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Biological contamination studies of lunar landing sites: implications for future planetary protection and life detection on the Moon and Mars

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Abstract: Chemical and microbiological studies of the impact of terrestrial contamination of the lunar surface during the *Apollo* missions could provide valuable data to help refine future Mars surface exploration plans and planetary protection requirements for a human mission to Mars. NASA and ESA have outlined new visions for solar system exploration that will include a series of lunar robotic missions to prepare for and support a human return to the Moon, and future human exploration of Mars and other destinations. Under the Committee on Space Research's (COSPAR's) current planetary protection policy for the Moon, no decontamination procedures are required for outbound lunar spacecraft. Nonetheless, future *in situ* investigations of a variety of locations on the Moon by highly sensitive instruments designed to search for biologically derived organic compounds would help assess the contamination of the Moon by lunar spacecraft and *Apollo* astronauts. These studies could also provide valuable 'ground truth' data for Mars sample return missions and help define planetary protection requirements for future Mars bound spacecraft carrying life detection experiments.

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The Committee on Space Research (COSPAR) of the International Council for Science (ICSU) was established in 1958 to promote international level scientific research in space. One of the continuing tasks of COSPAR has been to address planetary protection issues related to the Moon, Mars and other planetary bodies. The current COSPAR planetary protection policy states that space exploration should be conducted so as to avoid forward biological contamination of planetary bodies by outbound spacecraft that could jeopardize the search for extraterrestrial life. In addition, the Earth and its biosphere must be protected from potentially harmful organisms that could be present in materials or samples returned from extraterrestrial bodies (DeVincenzi & Stabekis 1983; Rummel *et al*. 2002). The COSPAR policy is viewed as an international consensus standard for compliance with Article IX of the United Nations Outer Space Treaty of 1967, requiring that space exploration should avoid harmful contamination of the Moon and other celestial bodies (United Nations 1967). Given the lack of knowledge of the Moon at that time, the successful crash of the Soviet *Luna 2* probe on September 14, 1959, which had not been heat sterilized, raised concerns within COSPAR about the forward contamination of the Moon. The greatest concern was that terrestrial bacteria on the spacecraft and equipment could cause irreversible changes in the environments of the Moon and interfere with scientific exploration. Although COSPAR acknowledged that the complete sterilization of a spacecraft was impossible, dry heat sterilization (115–200 °C) followed by ethylene oxide gas was determined to be the most efficient method for limiting the number of microbial spores on outbound spacecraft (Astafyeva *et al*. 1966; Murray *et al*. 1967). Beginning in 1961, NASA launched six lunar probes in its *Ranger* series designed to image the surface before crash-landing on the Moon. All of these probes failed and, among other problems, it was later determined that prolonged heat sterilization probably damaged some of the spacecraft electronics. Thus, NASA relaxed its use of dry heat sterilization on robotic lunar probes and later successfully completed the *Ranger 7*, *8* and *9* missions.

The human exploration of the Moon beginning with *Apollo 11* in 1969 left little doubt that, at least regionally, the lunar surface could be contaminated. *Apollo* crewmembers

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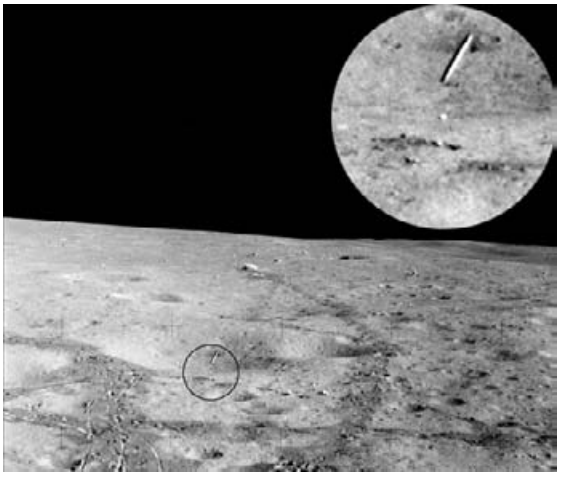

Fig. 1. Photo (AS14-66-9337) of the lunar surface taken during the *Apollo 14* mission. The enlarged region contains one of the golf balls hit by Alan Shepard; next to the golf ball is the Solar Wind Collection mast thrown as a javelin by Edgar Mitchell. It is unlikely that any organisms remain on the top of these objects due to intense UV exposure, but what about the bottom side? Are there any organic compounds present on the surface?

represented the primary source of organic contamination, although other sources existed as well. Most notable were the descent engine exhaust, Lunar Module (LM) depressurization, spacesuit materials and exhaust and leakage, human and food waste products and two golf balls (Fig. 1). To minimize the thrust required for lift-off from the lunar surface, all waste products were removed from the ascent stage and were stored in the equipment bays of the LM descent stage. To address planetary protection concerns, it was argued that even if the waste storage containers had leaked, microbial contamination would have been contained within the descent stage and not deposited on the lunar surface (Johnston *et al.* 1975). At that time the greatest focus on planetary protection was avoiding contamination of lunar samples with terrestrial micro-organisms during collection. Therefore, all tools and equipment used for sample collection were adequately sterilized by high-temperature bake-out under vacuum to remove volatile terrestrial contaminants from the hardware surfaces (Johnston *et al.* 1975). Based on the *Apollo* spacecraft bioburden at launch, the bioburden change in cislunar space and the survival of terrestrial organisms on the lunar surface, it was estimated that only 10^{-4}–10^{-5} viable micro-organisms per square metre of lunar surface were present at the times the *Apollo* samples were collected (Dillon *et al.* 1973).

The current planetary protection policy for the Moon related to forward contamination is not at all stringent (Category I, see Table 1) and the probability that terrestrial life can grow in the harsh environment on the lunar surface is very low. Even survival on the lunar surface is difficult to imagine with the Moon's nearly non-existent atmosphere, intense ultraviolet (UV), galactic and solar cosmic radiation, lack of liquid water and large temperature extremes. Nonetheless, it is likely to be the temperature extremes and the UV radiation that are the most significant. Experiments carried out on NASA's Long Duration Exposure Facility (LDEF) suggest that even after six years in space, a large fraction of spore forming bacteria will survive if they are not directly exposed to solar UV radiation (Horneck *et al.* 1994). These results certainly suggest that bacteria can be delivered to the surface of the Moon by robotic spacecraft. Based on a recent study, typical bioburdens of up to $\sim 10^6$ spores per square metre on uncleaned, unsterilized spacecraft surfaces have been measured (Venkateswaran *et al.* 2001). Although bacterial growth on the Moon remains unlikely, survival of terrestrial bacteria on non-UV exposed regions, such as the interiors of lunar spacecraft, the permanently shadowed south polar region of the Moon or below the surface cannot be ruled out. For example, terrestrial bacteria on the unsterilized *Lunar Prospector* orbiter that was deliberately crashed into a crater near the lunar South Pole may have survived impact and could remain viable in this permanently shadowed region.

One suggestion that bacteria might survive on the Moon came when the crew of *Apollo 12* returned to the Earth with selected components from the unmanned *Surveyor III* probe, including the television camera that had spent over two years on the lunar surface. Scientists working at the Lunar Receiving Laboratory (LRL) claimed to have isolated a colony of viable *Streptococcus mitis* bacteria from a sample of foam collected inside the camera housing (Mitchell & Ellis 1972). However, all of the other camera components, including an internal section of the electrical cabling, did not contain viable terrestrial bacteria (Knittel *et al.* 1972), nor was *S. mitis* found in the test camera that never went to the Moon. Meanwhile, it has been suggested that there is photographic evidence that these bacteria did not survive on the Moon, but instead were isolated due to laboratory contamination of the foam during analysis in the LRL (Rummel 2004). Nevertheless, the *Surveyor III* bacteria controversy illustrates the potential confusion associated with terrestrial biological contamination that can lead to false positive detection of life. Future microbiological investigations of the *Apollo* site materials that have remained on the Moon for over 30 years could help resolve the *Surveyor III* issue.

It also should be emphasized that even if bacteria delivered by lunar spacecraft are inactivated or sterilized on the Moon, due to the harsh surface conditions, organic compounds from dead cells will remain and could leave biomarkers in lunar samples returned to Earth. A 'typical' terrestrial microorganism such as an *E. coli* cell weighs approximately 10^{-13} g (dry weight) and is comprised of a complex mixture of organic compounds including protein (57%), nucleic acids (24%), lipids (9%) and other material (Neidhardt *et al.* 1990). It should be noted that, although dry heat sterilization kills most bacterial cells, their organic compounds will remain behind. Cleaning with a variety of organic solvents and degassing is also required to minimize the organic load of the spacecraft and sample path hardware. The lunar soil sampling equipment was cleaned to a non-volatile organic

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Table 1. *Current planetary protection requirements, including the Moon and Mars*

	Mission category					
	I or II	III	IVa	IVb	IVc	V
Mission type	Flyby, orbiter or lander	No direct contact: flyby, orbiter	Lander: no life detection instruments	Lander: life detection instruments	Lander: special region[a]	Earth return
Target bodies	e.g., Moon (I), comets/asteroids (II)	Mars	Mars	Mars	Mars	Mars (restricted) Moon (unrestricted)
Example past or *proposed missions*	NEAR (II), Lunar Prospector (I); *Rosetta* (*II*)	Mariner, MGS, Mars Odyssey, Mars Express	Pathfinder, MER, Beagle2 (IVa+)	Viking, *Mars Sample Return* (*MSR*)	*MSL, Phoenix, ExoMars, Next Decade Astrobiology Mission*	*MSR, Lunar South Pole Aitken-Basin Mission*
PP sterilization requirements	None or simple documentation	Cleanroom assembly, some bioload reduction	Microbial reduction	Sterilization of sample path hardware or contact parts	Partial or full sterilization required	Cat IVb for Mars bound craft, collection tools sterilized, no Mars cross-contamination; no restrictions for lunar spacecraft
Initial spacecraft bioload	Unsterilized $\sim 10^6$ spores m^{-2} 50–300 ng cm^{-2}	$<10^6$ spores m^{-2}	Pre-sterilization levels maximum: 300,000 spores/SC and 300 spores m^{-2}	Post-sterilization levels: 4-log bioload reduction[b]	Post-sterilization levels: 4-log bioload reduction[b]	Restricted Earth return same as Cat IVb; not controlled for lunar missions
Organic contamination levels	Not controlled, category II requires organic inventory	Not controlled, requires organic inventory	Not controlled, requires organic inventory	Not controlled, requires organic inventory; for Viking soils: <1–10 ppb[c]	Not controlled, requires organic inventory	Not controlled, for *Apollo* soils, up to 100 ppb

[a] Region where terrestrial organisms are likely to grow or has a high potential for existence of extant life forms.
[b] Original sterilization process designed for a 6-log reduction of *Bacillus subtilis var niger*, however it is only credited with a 4-log bioload reduction due to the the survival of more resistant bacteria.
[c] Based on Viking GCMS detection limits (Biemann *et al.* 1977).
SC, spacecraft; IVa+, additional chemical contamination control required for instruments.

level of 1 ng cm^{-2} (Johnston *et al.* 1975; Table 1) at the White Sands Test Facility (WSTF) in New Mexico. Based on the average dry cell weight for a single *E. coli* cell of $\sim 3 \times 10^{-13}$ g, at the 1 ng cm^{-2} level we calculate an organic load of the sampling hardware equivalent to $\sim 3 \times 10^{5}$ *E. coli* cells m^{-2}. Estimates of the total organic contamination to lunar samples from the *Apollo 11* and *12* missions based on spacecraft cleanliness was in the 0.1 to 100 part per billion (ppb) range (Flory & Simoneit 1972). It is important to emphasize that these levels were as low or lower than experimental blanks obtained in organic geochemistry research laboratories at that time. *Apollo* soil samples returned to the Earth were immediately analysed for bacterial and organic contaminants in the LRL. Although no viable organisms were detected in the *Apollo 11* and *12* samples (Holland & Simmons 1973), extensive amino acid analyses of lunar soils returned during the *Apollo 11*, *12*, *14*, *15* and *17* missions have been carried out, and indicate that terrestrial contaminants are present at concentrations up to 70 ppb in some samples (Hare *et al.* 1970; Harada *et al.* 1971; Brinton & Bada 1996). However, since these lunar samples were not analysed for organic compounds on the surface of the Moon, it remains unclear how much if any of the amino acid contamination in the lunar soils occurred during collection.

As of January 2004, NASA is planning to send a series of robotic orbiters, landers and rovers to the Moon, beginning in 2008, to prepare for future manned lunar missions by 2020 (Bush 2004). ESA, as part of its Aurora exploration program, is also planning similar lunar missions in the same time frame (Bonnet & Swings 2004). For these missions, *in situ* measurements that target key organic biomarkers in lunar soil samples as well as on spacecraft surfaces could be performed using highly sensitive instruments on landers and rovers, in order to determine the extent of terrestrial forward organic contamination providing a unique opportunity to evaluate planetary protection requirements for future life detection missions. 'Ground truth' experiments on the Moon would also be particularly useful for assessing the degree of organic contamination in lunar soil samples prior to their return to Earth, as well as the stability of organic compounds in sun-exposed and shadowed regions on the surface of the Moon. Furthermore, *in situ* experiments carried out at previous lunar landing sites such as *Apollo* could provide important information regarding the extent that extravehicular activities by the *Apollo* astronauts contaminated the Moon during lunar surface operations – including egress and ingress, deployment of instruments, sub-surface drilling and driving the Lunar Roving Vehicle[1]. At present it is not known whether or not past human contamination of the Moon is detectable in localized regions or limited to the *Apollo* landing sites themselves. Although the lunar surface environment may represent a worst-case scenario for the survival of micro-organisms and even terrestrial organic matter, lunar exploration provides a unique opportunity to use the Moon as a test-bed for future Mars exploration, where the search for evidence of life has become a primary objective.

The search for evidence of martian life requires robotic spacecraft with *in situ* life detection instruments and/or sample return capabilities. According to recommendations made by the US National Research Council's Space Studies Board, it is imperative that any Mars bound spacecraft carrying life detection instruments be sufficiently clean so that the integrity of the samples analysed is not drawn into question by terrestrial organic contamination (NRC 1992). The sensitivities of these techniques will be the major drivers for the sterilization and cleaning requirements required for future Mars-bound spacecraft. NASA's concern about the forward contamination of Mars and potential interference with biology detection experiments was evident by the extremely stringent sterilization requirements for the *Viking* missions to Mars in 1976. It was estimated that prior to terminal heat sterilization each *Viking* Lander Capsule (VLC) contained a total surface contamination of $\sim 300\,000$ aerobic spores or $\leqslant 300$ spores per square metre (NASA 1975), which in 1994 was set as the allowable bioload level for Planetary Protection Category IVa missions (missions without life detection instruments; see Table 1). Total bioloads for the 1996 Mars Pathfinder and 2003 Mars Exploration Rover and Beagle2 missions were also found to be within the allowable levels for Cat IVa missions (Barengoltz 1997; Newlin *et al.* 2004; Spry *et al.* 2004).

It is important to point out that these 'total bioburden' counts are likely to have underestimated the actual bioload of the landers, since only culturable spore-forming bacteria would have been detected with the swab-and-culture/heat-shock assay used to assess the spacecraft bioburden. Culturable, non-spore-forming bacteria as well as other non-culturable species present on spacecraft surfaces are both missed using this technique. Although it is now known that less than 1% of viable environmental species are culturable (Colwell and Grimes 2000), there is an apparent lack of data on the percentage of the actual spacecraft bioload that are non-culturable species. Direct counting methods using DNA-specific fluorochromes (Kepner and Pratt 1994) could be used to quantify the total number of both culturable and non-culturable bacteria on spacecraft surfaces. After assembly of the *Viking* spacecraft, the VLCs were then subjected to a terminal dry heat sterilization cycle that led to all portions of the spacecraft reaching at least 111.7 °C for 30 h which was credited with a 4-log reduction of the initial bioload to the level now required for category IVb missions (NASA 1990). The pre-launch bioload of the Viking spacecraft would have been reduced even further on Mars due to the biocidal effects of UV irradiation on sun-exposed surfaces (Schuerger *et al.* 2003). Nonetheless, even after the significant bioload reduction accomplished for *Viking*, non-volatile organic compounds (e.g., amino acids and nucleobases) derived from both culturable and non-culturable

[1] We acknowledge that it may be desirable to designate some of these sites as historical landmarks that should be preserved for future astro-archeologists.

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species would not have been destroyed during dry heat sterilization.

The two *Viking* gas chromatograph mass spectrometer (GCMS) instruments on the two landers were both successfully operated on the surface of Mars, but did not detect any organic compounds in martian fines above a few ppb (Biemann *et al*. 1977). The GCMS instruments did, however, detect trace levels of cleaning solvents, indicating that the rigorous *Viking* cleaning protocols were sufficient for the sensitivity of this analysis. The presence of a powerful oxidant in the martian regolith may have destroyed organic molecules in materials analysed by the *Viking* instruments (Klein 1979; Zent & McKay 1994). It is possible, however, that some organic compounds may have been present below the detection limit of the GCMS instruments. In particular, the *Viking* GCMS instruments were not optimized for the detection of several classes of organic molecules relevant to life, such as amino acids, nucleo-bases and carboxylic acid salts (e.g., Benner *et al*. 2000). These compounds would not have been identified by *Viking*, since they are best detected by higher-temperature GCMS techniques or after chemical derivatization to produce a species that is sufficiently volatile to elute through a GC column (Mahaffy *et al*. 2004). Based on a previous report it was estimated that there would have to be at least 10^5 micro-organisms in the samples analysed by *Viking* (corresponding to 5 parts per million in weight) in order for the GCMS to detect their pyrolysis degradation products (Anderson *et al*. 1972). A more recent study has also confirmed this estimate (Glavin *et al*. 2001). Therefore, even if one assumes as a worst-case scenario that all of the dead terrestrial spores brought by the *Viking* spacecraft ended up in the martian soil, it is unlikely that their organic compounds would have been detected by the GCMS instruments. Upcoming strategies for Mars exploration will require that *in situ* life detection instruments target a broader range of organic compounds in order to adequately assess whether any organic compounds, especially those that might be associated with life, are present in the martian regolith.

Along with the development of highly sensitive *in situ* instrumentation, future missions to Mars will require that all landers and rovers with biology or biomarker detection instruments be sufficiently sterilized and cleaned to levels potentially beyond *Viking* requirements to insure that the search for evidence of life on Mars is not compromised by false positive detections. The present state-of-the-art instrumentation for the analysis of non-volatile organic compounds that target key biomarkers have detection limits in the sub-ppb range. At this level, several thousand microbes per gram of martian soil should be detectable by these instruments (Glavin *et al*. 2001). A 2003 report by NASA's Organic Contamination Science Steering Group (OCSSG) concluded that a definitive search for the organic signatures of extinct or extant life on Mars could be carried out by maintaining terrestrial contamination levels below 1–10 ppb for relevant biomarkers (Mahaffy *et al*. 2003). Keeping terrestrial organic contamination at this level will require that future Mars astrobiology missions be cleaned to at least Viking post-sterilization levels, and it is likely that even more stringent sterilization protocols will be required for sample path hardware. In this case, science requirements will override any planetary protection requirements associated with concerns about the growth of Earth organisms on Mars (as was the case with *Viking*). Since traditional swab-and-culture techniques that assess the spore bioload on spacecraft surfaces do not take into account organic material from dead cells or unculturable species, highly sensitive *in situ* instrumentation currently being developed to search for organic compounds on Mars should also be used to test the spacecraft cleaning and sterilization procedures to be used on these missions.

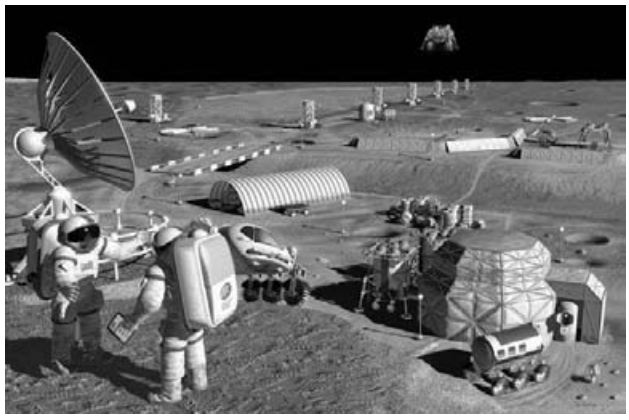

Fig. 2. Human and robotic activity at a future lunar (or martian) base provides ample opportunity to introduce biological and organic contamination. Painting by Pat Rawlings. (Courtesy NASA)

The use of sensitive robotic experiments to detect contamination that may still be present nearly 40 years after humans first explored the surface of the Moon may be critical to help establish a contamination baseline, but there are broader contamination challenges regarding a more sustained human presence on both the Moon and Mars. Such considerations should be kept in mind as we prepare for sustained human exploration (McKay & Davis 1989; Lupisella 1999). Human exploration could, in fact, confound the search for life on Mars, since the presence of humans will dramatically increase the amount of terrestrial organic material, potentially making the detection of indigenous organic matter exceedingly difficult, if not impossible. If we are concerned about human contamination unduly compromising the search for organic material and life, several interrelated questions arise: How much robotic exploration will be required before establishing a sustained human presence on the Moon and Mars? What are the criteria for robotically assessing the biological status of a location, region or entire body? How well will we be able to control contamination once humans are present? How might contamination be distributed as a result of a sustained human presence?

Future robotic and human missions to the Moon could provide a unique opportunity to carry out ground-truth

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experiments using *in situ* life detection instruments to help understand the extent of forward contamination by robotic spacecraft and human presence over a limited range of conditions and time (Fig. 2). Ultimately, these experiments will help guide future planetary protection requirements and implementation procedures for robotic and human missions to Mars. Using the Moon as a test-bed could also yield important information necessary for future long-term exploration of extraterrestrial environments. Nowhere else are there so many samples of environmental and construction materials that have been continuously exposed to space, while facing different conditions for different durations. These artifacts could provide a valuable insight into the structural stability and integrity of a variety of materials that could be used on future space vehicles, or for future lunar or martian outposts.

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