RESOLVING THE ISSUE OF EXTANT LIFE ON MARS

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ABSTRACT

A method is proposed to resolve the long-running issue of life on Mars. Based on the legacy of the Viking Mission Labeled Release (LR) experiment, the method exploits the sensitivity of ^{14}C respirometry. In 1976, the LR obtained positive responses at Viking 1 and 2 sites on Mars, but, for a variety of posited reasons, the consensus favored chemical or physical agents in the Martian surface material, not life. Since Viking, no life detection experiment has been sent to Mars. New information about the Martian environment and the discoveries of extremeophiles on Earth have re-energized interest in the possibility of living microorganisms on the red planet. The new method, the "Chiral LR" (CLR), combines the LR with the chiral specificities displayed by living organisms. The isomers of ^{14}C-labeled chiral substrates are separately injected onto soil samples, which are then monitored for the evolution of gas. Small self-sustaining probes are launched from the spacecraft in a manner protecting the original sterility of the probes. A strong preference for one chiral isomer over its sister in an on-going reaction with the soil is evidence of life. The new feature herein presented adds multiple controls to augment that supplied by a non-responding chiral isomer. The added controls increase the reliability of the overall result to the point where an abiotic agent is difficult to propose. The results may solve the life-on-Mars issue, and may, also, usher in a new era of comparative biology.

Key Words: Chiral Labeled Release experiment, Life on Mars, Viking LR, astrobiology, chirality of life, radiorespirometry

1. INTRODUCTION

The finding of extraterrestrial life is the Holy Grail of astrobiology. Discovery of life, even microbial life, on Mars or any other celestial object would answer humanity's age-old, plaintive question, "Are we alone?" Minimally, we would learn that life does exist beyond us. A positive result would raise the question of whether life began on Earth, on the other body on which it was detected, or on some third, still unknown object. Depending on how the question was asked and the reply, it might be determined whether we are related to the newly discovered life, or whether it originated independently. The most intriguing result would be if the life detected were of independent origin. This finding would strongly indicate that life is broadly distributed throughout the cosmos. This would herald the dawn of a new era in mankind's search for the origin.

2. BACKGROUND

A high priority for NASA has always been the search for extraterrestrial life. In describing the then-upcoming 1976 Viking Mission to Mars, NASA stated that the detection of life on Mars would be "perhaps the most important experiment in the history of science." However, despite this implicit urgency, the only direct experiments seeking extraterrestrial life remain those that were aboard the dual landers of Viking (Klein et al., 1976)[1]. The Viking life detection experiments were based on these assumptions: 1. if any life existed, there would be microorganisms (they seem necessary for the recycling of any more complex organisms that might or might not be present, 2. the life would resemble terrestrial life to the extent of being carbon-based (carbon bonds to more atomic and molecular species than any other element, including science-fiction's favorite alien life component, silicon); 3. the biochemical reactions would be aqueous (water being the ultimate solvent, and so commonly available).

One of the Viking life detection experiments, the Labeled Release experiment ("LR") (Levin and Straat, 1976)[2], was competitively selected for the Mars Mission in 1969, based on ten years' development and demonstration of the experiment's scientific rationale. Originally called "Gulliver," NASA re-named the experiment the "Labeled Release Experiment (LR)" to better describe its function. The LR dosed Martian soil with the ^{14}C-organic compounds shown in Table 1, all of the "Miller-Urey" type thought to have existed on primitive Earth, and, likely, Mars.

TABLE 1

VIKING LABELED RELEASE SUBSTRATES

Labeled Substrate	Structure and Label Position (*)	Concentration ($\times 10^{-4}$M)	μCi mL^{-1}	Specific Activity (Ci/Mole)
^{14}C-glycine	NH_2*CH_2*COOH	2.5	4	16
^{14}C-DL-alanine	*CH_3*CH (NH_2)*COOH	5.0	12	48
^{14}C-sodium formate	H*COONa	2.5	2	8
^{14}C-DL-sodium lactate	*CH_3*CHOH*COONa	5.0	12	48
^{14}C-calcium glycolate	(*CH_2OH*COO)$_2$Ca	2.5	4	16

The headspace over the dosed soil was then monitored for the evolution of ^{14}C-labeled gas as evidence of metabolism, and, hence, the presence of extant life. In the event of a "positive" response, a control experiment was to be conducted to determine whether the positive had been generated by a non-biological entity in the soil, not life. The control consisted of repeating the LR experiment with a duplicate sample of the soil. However the sample was first heated under a regimen thought destructive of microorganisms, but not of chemicals likely to cause a false positive. This NASA-devised control was part of all Viking life detection experiments. If the result of the control experiment was nil, or of greatly reduced amplitude compared to the initial result, this was viewed as confirmation of the presence of living organisms in the original sample. Conversely, a positive control would indicate that the "positive" was caused by a non-biological agent.

During one of the field tests of the LR instrument, a single drop of the labeled nutrient was placed directly on the ground. The air above it was trapped and monitored over time for the evolution of radioactive gas. Surprisingly, gas evolved immediately, with no lag phase as experienced with larger volumes of nutrient. Figure 1 compares this "moist" method to the "wet" standard method. Thereafter, the LR was designed as a moist experiment.

The LR approach is unique among life detection systems in that it is not based on static chemical or physical properties in the sample, but on the detection of on-going metabolism. The method is extremely sensitive, so that growth, which fails to register in more than 99% of soil microorganisms cultured by standard methods, is not required.

In the course of the development of the LR, thousands of laboratory and field tests were performed in compiling a library of responses for comparison with possible Martian results. The LR was shown to be effective in the rapid detection of a very wide range of microorganisms. Laboratory tests included pure and mixed cultures of aerobic, anaerobic and facultative bacteria, including autrophs and heterotrophs; chemotrophs; phototrophs; algae; fungi; lichen; halophiles;

and sulfur bacteria. Wild cultures were obtained from a variety of sources; and soils from extreme environments, including many supplied by NASA, were tested. No culture or sample that obtained a response from a standard method assay failed to respond in the LR test. A number of LR tests succeeded where standard methods failed. The negative LR controls verified the biological nature of the initial responses.

When laboratory tests were run with chemicals added to some soils, a chemical response occurred. This happened only in several cases in the field where the pHs of the soils were below 3. In every case, the added or indigenous chemicals were revealed by their surviving the heat-treated control. Thus, the very strong case for reliability of the LR was established. After a decade of instrument development, the LR was successfully incorporated into the biology package of Viking, which also contained the Gas Exchange (GEx) (Oyama et al., 1978)[3] and the Pyrolytic Release (PR) (Horowitz et al., 1977)[4] life detection instruments. The Viking sampling arm obtained the soil samples which were distributed to all three life detection instruments.

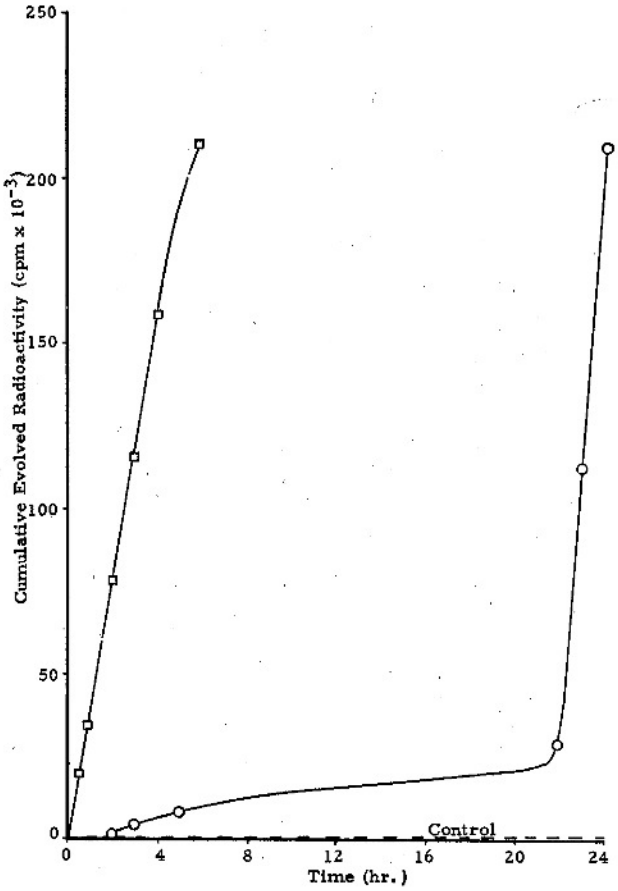

Fig. 1. "Moist" vs. "Wet" Mode. The rapidly-responding moist mode consisted of 0.5g Wyaconda (Md.) soil containing 10^7 organisms/g, 0.2ml nutrient including ^{14}C-formate, ^{14}C-lactate, ^{14}C-glucose, ^{14}C-glycine, and $^{35}SO_4$ (total activity: 20 μCi/ml). The slowly-rising wet mode consisted of 0.3g of the Wyaconda (Md.) soil, 10ml nutrient including ^{14}C-formate, ^{14}C-lactate, ^{14}C-glucose, ^{14}C-glycine, and $^{35}SO_4$ (total activity: 2 μCi/ml). The control utilized the anti-metabolite. Incubation temperature = 25°C.

3. VIKING ON MARS

Both Viking 1 and Viking 2 successfully made their 400 million mile journeys. The LR instruments operated flawlessly on Mars. Seen in Figures 1 and 2, both Viking landing sites, some 4,000 miles apart, produced strong responses and virtually nil 160°C controls. The results (Levin and Straat)[5] met the pre-mission criteria for the detection of life by the LR. In a further effort to distinguish between biological and non-biological agents, additional, more defining controls were executed by commands from Earth. It was shown that the causative agent was inactivated by temperatures as low as 51°C, and when previously active soils were sequestered at 10°C for sometime before approximately three months. Finally, to test the possibility raised that the LR reaction had been caused by soil energized by exposure to the virtually unattenuated ultra violet light reaching the surface of Mars, another experiment was improvised. At dawn, a rock was pushed aside by the Viking sampling arm, and a sample taken from where the rock had protected the soil from ultraviolet light over geological time. The LR test was run on the sample. The results were positive, with the response being very similar to those of the other active runs.

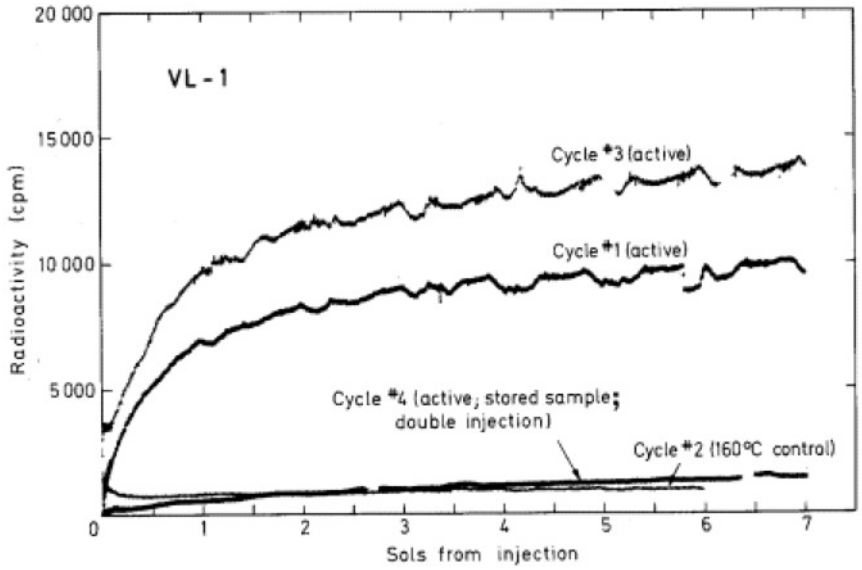

Fig. 2. LR results at VL-1. A fresh sample was used for of cycles 1 and 3; sample for cycle 4 was stored 141 Sols at 10-26°C prior to use. In cycle 2, a stored portion of the cycle 1 sample was heated for 3 h at 160°C prior to nutrient injection.

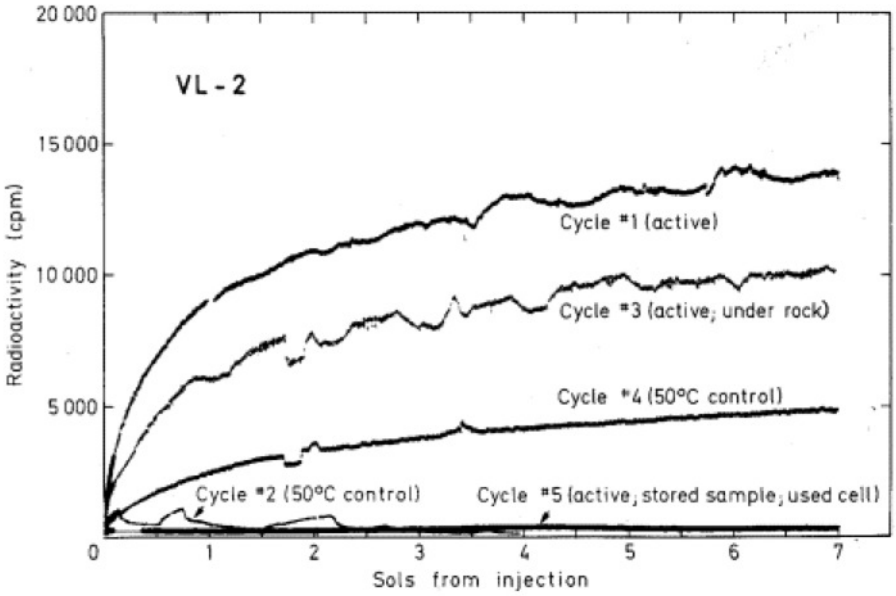

Fig. 3. LR results at VL-2. Fresh samples used for each cycle, except cycle 5 sample stored 84 sols at 7°C. The sample used in cycle 3 was obtained from under a rock. Attempted as 50^{o}C controls, cycles 2 and 4 were actually heated to 51^{o} and 46^{o}, respectively. Sample volumes were 0.5 cc, except cycle 5, which contained 2.2 cc, including old sample.

In additional *ad hoc* experiments, second injections of nutrient were added to active samples at the end of their seven-sol runs. On Earth, those additional doses usually produced new rises in gas production as the static metabolism was re-generated. On Mars, however, second injections produced temporary declines in the gas levels already present. A search of the library of terrestrial soil responses revealed that NASA-bonded Antarctic soil 664 had reacted to its second injection as had the Martian soils. It seemed possible that, by the seventh day/sol of the test, the microorganisms had died, and the decline in gas level was caused by re-adsorption of the evolved gas into the dampened soil.

Overall, the amplitudes and kinetics of the Mars LR results were similar to those of terrestrial results, especially close to those of soils in, or from, frigid areas. The instruments' performance and data were unquestioned, but the consensus was formed that the LR had not detected life on Mars, but had detected a chemical or physical agent that had produced false positive results.

The crucial factor in the formation of this consensus was another experiment aboard Viking. This was the Molecular Analysis experiment ("GCMS"), named for its component gas chromatograph and mass-spectrometer). It was designed to identify the organic compounds commonly expected to be on Mars. It had assumed that the surface of Mars contains organic matter in that Mars had been bombarded with organic-containing meteorites as had early Earth, and because like Earth, Mars continues to receive large quantities of interplanetary dust particles containing organic compounds. In addition, in the development of the Viking PR life detection experiment, it was found (Hubbard et al., 1973)[6] that organic matter was photosynthesized "in significant quantities" from simulated Martian atmosphere exposed to simulated Martian sunlight. Surprisingly, the GCMS (Biemann et al., 1977)[7] detected no organic compound

whatever in the same soils tested by the LR. Thus, the results of both the LR and the GCMS experiments were contrary to what had been anticipated by the most of the involved scientists.

4. NON-BIOLOGICAL THEORIES AND EXPERIMENTS

Many theories have been proposed and numerous experiments performed attempting to account for the Mars LR results abiologically. A review of the Viking LR evidence in light of the LR's strong performance on terrestrial soils, new data on the Martian environment, and the fact that no abiotic experiment had been reported to duplicate the Mars LR test and control results, led the writer, in 1997 (Levin, 1997)[8], to conclude that life had been detected. The discovery of perchlorates on Mars by the Phoenix Mission's TEGA does not contravene this conclusion. While perchlorates could produce an evolution of gas from the LR medium, they would easily survive the heating regimen of the LR control.

Over the more recent years, new data from Mars, including the report (Renno et al., 2009)[9] of current liquid water on the surface, the surprising findings of extremophile microorganisms on Earth, in our most Mars-like environments, the failed attempts to reproduce the Mars LR results abiologically, and doubts reported (Glavin et al., 2002)[10] about the sensitivity of the Viking GCMS to small amounts of organic matter have revived interest in the LR experiment. The Chiral LR (CLR) experiment is designed to test the 1997 conclusion.

5. THE CHIRAL LABELED RELEASE EXPERIMENT

The CLR experiment has been proposed (Levin et al., 2002)[11] as a means that can provide an unambiguous distinction between non-biological and biological agents. The key determinant is its ability to detect chiral preferences in the utilization of enantiomers of racemic substrates applied to the sample. Strong preference for L-amino acids and D-carbohydrates is a peculiar, still unexplained, property of all known living systems. On the other hand, such preferences have not been reported as naturally occurring in chemical reactions. Recent work in the laboratory has been reported (Castro-Puyana , 2008; Noorduin et al., 2009)[12,13] to achieve up to 10% enrichment of an L-amino acid. This is done by exposing a particular facet of a crystal mineral to a racemic mixture of the acid, and then eluting that crystal facet. The amino acid remaining on the eluted facet is slightly enriched, generally about one percent, in one of the isomers. More recent research (Viedma et al., 2008)[14] has significantly increased this enrichment, with the thought expressed that it might even lead to self-amplification. However, even if such abiotic selections occur, their presence in a compound would not produce a false positive in the CLR. A continuing reaction showing a strong preference for one enantiomer of a racemic mixture would constitute a clear distinction between living and non-living agents. This conclusion would be further confirmed by the demonstration that a duplicate sample tested after having been heated to life-destroying, but chemical-sparing temperatures produced a nil, or greatly reduced, reaction when tested in the CLR.

Many biologists believe that phototrophs evolved as the first form of life. Thus, they propose that phototrophs, but not necessarily heterotrophs, must exist on any planet harboring life. Based on experiments, as shown in Figure 4, it has become possible to incorporate the detection of phototrophs into the array of microorganisms targeted by the CLR.

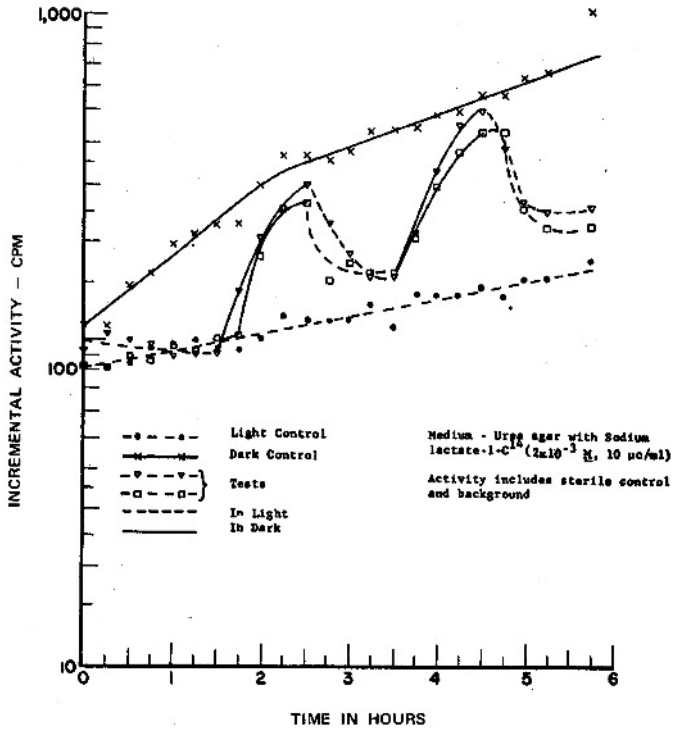

Fig. 4. Detection of Photosynthesis of Metabolically Derived $^{14}CO_2$ from Chlorella Pyrenoidosa. A white LED was incorporated into the CLR with means to pipe the light to the soil sample. Should any microorganisms be present in the sample and evolve labeled gas, turning the light on and off at two-hour intervals would produce a correlating modulation in the signal amplitude.

The CLR instrument (Levin et al., 2007)[15] consists of individual, virtually self-sustained probes of the smallest dimensions and weight practicable for deployment on Mars. The result sought is a miniature, low-weight, low-energy-requiring instrument that could be used in multiples. They could constitute an entire package for a small mission, be an integral part or be piggy-backed onto a larger planetary lander, or be deployed from an orbiter. The probes would be packaged in a canister that would be heat-sterilized before launch, thereby avoiding the necessity to sterilize the entire spacecraft. On deployment, the probes would be ejected through the canister cover, thus maintaining their sterility.

The aim is for a design permitting 10 or more CLRs and support equipment to fit within the weight and space limitations of small scale missions, such as Scouts, perhaps constituting the entire science package. The deployment of multiple units provides two types of redundancy. Should one unit fail to land safely, its duplicate would be available. Alternatively, another of the units with different substrates could obtain the definitive result sought. The units are thus independent and mutually supportive**.** As presently conceived the individual CLR probe is depicted in Figure 5.

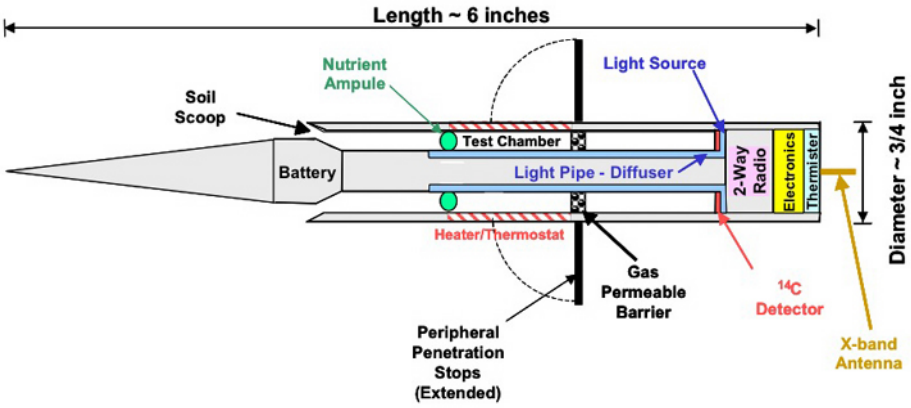

Fig. 5. Chiral LR individual probe. Depending on loading, probes can serve as test and/or control units.

CLRs would be launched in the morning to take advantage of the rising temperature. Using its integral battery, the temperature of the CLR is raised to 10°C just prior to launch. This ensures liquidity of the nutrient so that it can moisten the soil. A weather vane platform allows for launches to be upwind, away from possible terrestrial contamination of the landing site. If desired, the vane platform could be rotated to effect launch in a desired direction should an attractive site be seen. The CLR is propelled by a squib. The launch angle allows for a horizontal distance of approximately 100 feet. The CLR's aerodynamics land it nose first, with the penetration stops preventing it from going too far into the ground. The impact of landing drives soil into the two sample chambers, breaking the ampoules and allowing the substrate solutions to moisten the soil samples. The soil samples are then held at 10°C, the temperature, at which the Viking LR samples were maintained (for possible comparative purposes), for five sols. Any labeled gas arising from the soil sample passes through a permeable barrier installed for the purpose of preventing the beta detectors from seeing the liquid and counting its radioactivity, and also preventing dust or aerosols from carrying radioactive material to the detector. As in the case with the Viking LR, the radioactive gas in each CLR's headspace is measured every four minutes for the first four hours, to obtain early kinetics, and every 16 minutes thereafter. A preference in the utilization of one stereoisomer over its mirror image would be strong evidence for life, very likely accepted by a consensus of astrobiologists.

In the herein reported new iteration of the CLR, an important innovation is added. It is the use of multiple controls. In the past version, each pair of the chiral substrates was viewed as its own control, relying on the general acceptance that very strong evidence for extant biology would be indicated by a preferential response to only one of the mirror-image molecules. However, an alien life might not exhibit a chiral preference. In this case, the CLR would register a false negative, unwittingly missing its paradigm-shattering quest. Hence, the Viking heat "sterilization" is now included as a second control, but applied in step-wise heatings to determine the thermal endpoint of the active agent. In addition, the imposition of other environmental conditions would serve as important controls. These could include moisture, humidity,

atmospheric composition and the like. Further controls consist of anti-metabolites added to the samples. While these are more Earth-centric, a variety of them could help confirm the test and other control results. A reaction to only one of them could supply strong additional evidence. Toxic metals, cyanide, antibiotics and enzyme inhibitors are control candidates, as is the de-coupling agent 2,4-dinitrophenol. Pre-mission studies would establish priorities for the test and control probes.

The new CLR, thus, inverts the normal concept of an experiment. Multiple controls are applied to each test. A positive result combined with more than one nil control would be difficult to explain away abiotically. Availability of instrument weight would determine the final package. A chiral preference supported by multiple controls could indicate not only that extant life had been found, but whether life were related to terrestrial life or were of independent origin. Results from the various tests and controls could begin a study of comparative biology with terrestrial forms.

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