A CHIRAL LABELED RELEASE INSTRUMENT FOR IN SITU DETECTION OF EXTANT LIFE. A. D. Anbar[1] and G. V. Levin[2], [1]School of Earth & Space Exploration, Arizona State University, Tempe, AZ (anbar@asu.edu), [2]Beyond Center, School of Liberal Arts and Sciences, Arizona State University, Tempe, AZ (gilbert.levin@asu.edu).

Introduction: Recent findings challenge the consensus interpretation of the chemistry and biological status of the Martian near-surface environment that has guided the community since Viking. That interpretation posits that the Martian soil contains an abiotic oxidizing agent that can account for (a) the absence of detectable organic compounds and (b) the positive response of the Viking Labeled Release (LR) life detection experiment. The erosion of this consensus provides a compelling motivation to revisit the possibility of extant biology in the Martian near-surface. This motivation will increase if the Mars Science Laboratory (MSL) detects organic compounds in Martian soil.

To resolve whether the LR response was biotic or abiotic, we propose a focused and flexible instrument concept and design, based and improving upon the LR heritage. This instrument could be mated to a range of mission concepts and lander architectures, a key consideration given the budgetary environment facing the NASA Mars exploration program. The proposed instrument addresses key issues in "Challenge Area 1 (Instrumentation and Investigation Approaches)" of the Mars program reformulation effort.

Crumbling Consensus: The only direct attempts to detect life on Mars to date were carried out by the Viking Landers. The Labeled Release (LR) experiment [1], a major component of the Viking science package, worked flawlessly on both landers [2]. Below we summarize the LR results and consensus interpretation, and the recent work that throws them into question.

The Viking LR. In this experiment, ^{14}C-labeled amino acids and carbohydrates in aqueous solution were applied to Martian soil samples. Oxidation of these substrates by metabolic processes, such as respiration or fermentation, was assessed by monitoring for the evolution of radioactive ^{14}C-labeled gas in the overlying space with a β-detector. The evolution of such gas would be suggestive of metabolism and, hence, the presence of extant life. Any such evidence was tested by pre-heating a duplicate sample of the soil to destroy living organisms, but not chemicals which might have produced the reaction.

On both Viking landers, positive responses were obtained. The controls established that the active agent detected in the Martian soil was destroyed at 160°C; was greatly impaired at 46°C; essentially destroyed at 51°C; and fully depleted after storage in the dark at approximately 10°C for 3-4 months. These results were consistent with microbial metabolism [3]. However, "false positives" arising from abiotic oxidation were deemed the more plausible hypothesis by most researchers [4]. This conclusion was reached for two reasons.

First, the Viking Molecular Analysis Instrument (a thermal volatilization gas-chromatograph mass-spectrometer – GC-MS) detected no indigenous organic matter in the Martian soil [5], suggesting that the Martian soil harbored a strong, unidentified oxidant.

Second, many researchers found the results of the LR control experiments unpersuasive in differentiating biotic from abiotic signatures. The complex and contentious history of the interpretation of the LR experiment has been discussed in detail elsewhere (e.g., [6], [7]).

Viking revisited. The GC-MS findings must be interpreted with care for at least two reasons, one of which only emerged recently.

First, as long recognized, the GC-MS experiment was many orders of magnitude less sensitive than the LR; the LR was capable of detecting the activity of as few as 30 bacteria, even in lag phase, whereas the GC-MS required organic carbon equivalent to $>10^6$ microorganisms [8].

Second, some recent studies suggest that the Viking GC-MS experiment underestimated the organic content of the tested soils and may have even detected organics indirectly ([9] and references therein). The basis of these studies is the detection by the Phoenix Lander of substantial quantities of perchlorate in Martian soil [10]. Although not an oxidant at ambient Martian temperatures, perchlorate will promote combustion of organic compounds when heated to 500 °C. Such heating prior to analysis was part of the protocol of the Viking GC-MS experiments and may have compromised the detection of organics by both the Phoenix TEGA and Viking GC-MS. Chlorohydrocarbons detected by the Viking GC-MS may have been byproducts of such reactions, in which case they are evidence of ppm-levels of organics in soil at the Viking sites.

With the possibility of significant organic content in Martian soils revived, and soon to be tested by MSL, the motivation for future life-detection experiments at Mars is renewed.

The Chiral LR Instrument: The use of chiral substrates would greatly enhance the ability of an LR

experiment to differentiate biotic from abiotic responses, as explained below.

Chiral metabolism. One of the deepest characteristics of all known life is homochirality, a property by which organisms show selective preference for only one of two forms of a chiral compound. Thus, left-handed ("L") amino acids and right-handed ("D") sugars dominate known life. Metabolic redox reactions exhibit this effect [11], as has been demonstrated in Mars analog environments on Earth ([12] and references therein).

In contrast, no known non-biological redox reactions distinguish between chiral isomers. While chiral selectivity has been seen in some syntheses reactions (e.g., [13]), it arises from selective adsorption to minerals, is compound-specific, and the enrichments are relatively small. Enantiomeric excesses are seen in some amino acids – and inferred in aldehyde precursors – in some meteorites (e.g., [14]). These excesses reflect interstellar chemistry and have little bearing on life detection experiments.

Thus, the observation of chiral-selective oxidation (or reduction) would be a strong marker of biotic, rather than abiotic, chemistry. To be sure, this preference is not absolute. Some organisms produce racemases and isomerases, which are enzymes enabling them to metabolize D-amino acids and L-sugars. However, because the onset of the consumption of the "wrong" enantiomer displays a lag due to the need to express racemases, the CLR can even differentiate true negatives from false negatives. Additional controls could also be used to discriminate false from true negatives, as described below.

The "TWEEL" CLR. The use of chirality in the search for extraterrestrial life was incorporated into the earliest versions of the LR [15]. Although chiral isomers were included in the Viking LR, they were not separated, so it was impossible to distinguish which, if any, gave rise to the positive response. This issue is addressed in the "Twin Wireless Experiment for Extraterrestrial Life" (TWEEL; Fig. 1).

We envision that a CLR-bearing mission would include a number of individual TWEELs, each consisting of a small penetrometer that contains a β-counter, nutrient ampoules, and associated hardware. A key design goal is to scale to the smallest dimensions and weight practical so that an array of multiple units could be deployed on a single mission.

In our baseline concept, each TWEEL includes two test chambers. The nutrient ampule in each chamber would be either the D- or L- version of the same single substrate. We propose to use ^{14}C-labeled enantiomers of the chiral Viking LR substrates (alanine and lactate) and will investigate the possible use of glucose or other sugars despite the challenges of protecting these substrates from degredation during pre-flight heat sterilization.

Launched from a lander or rover, or from orbit, each TWEEL falls to the Martian surface nose-first, scooping up soil in its twin sample chambers through the impact of landing. The substrate ampoules are smashed by the incoming soil, and the substrate and soil mixed to initiate the test. Any gas evolved enters the counting chamber after passing through a gas-permeable barrier, which keeps out any radioactive dust and aerosol. The amount of ^{14}C-labeled gas evolved is monitored continuously and cumulatively.

Each of the chiral substrates is its own control in that extant biology would be strongly indicated by a preferential, continuing, metabolic-type response to only one of the mirror-image molecules. False positives are therefore highly unlikely. However, to test for false negatives, one of the chambers on some TWEELs could be replaced by an independent control, using an ampoule that is not broken by the incoming soil, but instead is remotely broken on command after heat sterilization of the soil in that chamber.

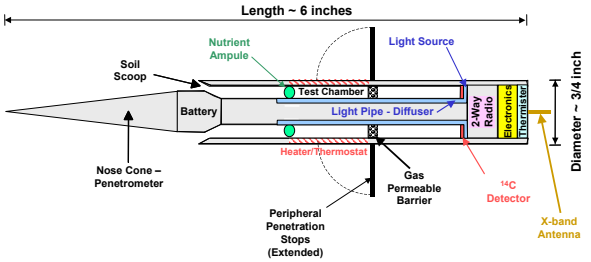

Fig. 1. Twin Wireless Experiment for Extraterrestrial Life

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