THE LADDER OF LIFE DETECTION. M. Neveu1,2, L. E. Hays3,4, M. A. Voytek2, M. D. Schulte2, and M. H. New2. 1NASA Postdoctoral Management Program Fellow. 2NASA Headquarters, Washington, DC 20546, USA. 3Jet Propulsion Laboratory, Pasadena, CA 91109, USA. 4Center for Life Detection, NASA Ames Research Center, Moffett Field, CA 94035, USA. (marc.f.neveu@nasa.gov).

Introduction: Life detection includes all measurements seeking to find life, including searching for biosignatures (features that evidence ongoing or past biological processes) and establishing context (properties and processes inherent to the sample’s current and past settings). The Viking experiments remain the only direct, in situ attempt at microbial life detection beyond Earth [1]. Since, our knowledge has much improved, in part due to searches for and studies of life in extremes of time [2-7], space [8,9], and environmental conditions [10-12].

Life-detection missions are currently a top priority at Mars [13], ocean worlds [14], and on exoplanets [15,16]. To guide these searches, it is crucial that we apply lessons learned from past efforts to detect life. To do so and help establish standards across different fields of science, we have assembled a Ladder of Life Detection (available at https://astrobiology.nasa.gov/research/life-detection/ladder [17]). This tool, meant to be challenged and upgraded by the community, lists features of life and assesses their potential for providing conclusive evidence for life through the criteria listed below.

Criteria for life detection:
• Sensitivity includes all aspects of instrumental performance (e.g. selectivity, response time, dynamic range [18]). The instrument must detect the feature of life within set performance thresholds, to avoid instrumental false negatives. Positive controls test for this criterion.
• Discrimination from contamination interfering with the measurement, to avoid instrumental false positives. Contamination signals are measured on negative controls (abiotic samples). Indigenous signals must also be distinguished from contamination by hardware or other samples. This is assessed with blanks.
• Replicability: Three replicate measurements per sample are the burden of proof in microbiology and chemistry. As many samples as needed to capture the heterogeneity of the setting should be analyzed.
• Detectability: Physical, chemical, or geological conditions in the sample’s current environment must not prevent the measurement to be made. Environmental interference can include reaction of organics with oxidants upon heating [1] or ionization suppression by salts. It can be remedied by sample preparation (e.g. derivatization). This criterion and the next both aim to avoid false negatives.
• Survivability. The feature of life must not have been destroyed (e.g. by photolysis or racemization) in the environments encountered by the sample between its synthesis and its measurement. This can be assessed by comparing the residence time of the feature with its degradation timescale [19]. This depends on factors such as burial, oxidation state, availability of fluids, temperature, etc.
• Reliability is the propensity for the feature to be produced by life and distinguished from abiotic backgrounds from any of the environments encountered between feature synthesis and measurement. This criterion aims to avoid false positives.
• Compatibility. The feature must be consistent with what is known of life. This criterion assesses the feature’s genericity vs. specificity to life on Earth. As an upper bound on genericity, we suggest life based on carbon-based organic molecules (this includes e.g. proposed “arseno-DNA” [20]).
• Last-resort hypothesis [21]: The measurement (alone or part of a set) must preclude an abiotic origin to a given statistical significance. Astrobiologists have informally adopted this criterion as a standard [9].

Features of life: The Ladder details the propensity for each feature to meet the above criteria. We qualitatively combine this into a subjective “likelihood” that the feature will be diagnostic of life. Features below are loosely ranked from highest to lowest likelihood. Again, we encourage the community to refine this ordering.
• Darwinian evolution is an essential feature of life as we know it, but takes place over many generations of organisms as conditions change. There is no universally accepted means to detect or measure Darwinian evolution (no “Darwinometer” instrument). It is not practical to detect in unexplored natural environments in the time frame of relevant investigations [22].
• Growth and reproduction, despite being central traits of life, are not unique to it [23]. They are evidenced by morphologies of concurrent life stages or a reproductive form, such as cell or cell-like structures in multiple stages [24], including motility. This assumes a search for life compartmented in cells.
• **Metabolism:** Organisms derive energy from their environment and convert it to forms used for growth, reproduction, or repair. Conversion is not fully efficient, so metabolism also results in waste. Both conversion and waste intermediates or products can evidence ongoing biological activity, as their abundance can deviate from thermodynamic equilibrium or kinetically limited abiotic steady states. Such deviations include elemental or isotopic fractionations, co-location of oxidants and reductants, or a response to substrate addition. These alone need not be due only to biological activity.

• **Functional molecules** include polymers that support information storage or transfer (e.g., DNA, RNA) and functions such as catalysis (proteins), specific structural preferences in organic molecules (e.g., homochirality, repeating charge), and pigments (detectable by remote sensing). These “suspicous biomaterials” can be mimicked by abiotic processes [25] or prone to contamination, but they can be sensitively measured with existing techniques.

• **Potential biomolecule components** comprise complex organic compounds such as small oligomers (e.g., peptides or nucleic acids), PAHs, and simpler single amino acids and lipids (including degradation products of biomolecules, such as hopanes [24]). They are less reliably attributed to biology than the above functional molecules (e.g., [26]), but are more likely to be detected.

• **Potential metabolic byproducts** are distributions of species that differ from those resulting solely from abiotic thermodynamic equilibrium or kinetic steady state. They include distributions of elements present at trace levels in biological matter, but which can carry essential metabolic functions at the active sites of enzymes [27], and patterns of complexity in mixtures of organics [28].

• **Biofabrics:** Microbial communities can affect the morphology of their environment, creating laminations (as in stromatolites), mounds, or microbially induced sedimentary structures [29]. The interpretation of both biofabrics and cell-like features is considerably strengthened by elemental, isotopic, or mineralogical mapping of the morphologies [30].

**Applications:** Interpreting measurements is the crux of life detection. Sets of measurements meeting the first seven criteria have been made several times [1-3,8,11], but only rarely [11] has the biological interpretation been widely accepted. The Ladder can be used to help formalize interpretation by identifying sets of measurements that together statistically significantly diagnose a biological vs. abiotic origin [31]. We will present an example decision framework [17] based on a dozen rules that cover all possible sets of measurement outcomes. An example rule is “homochirality+morphologies => unlikely abiotic => likely biological”, so any set with additional features detected, such as “homochirality+morphologies+complex organics”, would also point to life. Simple as it is, this framework is an accurate lens through which to examine past attempts at detecting life [17]. It also suggests that ongoing efforts in instrumentation, mission formulation, contamination, and planetary protection could indeed enable the detection of life, if present.

Beyond measurement interpretations and payload design, we anticipate that the Ladder will stimulate discussion and spur progress in elaborating strategies for life detection. This will undoubtedly lead to changes to the Ladder itself as our understanding of life and technological abilities improves.

**References:**

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