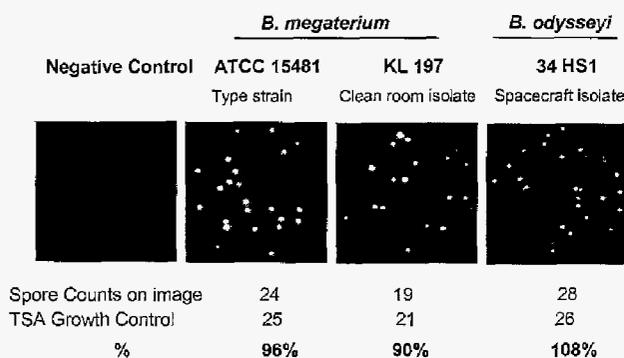


Figure 7. - Microstar images of three representative strains of spores. All gave comparable results as the growth control after incubated 5 hours on ADase treated TSA.

After applying the reagents, images were collected to determine the background noise associated with the environmental sample as well as any background signal from dead microbes. As shown in Figure 8, no background signal was detected using HS reagent.

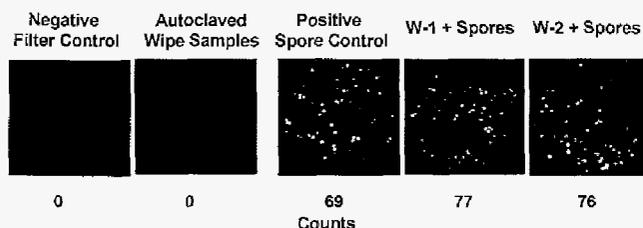


Figure 8. - Microstar images of three representative strains of spores. All gave comparable results as the growth control after incubated 5 hours on ADase treated TSA.

The other half of the samples were heat-shocked at 80°C for 15 minutes, to kill non-spores, then spiked with a known amount of spores. The spiked samples were filtered onto membrane, incubated on TSA for five hours. After apply the reagents, images were collected and compared with a positive control (a sterile distilled water sample spiked with same amount of spores). Both the intensity of the spots and

the number of spore counts did not decrease in the wipe samples (Figure 8). These results indicate that there are no environmental inhibitors in the wipe samples that interfere with this assay. The RMDS spore assay is suitable for evaluating spore contamination in clean room environments and may be used for NASA planetary protection applications.

4. CONCLUSION

Previously, the Millipore MicroStar Rapid Micro Detection system (RMDS) is used as a method to quantify microbial contamination with the detection and enumeration of viable microorganisms [4], and not for spore detection. This study evaluated the Millipore system extensively to determine whether it can be used for spore enumeration, whether it can be compatible with the NASA sampling method, and whether it can be recommended for use by NASA. It was demonstrated that by hybridizing the Kikkoman HS chemistry with the Millipore system, it was possible to enhance the sensitivity of the system, thus leading to a more rapid detection of bacterial endospores. It also established that it performed successfully on a small selected set of spore forming strains isolated from spacecraft assembly facilities by virtue of the fact that spores were detected, thereby allowing it to be used specifically for spore detection. This study demonstrated that the Millipore system is compatible with the NASA sampling method. Furthermore, the study demonstrated that it was possible to achieve reproducible spore enumeration in only 5 hours as compared to the 72 hours required by the NASA standard method [3]. Because of this study, the Mars Program has embarked on a validation study to critically evaluate this method for possible use as an accepted alternative to the NASA standard method.

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would also like to acknowledge the efforts of Jack Barengoltz for technical discussions and creative guidance.

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7. BIOGRAPHY



Fei Chen received a B.S. in Biochemistry from Beijing University in China and a Ph.D. in Pharmaceutical Sciences from University of Southern California. She was a senior research fellow at California Institute of Technology before she joined the Biotechnology and Planetary Protection Group at the Jet Propulsion Laboratory. Her current research is focused on developing and evaluating advanced technologies for cleaning and detection of trace levels of bio-signature molecules and spores on spacecraft materials. These technologies will help to protect life detection research of future mission. Her research interests also include applying cutting-edge molecular techniques to study microbial diversity in spacecraft assembly facilities, and studying the survival and reproduction of microbes under Martian conditions.



Gayane A. Kazarians received a B.S. degree in Microbiology from the University of California at Los Angeles in 1984. Following graduation, Gayane has been a member of two Jet Propulsion Laboratory investigator teams that participated in

radiology spaceflight experiments using *C. elegans* as a biological dosimeter. Gayane was involved in the development of a NASA sponsored Radiobiology facility at Loma Linda University's Chan Shun Pavilion, Loma Linda, California. In addition, Gayane has actively been busy with the evaluation of biochemical methods used in the assessment of microbial burden of spacecraft surfaces for planetary protection purposes during spacecraft assembly, test, and launch operations at JPL.

evaluation of methods for the killing and removing of microbiological contamination from spacecraft surfaces. An integral part of such study is the development and/or adaptation of methods for the detection of microorganisms and bio-signature molecules such as the one described here.



Kasthuri Venkateswaran's 25 years of research encompass marine, food, and environmental microbiology. Dr. Venkateswaran has applied his research to better understand the ecological aspects of microbes, while conducting field studies in several extreme environments such as deep sea, spacecraft assembly facility clean rooms as well as the space environment in Earth orbit (International Space Station). Of particular interest are microbe-environment interactions with emphasis on the environmental limits in which organisms can live. The results are used to model microbe-environment interactions with respect to microbial detection, and the technologies to rapidly monitor them without cultivation. The bioinformatics databases generated by Dr. Venkateswaran are extremely useful in the development of biosensors. Further, these models or information in database are extrapolated to what is known about spacecraft surfaces and enclosed habitats in an attempt to determine forward contamination as well as develop countermeasures (develop cleaning and sterilization technologies) to control problematic microbial species. Specifically, his research into the analysis of clean room environments using state-of-the art molecular analysis coupled with nucleic acid and protein-based microarray, will allow accurate interpretation of data and implementation of planetary protection policies of present missions, helping to set standards for future life-detection missions.



Roger G. Kern, Ph. D. in Microbiology from U.C. at Davis, is a researcher with specific expertise in the molecular biology, biochemistry and genetics of microorganisms. He is a member of JPL's Biotechnology and Planetary Protection Group. Since arriving at JPL in 1988, he has been task manager on DARPA, DOE sponsored tasks focused on chemoautotrophic growth as it relates to polysaccharide production, and served as molecular biologist associated with two recent flight radiation biology experiments on board STS—42 and 76. Dr. Kern has authored more than 15 papers in bacterial genetics and biochemistry. The primary focus of his research in recent years has been on has been the study of technical issues related to the prevention of forward contamination by spacecraft bound for the surface of Mars. This has included the study of the diversity of microorganisms associated with spacecraft and their assembly environment as well as the