DIAGNOSING LIFE

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ABSTRACT

A sure definition of life is a necessary but often overlooked step to finding life, particularly in an astrobiological context. We attempt to define life using a series of diagnostic criteria that establish a confidence level that a given system, species, or detected phenomenon is alive. These criteria have been laid out and defined in a codebook, which is then applied to eight possible life detection events in the history of astrobiology as case studies. This allows us to determine the confidence level that life was detected in those events. We discuss improvements and changes that can be made in future versions of these criteria. Finally, we also discuss the fact that our confidence levels are necessarily low for most of our case studies and how this indicates the need for further study and a minimum level of evidence to confirm the findings of life in an astrobiological context. The appendix includes the codebook itself, as well as a key terms section to hopefully reduce semantic confusion and user bias in the use of our criteria.

KEY POINTS

- Life as we know it does not follow a singular format or definition.
- We try to diagnose life using a DSM-style codebook.
- It may not be possible to diagnose something as living, but we can determine a confidence interval for its possibility of life.

1. INTRODUCTION

As it currently stands, we do not possess a clear means of defining what constitutes as alive. Many methods in the past have attempted to identify specific criteria that living systems possess, with the most popular being that life is ordered, capable of reproducing, growing, utilizing energy, maintaining homeostasis, responding to stimuli, and adapting. These categories have time and time again run into issues with objectively non-living systems possessing these established characteristics to some vague capacity (e.g., fire, crystals, stars), or for lifeforms that are clearly alive to fail in some of these categories (e.g., infertile organisms).

This codebook takes a different approach by turning life detection into a confidence measurement rather than a simple “yes or no”, and provides more clear lines that properly define what each criterion means and what evidence constitutes that a potentially living system in question possesses these characteristics, and to what capacity. In terms of the primary criteria living systems possess, we have identified a total of 8, that being life is detectably counter-entropic, possesses some degree of information storage, metabolism, and spontaneous teleology, as well as has the capacity to perpetuate, age/die, utilize its environment, and create. These primary categories are then further broken down into secondary and tertiary subsections, which are used to determine how well an observed system performs and to what capacity it shows signs of possessing certain characteristics we have identified to be ubiquitous to living systems.

2. METHODS

To diagnose something as living, we introduce the idea of a diagnostic codebook, following the same format one may expect to find in the Diagnostic and Statistical Manual of Mental Disorders. Within this codebook, attached in Appendix A, there are a total of 8 subsections and 62 individual codes. Each of these sections breaks down a specific criterion we have deemed necessary for life, with subsections further breaking down each requirement. Each requirement has its own specific grading material and threshold for life, with all being on a scale from 0 to 1, where 0 is no confidence that a species is alive and 1 being 100% confidence that a species is alive. If no information is available, a zero is assumed. The overall score is then the average of the scores for each criterion - though it is important to explain the scores for each criterion independently to provide additional context.

Within this paper, the terms species, system, and phenomenon can be used to describe almost anything, from a rock to any type of organism, and anything in between, and are used interchangeably. This avoids connotation with things that may already be living, but it helps organize by lumping together things with the same chemical building blocks and genetic material, and it helps with categorization. Each species can individually be evaluated against the codes of our codebook, where each individual or species can obtain a score to determine the confidence that that species or individual is alive. Using this system, a species can still be alive even with a final score of 0.1; however, there would be little confidence in this measurement.

Each of the following sections describes a specific section of the codebook, what its section means, how a species is graded, and why this has been chosen as a requirement for life. The complete codebook is attached as Appendix A and remains a working and living document, much like the actual DSM, which receives periodic updates. As more research is done, data is collected, and more species are found, more diagnostic material and codes can be added or removed to improve our confidence scores and allow them to reflect new data.

As for the coding structure of the categories and how secondary and tertiary characteristics are weighed relative to each other, a total of 4 unique coding systems were developed for this codebook:

(1) The first system is used by the "Counter-Entropic, Metabolism, Perpetuation, Aging/Death and Spontaneous Teleology" criteria. This code works by dividing the processes of these criteria into secondary categories, each weighted by the respective number of tertiary codes assigned to them, and each tertiary element being assigned either yes or no, which correlates to a 1 or 0, respectively. If the system being observed shows signs of possessing the characteristic described by a tertiary element, then it gets a 1 for that element. The secondary code's score is based on how many of its tertiary elements scored a 1, divided by its total number of tertiary elements. The scores of the secondary categories are then multiplied together to get a total confidence score of whether the criteria being tested are present. This means that if the system in question fails to pass one or more of the secondary categories, it gets a total score of 0 across the board. This is to eliminate uncertainty related to fringe cases, significantly lowering the possibility of false positives, as well as reinforcing the idea that a true living system would

possess at least some aspects of all secondary categories within the primary criterion they fall under.

(2) The second coding system devised is used only by the “Information Storage” criteria. It differs from the structure used throughout the codebook to account for the diversity of mediums that could be utilized by systems. To account for this, tertiary coding records four responses for each binary question, one for each primary category. Within the tertiary categories, a 1 in any medium is recorded as a 1 in the overall categories. The secondary categories calculate an average from the respective tertiary categories. An average is again used to determine the primary evaluation from the secondary categories instead of multiplying them together.

(3) The third coding system is used by the “Utilizes Environment” criteria. It differs from the primary coding system that the majority of other criteria use due to the fact that it lacks tertiary components. Scores using this coding structure are determined only by a simple yes/no question within its secondary elements, correlating to a 1 or 0, respectively. The final score is calculated by totaling all secondary elements that scored a “yes” and dividing it by the total number of secondary elements.

(4) The fourth, and final coding system is used by the “Life Creates” criteria. Like the majority of the other criteria, secondary codes are also broken down further into tertiary codes. Each tertiary code can be answered yes/no, but rather than a “yes” correlating to a 1, it is instead correlated to a designated number of points ranging from 1 to 5, depending on how heavily that tertiary element is weighed relative to the rest. An answer of “no” still correlates to a 0. Each tertiary code can be answered independently, and it is possible for a system to meet multiple tertiary codes within a given secondary code. The final score for this criterion is the sum of all the tertiary code scores, divided by the score of a human (18), thereby giving a number between 0 and 1.

Once a score has been determined for all 8 categories, their values are averaged to get a final confidence score regarding how certain we are that the system being observed is alive.

2.1 DSM Style Codebook: Diagnostic criteria for Life

CODE A: Information Storage

In this model, we posit that a living system depends on information storage. Therefore, in determining whether a discrete system is potentially life, information storage mechanisms are an effective heuristic. In Earth organisms, a number of distinct information mechanisms are observed. Cells utilize the durable chemical method of Deoxyribonucleic Acid, as order rises, many multicellular eukaryotes adopt the use of nervous systems for storing and processing information. Additionally, many of these organisms diffuse information in networks such as social groups. By diffusing information through this network, organisms are able to outsource certain processing functions as well. Technological systems are an extension of this, in which humans externalize storage of information in tools such as writing and global social and digital networks. External processing here is taken to the currently known extreme of computer processing, which processes data too large for the network to parse.

To define and clarify these measures, we utilize four primary criteria, which are split and assigned a value from a set of binary questions in each of five secondary subcategories. The primary categories are Internal Chemical Storage, Internal Structural Storage, External Storage, and External Processing. These primary categories are defined to account for storage mediums in living systems and to permit for unknowns and variation. The secondary categories are measures of functionality, Distinguishable States, Stability, Readability, Writability, and Propagation. These allow our value measures of information storage to be more agnostic to storage systems, enabling their use across unrelated evolutionary trees. The tertiary subcategories are the binary questions used to generate the values for a sum analysis.

To elaborate on the binary information storage codebook, the primary code categories must first be observed and identified within the system. Then, for each secondary code category, distinguishable states are quantified as the number of distinct configurations the storage medium (the main category) can assume, based on how an individual can tell them apart. Stability is how long a system can maintain those states without external assistance, measured relative to the system's timescale. Readability is the ease with which a system’s states can be read or decoded, accounting for the specificity to the organism and error rate. Writability is the degree to which new information can be encoded into storage, as measured by speed, precision, and the agent performing the encoding. Propagation is the ease with which the storage medium can be replicated and passed on, accounting for fidelity and the number of copies. The tertiary code further breaks down these categories into defined groups to allow detailed analysis for each primary category.

The code works by assigning a 1 or 0 to each tertiary question, corresponding to the primary code at the top. Each tertiary code receives an aggregation score of 1 if at least one category scores 1. These aggregation scores are then summed and divided by the number of tertiary codes in the secondary code, resulting in the secondary score. These secondary scores are then summed and divided by the total number of secondary categories to yield the final confidence value.

CODE B: Life is Counter-Entropic

This model proposes that life depends on reactions that are counter-entropic. Entropy is the measure of a system's thermal energy per unit temperature that is unavailable for doing useful work [1]. This can also be quantified in terms of molecular "disorder", which can be quantitated through entropy change (dS), heat transfer (deltaQrev), and temperature (T):

$$\mathrm{d}S = \frac{\delta Q_{\mathsf{rev}}}{T} \quad (1)$$

Within a closed system, entropy always increases (or in less common cases, breaks even). The universe gravitates towards chaos, following the second law of thermodynamics:

$$\mathrm{d}S_{\mathsf{uni}} \geq 0. \quad (2)$$

Spontaneous reactions heavily favor the formulation of products by releasing overall free energy within the system [2]. On the other hand, a nonspontaneous reaction does not favor the formation of products under standard conditions. They are endothermic and require energy input to occur.

Gibbs free energy is the measure of energy change within a system. The equation combines enthalpy (H) and entropy (S) into a singular value, factoring in internal energy (U), pressure (P), volume (V), and temperature (T):

$$G = H - TS \quad (3)$$

$$G = U + PV - TS \quad (4)$$

Under constant temperature and pressure, the value of ΔG determines the spontaneity of a reaction. The sign determines the direction of a reaction, with $\Delta G < 0$ defining a reaction as exergonic, $\Delta G = 0$ defining a reaction at equilibrium, and $\Delta G > 0$ defining a reaction as endergonic. Exergonic reactions are spontaneous in the direction written, whereas endergonic reactions require an input of energy (usually in the form of heat) in order to occur. Higher-order systems tend to maintain a lower degree of entropy, balancing endergonic, unfavorable processes with exergonic, entropy-raising reactions.

As an example — within known, carbon-based, biological systems, cellular metabolism is heavily dependent on specific, replicable pathways; glycolysis and gluconeogenesis being two universally conserved reactions. They are the basis of glucose homeostasis, allowing an organism to either produce energy or conversely, synthesize energy storage [3]. Glycolysis is a predominantly exergonic energy conversion process that facilitates the catalysis of glucose into Adenosine Triphosphate (ATP) [4]. Free energy is released, resulting in an overall thermodynamically favorable reaction. Gluconeogenesis, on the other hand, is a largely endergonic energy conversion process (which occurs in the exact order that glycolysis does, but reversed) that facilitates the formation of glucose. Three key steps, which are largely spontaneous in glycolysis, now require an input of energy [5]. The input of reactions must result in an overall thermodynamically favorable pathway. Both reactions have spontaneous and nonspontaneous components, but lead to a decrease in entropy within the closed system of the organism.

The categories that we have chosen to represent this with are *having specific and replicable reactions*, and *containing coupled reactions in systems that are integrated within their environment*. Reactions within a biological system can be widely defined through two subcategories: exergonic and endergonic. Exergonic reactions are commonly seen in both abiotic and biotic settings due to their spontaneous nature, releasing energy in the form of heat and light through the breakage of bonds. Combustion reactions can be observed in abiotic conditions, where a source reacts with oxygen to produce energy [6]. Hydrolysis reactions can be observed in biotic conditions, with a prevalent case being the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). The high-energy phosphoanhydride bonds are broken in exchange for energy within many cellular processes, such as the active transport of molecules across cellular membranes [7]. Endergonic reactions, on the other hand, are much rarer abiotically than their exergonic counterparts. The formose reaction (also known as the Butlerov reaction), is the formation of sugars from formaldehyde. It is purely autocatalytic, possibly driven by light or a free radical process through an unknown mechanism [8]. While extremely uncommon in abiotic nature, it is ubiquitous in biological systems. Fatty acid (FA) synthesis, for example, is frequently observed in the excess of glucose. In just one of the many steps required, malonyl-ACP is linked together to form a 16-carbon intermediate (palmitoyl-CoA) [9]. Amphibolism can be described as a reaction consisting of both exergonic and endergonic traits, requiring a much higher level of complexity. This is extremely uncommon in abiotic environments, but can occur in select conditions. The reverse tricarboxylic acid cycle (rTCA) is a sequence of chemical reactions that produces carbon compounds through the use of energy-rich reducing agents. Though mostly observed in ancient bacteria and archaea, non-consecutive steps can be catalyzed by minerals and ions, making the entire process plausible under entirely abiotic situations [10]. Biological systems, especially ones of higher complexity, frequently use amphibolic reactions depending on their needs. Relating to the previous example, glycolysis can be interchanged with gluconeogenesis based on the organism's needs. Certain "irreversible" steps conducted during glycolysis are circumvented through different enzymes, lowering the activation energy enough to a point where the entire reaction is still thermodynamically favorable.

The coding system that we have chosen to represent our classifications divides our criteria into two secondary and subsequently, three tertiary categories; the tertiary category assigned a value of 1 or 0 corresponding to yes or no, respectively. If the system of interest exhibits the characteristics described within the subcategories, it will be assigned a value of 1. This is factored into the secondary category, delegating it a score out of three. The scores of the secondary categories are simply multiplied together to represent the section as a whole.

Specific and replicable reactions are defined as a specific reactionary pathway that occurs with the same mechanism on a consistent basis. Biochemical reactions are incredibly complex, but always require the same reactants, and produce the same products. The reaction mechanism is identical upon each repetition, consisting of the same intermediates. Though this is observed in both abiotic and biotic systems, they tend to occur in regular intervals in response to prompted environmental stimuli. This leads us to our final criterion, the coupled, overall low entropy reactions occur in systems that are integrated within their environment. Systems are sorted into two categories: open and closed. An open system allows energy to be transferred in between the system and its environment, while a closed system cannot facilitate any form of energy transfer into its surroundings [11]. Our criteria accounts for both, allowing for an interpretive perspective of the object. In the case of a closed system, the coupled reactions of interest must be entirely observed within the object. Though this may seem fairly arbitrary, biological systems are generally defined as open systems, which gives the sub-category of an open system a much higher weight. The open system of interest has a responsive relationship with its exterior surroundings, with the prompted reactions of interest exchanging energy and matter with the environment in order to lower the system's entropy. Ordered systems tend to maintain a lower entropy, and living organisms are highly ordered.

Across these examples, a consistent pattern emerges that extends beyond specific biochemical systems. The significance of these processes is not rooted in the individual reactions themselves but rather how they are sustained and organized overtime. While both biotic and abiotic systems can show exergonic or even sometimes coupled reactions, these are typically isolated, short-lived, or lack regulation. Living systems, on the other hand, integrate exergonic and endergonic processes into coordinated and continuous pathways that reliably maintain their internal order. This order allows for the persistent reduction of entropy in the system rather than incidental release. As a result, the defining characteristics of life in our system is not the presence of energy-releasing and/or consuming reactions but rather the sustained coupling, regulation and repeatability of these processes within a system that activity works to maintain its structure over time.

Taken together our criteria supports the idea that life is able to be understood fundamentally as a system that persistently resists entropy with organized, energy-dependent processes. While the second law of thermodynamics dictates that the total entropy must increase, living systems are able to distinguish themselves by locally decreasing entropy through the coupling of both non-spontaneous and spontaneous reactions, which results in an overall thermodynamically favorable pathway. This dynamic is not limited to carbon-based biology which is important in the search for life, but rather reflects a broader principle. Any system that can sustain ordered structure over time must do so by continuously exchanging its energy and matter with its surroundings, as well as relying on consistent and repeatable pathways that reliably produce structured outcomes. Some of these aspects can be observed in abiotic systems, like spontaneous energy-releasing reactions or rare instances of autocatalysis, the defining feature of life here is the sustained integration of the mechanisms into a cohesive system that maintain low internal entropy. Rather than defining life by its specific chemical makeup, our model emphasizes its thermodynamic behavior, which in turn, highlights life as a process that is detectably counter-entropic, and maintains order not in isolation but through continuous interaction and contribution to the increasing entropy of the universe.

CODE C: Metabolism

As demonstrated, life can arguably be described as a low entropy system within a higher entropy environment, and as per the second law of thermodynamics, the overall entropy of an isolated system will continue to increase over time; in an attempt to regulate and maintain the homeostasis of a living system, life will harness its environmental free energy via a process defined as *Metabolism*.

For this section of the code, we are using the first coding system discussed in our methods section and have divided the process of metabolism into 3 categories, each further divided into tertiary elements that can be identified within the system being observed by either yes or no, which correlates to a 1 or 0, respectively. The resulting scores of the 3 categories are then multiplied together to get a total confidence score of whether a metabolism comparable to those found in living systems here on Earth is present within the system being observed. If the system in question fails to pass one of the 3 categories, it gets a total score of 0 in order to reinforce the idea that the metabolism of a true living system would possess at least some aspects of all three categories.

As shown by the definition of Metabolism, the first category stipulates that ***life must capitalize on its environmental free energy***, whereby energy available to do work already existing in the external environment is concentrated within the living system for exploitation. A common example amongst Earth life would be photosynthesis/chemosynthesis, where environmental energy in either the form of light or redox potentials are utilized by the cell to perform carbon fixation, or in aerobic respiration where free energy is released via the coupling of redox potentials between species sourced from the environment. In order to further break down this coupling of environmental free energy, we have identified the following tertiary codes: *1) an observable generation of waste products* necessitated once again by the second law of thermodynamics. It can also be presumed that the mechanism, whatever it may be, which utilizes the environmental free energy will require some degree of *2) catabolism* and *3) anabolism*, storing and releasing energy in the form of bonds, as well as producing essential biomolecules.

In order for energy to be used to do work, the laws of thermodynamics state that energy must flow from high to low concentration. In order for life to utilize free energy, it must *4) generate a space of high energy concentration* and use its surrounding environment as the final energy sink in order to perform work. In essence, they must produce more free energy than their surrounding environment.

Fick's Law of diffusion states that species will naturally flow from areas of high to low concentration, and hence why 5) *active transport*, which is diffusion in the opposite direction described in Fick's Law, is listed as the last tertiary code, as it requires energy input to move species from an area of low to high concentration, a process almost exclusively observed in living systems. [12]

The second category encompasses system ***Regulation***, which is relevant to metabolism through: *1) Selection*, which we have defined as the consumption, production, and concentration of only a select few species relative to the number of possible species the environment can produce with the same building blocks. The second category accounts for the fact that a metabolic pathway will have a temporal reliance in that not all its processes will need to be functioning at a given time, and so it will maintain the ability to activate and deactivate said processes when necessary, defined by the term *2) Feedback*. Lastly, these processes will occur through a *3) Structure,* which entails a sustainable pathway that encompasses more than one phase/step, indicative to how life on earth utilizes reaction chains to regulate consumption/production of metabolites.

Finally, the last category addresses ***Stimuli*** which is necessarily enabled by Metabolism. Under stimuli fall two tertiary codes: *1) Preference,* which describes how a living system is likely to select for areas/circumstances where essential bio-species are prevalent and harnessing environmental free energy is easiest. To define what we mean by this a little further, it can also be described as a selection based on specialization. Living systems will exhibit some form of selection towards whatever environments they inhabit or what specific physical/chemical species they utilize for specific functions. If the system is removed from a space where these selections become less favorable (different environment or different species availability), but not to the point where there are signs of complete loss of function, and the system undergoes change to either facilitate regression back to favorable conditions or partake in alternative, more favorable pathways within the new environment which later cease, reverting back to the original, more favored pathways after being placed back into its original environment, then it is demonstrating metabolic preference. It also becomes necessary that a system can change how it interacts with its environment in response to changing environmental factors, described by *2) Response*.

Although a potential biological system may not necessarily meet each criterion detailed within the tertiary code, it would necessarily result in a non-zero final score in order for one to consider the observed system to be performing metabolism to some extent- the closer the final score is to 1, the more certain we can be that the system is showing signs of partaking in metabolic activities similar to that of a living system, as metabolism serves as the basis of which every other life function is built.

It is possible for non-zero scores to be observed within abiotic systems. An example may be found when considering a *computer* which, in its basic form, can be asserted to produce waste in the form of heat, show evidence of regulation in the form of feedback and structure within its internal systems and demonstrate arbitrary forms of stimuli by responding to temperature changes. These aforementioned criteria would award a 0.0667 (out of 1) to the computer, demonstrating relatively low confidence, as would be expected for an abiotic system.

CODE D: Life Perpetuates

The goal of living things is to perpetuate and maintain a continuous presence over an extended period of time. Here, we use the word perpetuation to refer to the longevity, continuation, and storage of information via genetic information within a given species. All life we have observed exists in a system in which it must interact with the ecosystem to survive. At the most basic level, in order to be considered life, a species must be identifiable as thermodynamically different from its surrounding environment. Thus, living organisms require the ability to perpetuate themselves in order to maintain the cycle of countering entropy.

Longevity is integral to a living system's ability to perpetuate itself [13]. Pross describes this phenomenon as “dynamic kinetic stability.” A system must have existed and seem as though it will continue to exist in order to be considered alive. Each species needs to survive for a specified period long enough to confirm that it can persist across a range of conditions that may arise as the ecosystem and environment evolve around it. The species should also be able to adapt and heal from any damage incurred. This includes, but is not limited to, injuries, illnesses, and other damages that can result from conditions such as starvation or dehydration. In life, we can observe this as the ability to repair the cell wall or to find mistakes during the DNA replication process. Taking this a step further, a species may be able to improve its own health through choices such as using better food sources or seeking out more favorable environments. Furthermore, the species should be able to store information in its DNA, genetic material, or other system that allows it to reproduce with adaptations to its environment.

Lastly, the species must be able to reproduce [14], either by creating exact genetic copies of itself through asexual reproduction or by producing new individuals with unique genetic signatures through sexual reproduction. Larger population sizes increase a species' strength and its opportunity for perpetuation. A virus can do this by hijacking a cell's machinery; a cell can do this through mitosis, or sexual reproduction can occur, creating a stronger, more diverse population.

These three categories are split into three secondary codes within the codebook: thermodynamic difference, longevity, and reproduction. For thermodynamic difference, the species can obtain a score of 0 or 1, with 1 indicating that it is clearly different from the surrounding environmental equilibrium. Under longevity, there are three codes: the first is that which has existed for a defined period, whose duration can change based on the observed environment and datasets obtained. Under this code, a species can get a score from 0 to 1, with a fractional score possible based on how long it has existed as a fraction of the given time period, and 1 being the maximum if it has existed for at least as long or longer than the specific period. Next, the criterion of the ability to heal from damage. This is scored from 0 to 1 as a yes or no answer. Lastly, under longevity is the ability to increase health, which is a 0 or 1. A value of 1 is not necessary to be considered life, but can be used to increase the average score. Under reproduction, there are two sub-criteria: can create copies or can create iterations, where copies are exact genetic copies, and iterations are unique. Each is either 0 or 1, but the maximum for this category is 1; they will not lose points if they can only do one type of reproduction.

In this category, a species can achieve a score higher than 1, e.g., a eukaryotic cell that can reproduce both sexually and asexually, thereby achieving a reproduction score of 2. This is mathematically corrected by the presence of the minimum score for life defined for each secondary code. Once an organism has achieved this amount of points, it resets to 1; therefore, a species cannot achieve a final score greater than 1, but can achieve a non-integer score between 0 and 1, with 1 being 100% certainty that a species is alive and 0 being 0% certainty that a species is alive.

CODE E: Life is Capable of Dying

A common thread between most, if not all, known living organisms is the propensity for the organism to die. When trying to categorize whether something dies, it is helpful to start with a definition. The working definition that we are working with is the loss of the ability to be counter-entropic through the voluntary or involuntary cessation of metabolic activity. This is distinguished from the concept of destruction, which can be confused with death, as just about everything can be destroyed when submerged at the center of the sun, or perhaps, in magma/lava.

The idea that life is capable of aging and dying is divided into the secondary codes of aging and dying. Aging is divided into two tertiary categories: continuous growth and deterioration. Continuous growth is defined as something continues to increase in size/develop over time, and deterioration is that something shows physical signs of wear or aging physically. Dying is defined as the ability for something's internal systems to halt without the mass destruction of the system. For application of this code, something can score either 0 or 1 in continuous growth, deterioration, and dying, with continuous growth and deterioration being averaged together and then multiplied by dying to find the final result of this code.

The first application of this codebook was done on a virus. In the secondary category of aging, a virus scored a 1/2 as it falls into the tertiary category of showing visible degradation. For the secondary category of dying, it scored a 0 as internal system failure is not a factor in a virus's continued existence. Multiplied together, the result 0 of this code would imply that a virus is more likely to be not alive rather than alive

The second application of this codebook was on a rock, which scored a 0.5 in the Aging and Death category. This reflects that a rock can break or change over time, but this change comes from environmental factors rather than biological development. This category was scored through three tertiary codes: continuous growth, visible deterioration, and molecular activity irreversible ceasing. When zooming into the continuous growth part, the rock scored a 0 because there is no internal biological process happening. While sediment and minerals can collect around or on the rock, it is not the same growth as metabolism or cell division. Any increase to the size of the rock comes from outside sources that have assumed over time and not the rock actively growing.

For visible deterioration, the rock scored a 1, because a rock can clearly show the physical deterioration over time. It can crack, erode, or break apart because of outside factors like wind, pressure, ice, heat, or other environmental forces. Again, these are physical changes that are happening to the rock, but they are not biological. Finally, when looking at molecular activity irreversible ceasing, the rock had a score of a 0. This is due to the rock not having any cells, metabolism, or any internal molecular functions that can permanently stop. Since the rock is nonliving, it cannot die in the same way a living organism can. Overall, the rock's final score with this tertiary code was a 0.5, it can physically deteriorate, but it does not grow or undergo any death. This shows that there may be more information needed in this section of the code book since we can agree that a rock is not actually alive. The rock should have scored a zero overall.

CODE F: Spontaneous Teleology

Here we explore that life requires spontaneous teleology as an autonomous system [15]. Life with a 100% confidence level reaches a value of 1 on our scale through key characteristics that non-life distinctively does not achieve. We will define teleology as goal-directed behavior that a system performs specifically in its favor [16]. The living system is acting specifically in its self-interest, leading to functional goals like reproduction, metabolism, and survival, that we recognize in biology. An autopoietic unit is a system capable of self-maintenance from an internal organizational process [17]. Modern interpretations structure autopoiesis as the foundation of teleology, although a system that is not fully self-maintaining can still exhibit a directed goal, like a virus. This causes us to place a virus at a value of at 0.33, equaling a 33% confidence, still relatively low in confidence.

A living system also has an apparent drive for survival, a continuation of the system through intentional targeting. Acting out of equilibrium with its environment, life strives to maintain its own existence with an active selection of endpoints. This means that life would have to follow a maximum version of the action principle to be able to achieve the intended functions. This contrasts from nonlife that follows the principle of least action, governing inert matter [18].

This also indicates that an intentional endeavor or received action to the system can advantageously or disadvantageously affect the living system [19]. Life is also able to exploit resources, through the capacity of self-interest, like being selfish or greedy. Nonlife cannot in the same as life, be harmed nor receive benefit. So any system that cannot intentionally improve itself in its favor, is nonliving, and a value of 0 in our scale, corresponding to a 0% confidence level.

To be spontaneous, the living system would need to exclude external consciousness from directing its design, goal, or intent. Therefore, this rules out systems like technological intelligence, since they are externally directed by a living system, positioning at a value of 0.

CODE G: Life adapts and utilizes its environment

Life can adapt to and utilize its environment for its benefit in many ways. It can use the environment inefficiently, efficiently, or even control it in some cases. Overall, life uses its environment in some way, i.e., metabolism and creation, to live. This is why the code for this criterion has only one secondary code with three tertiary codes and will be averaged, which differs from how the rest of the criteria will be calculated.

The first tertiary code is “adapts but inefficiently utilizes its environment.” This means that it adapts to its environment but does not necessarily use it effectively or efficiently. Two examples of this are cyanobacteria and viruses. Cyanobacteria flourish in the oxygen they produce from their environment; however, they can eventually overproduce it and make the environment toxic to themselves. With viruses, the host they infect is their environment, and they cannot perform basic functions like metabolism without it. This code is the baseline for life, as some of the lifeforms we are still hesitant to classify as living meet this criterion, and if it were used any less, it would be unlikely that it would remain alive.

The second tertiary code is “adapts and efficiently uses its environment.” This means that the organism maximizes resource use and minimizes waste. The use of the environment is more sustainable, resembling a closed-loop system. Some examples of this are nest building, neutral or mutual symbiosis, and the Goffin’s cockatoo. Nest building involves gathering materials from the environment to make a safe structure for offspring to hatch and grow. In the case of symbiosis, it creates a sustainable, nearly closed-loop system that minimizes waste and maximizes resource use. The Goffin’s Cockatoo eats the seeds of the sea mango, which are hard to get through due to the fruit's hard and fibrous pit, by creating sticks and wood splinters to gain easier access. This code shows effective and efficient use of the environment, which increases confidence that what is being observed is alive.

The final tertiary code is "adapts and controls the environment." This means that it fully utilizes the environment, sometimes for the goal of altering or cultivating it. A few examples of this are beaver dams, social utilization such as domestication, and farming. Beaver dams are a good case of controlling the environment as they transform river systems into deep water ponds that provide protection from predators and underwater access to their shelters. They also improve water quality, create habitats for many other species, and increase climate resilience. Social utilization, more specifically domestication, is also a good example of controlling the environment. The domestication of cows, sheep, dogs, etc. allows for the ability to cultivate food, materials, and protection in an easier, more controlled manner than searching for it. Similarly, farming is a good display of intentionally modifying the environment to produce food and materials. This code shows full utilization of the environment that gives the highest confidence levels for life.

There are, however, a few abiotic processes that might mimic this criterion. In general, light, temperature, and water influence the environment and affect what processes happen within it, but are easily ruled out when considered alongside the other criteria of the codebook. This also applies to chemical redox reactions and physical changes caused by erosion or deposition. Overall, abiotic processes should not meet or reach high confidence levels within the codebook, despite occasionally mimicking certain criteria.

CODE H: Life Creates

"Life can Create" is the idea that the existence of life indicates some detectable output from that life. It could be biosignatures (secondary code 1), more of the same species (secondary code 2), or "useful" items (secondary code 3). This last in particular is strongly indicative of life, while many things can create basic byproducts, only life can create more complex things, such as culture or technology — though it is not present in all life. In other words; many systems will create byproducts of life while at a more complex level would be creating life itself — for which non-biotic sources are known to exist, but be exceedingly rare. At the most complex level, life can create culture or technology - a sure sign of sapience and therefore life.

The first secondary code is the weakest indicator of life in this criterion, and so is worth the least amount of points. Byproducts of life can also be created by many geologic and chemical processes. But creating more of itself — particularly on its own, can only be done by life, or abiogenesis. The final secondary code, is a sign of intelligence and/or sapience — which has no known abiotic sources, and so is a strong indicator of life's presence.

For this criterion, it is important to note that the three secondary codes have (largely duplicated) tertiary codes. Each tertiary code should be answered with a yes or a no — a no indicates 0 points and a yes indicates the number of points listed. Each tertiary code can be answered independently, and it is possible for a system to meet multiple tertiary codes — i.e., some species of fish and reptiles can reproduce both sexually and asexually (through parthenogenesis) — so they can answer yes to both the 3b and 3c tertiary codes. The final score for this criterion is the sum of all the tertiary code scores, divided by the score of a human (18) - which we know is alive - thereby giving a number between 0 and 1.

3. CASE STUDIES

Here, we apply our codebook to a range of well-known astrobiological papers across diverse subjects to determine whether it can be used in practical applications and to observe its potential limitations. Each case study has at least two distinct evaluators, allowing for an array of opinions, more reliable scores, and confirmation of interrater reliability. In the case where the two evaluators determined different scores for the same case study, both of their opinions are presented, while if both agreed their combined reasoning is given.

Many of these papers may not report observations of specific species, which may pose a challenge and yield many inconclusive or negative results; however, this important exercise will help us identify how the codebook can be modified to evaluate biosignatures or other datasets received during missions and will help to show how little evidence we have for many potential life detections.

3.1 Viking Lander Labeled Release Experiment (1976)

As a part of the biological experiments conducted under NASA's Viking program, the Labeled Release (LR) experiment is unique in that it was designed to test for extant Martian life via signs of metabolism, a result it would hope to achieve by employing the radio respirometric method which entailed adding 0.115mL of various aqueous nutrient solutions each containing 14C-labeled organics to a 0.5 cm^3 sample of martian surface soil collected from two separate sites [20]. In the event of metabolizing microbial life, the LR experiment hypothesized that oxidation of the organic substrates carbon would result in radioactive gas of particular counts per minute (cpm).

Primary investigators interpreted the data from the LR experiments as being “positive for extant microbial life” [21], and so to test for the validity of these claims the data presented by the LR’s interim results paper [20] was evaluated using the Diagnosing Life codebook.

Firstly, considering Information Storage, it was notable that nutrient injections to each experimental soil sample resulted in a plateau at 10,000 - 15,000 cpm against background (~490-660 cpm), confirming a radioactive gas release. This difference to background would create distinct, recognizable configurations, scoring the LR data 2/3 points under *Distinguishable States*. However, no evidence collected could abide by the implications of *stability, readability, writability,* or *propagation*, ultimately giving the LR data a 0.133 under this particular category.

Results collected from both landing sites (which were 4000 miles apart) were identical in both kinetics and magnitude, suggesting that the reactions which resulted in gas release were indeed specific and *replicable* in the sense they were repeatedly observed by applying the same experimental method. Additionally, the phenomenon represented by the data reflect an open system in which cause-and-effect occurrences were observed, however there is no evidence that suggests this occurred for the ‘purpose’ of countering entropy. And so, although the data observes a state of matter arguably with a *higher* entropy above the background, the LR data scores a final 0.667 under counter-entropic.

Soil samples heated to 50℃ and 160℃ then allowed to cool exhibited *significantly* low counts of radioactive gas release once injected with the nutrient compared to samples that were unheated, which were stable at temperatures of around 18℃. Notably, once heated to 160℃ the sample would plateau at 500 cpm above background, resembling control samples which were sterile and displayed radioactive activity of 300-800 cpm. Such a phenomenon could indicate death via molecular inactivity that would otherwise be observed in non-heated samples- this coupled with the development of radioactive activity over time (eluding to a sense of growth) awards the LR data a 0.500 under Aging and Death.

When assessing the phenomenon under Metabolism, the noted gas release observed in the experiments would be fairly representative of waste production and catabolism through oxidation of the 14C. Additionally, production of the radioactive gas would indicate selection of the carbon positions, implying a form of feedback- further suggested by the fact the in most cycles (except one), a pattern was observed where a second nutrient injection would result in a sharp increase in radioactive activity, followed by a 30-35% decrease, soon followed by a gradual increase once again. Given these criteria, the LR experiments score a 0.133 under Metabolism.

When evaluating the Viking LR experiment with our Life Perpetuates Itself codebook, overall, it scored a 0; however, it is important to note that this is because of the exclusionary system. Reproduction scored a 0, but it is feasible to assume there is potential for this species to have the ability to reproduce, which was not within the scope of the experiment, and thus there is no evidence to assume so. Assuming it could reproduce, the confidence in the perpetuation category would increase to 0.250, which is quite notable. In the Longevity category, it scored a 0.500 since whatever species caused the increase in radioactivity existed for a period of time, which is clearly observed in the experiment as it was run over a series of sols. There is also a thermodynamic difference present, as something was present to increase the radioactivity.

Spontaneous Teleology is the goal-directed design of systems. In the Viking LR experiment, it is difficult to determine if the observed species has a goal, since the only observable property is the increase in radioactivity, hence the overall score of 0. However, it does score points because there is an observable disequilibrium as well as evidence to suggest the system sustains itself, as the system lasted at its longest 51 sols, after which the system was purged [18], implying that it could have perpetuated longer.

In this experiment, it is clear that whatever is in the Martian soil is using its environment. When the nutrient substrate was deposited into the soil, the radioactivity increased implying the soil utilized the substrate distributed into the soil. In regards to the codebook, it is unclear whether the species is using its environment efficiently. In this category, the Viking LR experiment scores 0.333.

It is difficult to observe whether the Viking LR experiment observes a species that creates. It scores 1 point in the category for creating other molecules, but does not score in any other categories, hence the score of 0.0556. The only available metric in the LR experiment was the increase of radioactivity, implying a different chemical species is being produced, but beyond that it is impossible to determine if the species being observed is capable of reproducing or creating objects.

By applying the framework offered by the Diagnosing Life Codebook, an average score can be deduced resulting in a 22.8% confidence that the results of the Viking LR experiments show signs of biotic activity , the highest score amongst the subsequent case studies. The Viking LR experiment is unique among the other case studies as it actually is testing to see if something living is present in the martian soil, as opposed to the other case studies, which tend to focus on less concrete biosignatures, such as atmospheric makeup or presence of organics..

3.2 Possible Relic Biogenic Activity in Martian Meteorite ALH84001 (1996)

McKay et al. (1996) [22] examined a Martian meteorite found in Antarctica, ALH84001, using many different instruments to analyze the polycyclic aromatic hydrocarbons (PAHs) and carbonate globules found within it. They used a microprobe two-step laser mass spectrometer for spatial distribution and abundance of PAHs, SEM stereo imaging for surface texture of the globules, Transmission electron microscopy (TEM) and energy dispersive spectroscopy for internal structures, and electron microprobe for chemical mapping. After analyzing the meteorite, the globules contained magnetite and iron sulfides similar to those produced by terrestrial bacteria, as well as microscopic ovoid and elongated structures that resemble fossilized microorganisms.

This suggests potential primitive life on early Mars; However, when compared to the codebook, it is difficult to get a clear picture. Given that the meteorite would be a fossil of a Martian microorganism, there is no distinguishable information storage, aging besides death, or way to detect counter entropy. Assuming the microorganism metabolizes iron to produce the magnetite and iron sulfides, it still achieves a 0 in the category due to stimuli. Similarly, it shows thermodynamic differences and existed for a period of time, but there is no known reproduction or other forms of longevity. This is the case for all categories of the codebook. It only checks one of the many parts of a single criterion, ultimately scoring an overall 0. Naturally, the meteorite itself is not alive, but it has next to no indication that what it contains is worth looking into, either due to limited, weak signals that can be mimicked by abiotic processes.

When considering this case study, it is important to acknowledge that the technology that was used, microprobe two-step laser mass spectrometer, transmission electron microscopy, etc., is now outdated. The paper *Magnetite morphology and life on Mars* by Peter R Buseck [23] highlights the problems with the statistical method and mineralogical terminology used in the McKay paper [22]. Buseck's team argues that the previous research incorrectly identified these minerals as biological when comparing their shapes to terrestrial bacteria. Buseck's team used advanced electron microscopy to demonstrate the three-dimensional morphology of the bacterial magnetite. This showed that they were highly varied and did not match the Martian samples. Ultimately, the paper [23] concluded that the existing physical evidence is inadequate to confirm past life on Mars. When considering either paper with our codebook, we still would end up with a score of 0, which further proves the effectiveness of the codebook regardless of the statistical methods that were used in this case study.

3.3 Seasonal Variations of Methane on Mars (Webster et al. 2018) [24]

Measurements of methane in the atmosphere of Mars have stumped scientists for years, as, typically on Earth, methane is a biosignature and an almost sure indicator of life. On Mars, methane has been detected by multiple instruments, both space- and Earth-based, at levels higher than expected, indicating that something else is producing it. However, on Mars, methane has also been measured at different levels, depending on location, season, and time. This suggests there may be something causing seasonal variations, which could be a more reliable indicator of life, similar to CO_2 in Earth's atmosphere. (Liu et al., 2024) [25]. Despite conducting multiple experiments, both with modeling and in laboratory settings, Webster et al. were unable to explain these variations, but offer the explanation that it could come from a myriad of different sources including exogenous material, rock cycles, the pressure cycle on the surface of Mars, or releases of material from subsurface Mars, ultimately stating, "The amplitude of the seasonal cycle indicates that there remain unknown atmospheric or surface processes occurring in present-day Mars."

Evaluating these seasonal variations in methane using our codebook, we find that the biosignatures alone cannot be determined to be alive; however, they could be considered evidence for life. This highlights a shortcoming of our codebook: it cannot be used to evaluate a biosignature unless that biosignature is evaluated as a species we are trying to determine is alive or not. However, we continue this exercise nonetheless. The scores for each code are given as follows: A: 0.2, B: 0.83, C: 0, D: 0, E: 0, F: 0, G: 0, and H: 0.1, giving it a final score of ~0.1.

This means there is roughly a 10 percent chance that these biosignatures are produced by a living thing; however, this is a very rough estimate based on a preliminary codebook that was not intended to evaluate biosignatures. Furthermore, the original paper by Webster et al. provides significant explanation and evidence that it is unlikely these variations come from a single source, but rather are produced by a myriad of different processes. Initial ideas about these variations have been disproven and withdrawn (Fonti et al., 2015) [26]. As a result, it is just as likely that an internal process on Mars is causing these variations as that life is.

A secondary analysis performed independently yielded similar results. The analysis of Methane variation on Mars yielded A: 0, B: 0.16, C: 0, D: 0, E: 0, F: 0, G: 0, and H: 0. These values are explicitly conservative, as 0 is regarded as the default for a lack of information. Further, the codebook was designed for observations from a discrete system. Extracting information from a planetary scale is outside of the scope of this coding. H in particular, which is oriented around biosignatures more than the other measures on the scale illustrates this issue. As it is dependent on the direct observation of the systems at work, using a planetary scale system results in a scale of 0, even when applied to the seasonal cycles of Earth atmospheric gases, which we know to have biological origin. This is a demonstration of a success of the system, not a failure. Returning 0 in all but one category is appropriate, as our observations are of an open system which is unlikely to be alive. Identifying the origin of these methane variations and conducting an assessment of that specific system would likely dramatically improve the values returned. Until the origin of the variation can be observed as a discrete system, the scale will continue to report that the planet Mars is unlikely to be alive.

A tertiary analysis determined that methane variation is not sufficient to conclude that life exists on Mars. The scores were quantified as follows: A: 0.10, B: 0.83, C: 0, D :0, E: 0, F: 0, G: 0, H: 0, finalizing with an overall average of 0.11. These scores are reflective of previously conducted analyses, demonstrating consistency between all trials. Results align with the conclusions provided by Webster et al.; the observed methane is produced through abiotic means, such as UV photolytic processes, sublimation of carbon dioxide, and potentially micro-seepage release.

3.4 Organic matter preserved in 3-billion-year-old mudstones at Gale crater, Mars, by Eigenbrode et al. (2018) [27]

In this paper by Eigenbrode et al. (2018) [27], the authors detected in situ biosignatures within lacustrine mudstones in Gale Crater, specifically at two sites: Mojave and Confidence Hill, using the SAM (Sample Analysis at Mars) instrumentation on the Mars Curiosity Rover. Using pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) and evolved gas analysis (EGA), the drilled samples were heated to 860 °C, isolated, and analyzed by mass spectrometry. Upon examination of the mass-to-charge ratio profiles with temperature ranging from 500 °C to 820 °C, several thiophenic compounds such as thiophene (C_4H_4S), 2- and 3-methyl thiophene (C_5H_6S), methanethiol (C_2H_6S), and dimethyl sulfide (C_8H_6S) were identified. Additionally, aromatic and aliphatic compounds such as benzene (C_6H_6) and toluene (C_6H_6Cl), along with carbonyl sulfide (COS) and other sulfuric organics. Notably, these organics were preserved in 3-billion-year-old mudstone, biologically indicating a potential once-habitable lake with microbial life remnants protected by sulfurization, whereas, abiotically, they suggest meteoritic impacts or chemical sulfurization.

Eigenbrode et al. (2018) [27] do not explicitly state a biological origin, but note that if life were the source of the organics, the compounds do not look particularly biological. The lack of similarity to complex biomolecules was suggested to have been degraded from diagenesis and ionizing radiation. Additionally, the authors elaborate on the complexity of the compounds upon pyrolysis, being consistent with the chemistry of meteorites and geological organic matter. Indicating rather than evidence to imply biological life, it implies a lack of evidence to count the possibility of life out.

The significance of these findings lies not only in the presence of organic material, but in the mechanisms that enabled its preservation despite Mars' harsh surface conditions. The organics detected are likely derived from large refractory macromolecules, structurally similar to kerogens or coals on Earth, and are inherently resistant to degradation, allowing for preservation. Most notably, sulfurization accruing through early diagenesis appears to have played a central role in aiding preservation. Via these reactions, reduced sulfur species (e.g., H_2S and HS^-) produce chemically cross-linked compounds and sulfur-bearing heterocycles(thiophenes). As a result, the compounds are now more resistant to oxidation and thermal breakdown. Additional preservation pathways include adsorption onto mineral surfaces such as clays and iron oxides, which physically shield organic matter, create localized reducing microenvironments, and provide low sediment permeability, which limits exposure to reactive fluids. Together, these processes allow organic material to persist for billions of years, albeit in a chemically altered, fragmented, and partially homogenized state.

The origin of the organics remains unknown, as both abiotic and potentially biological processes could be credited for their formation. Potential abiotic sources may include meteoritic delivery of organic material, hydrothermal synthesis, and geological processes within the Martian crust, all of which are capable of producing aromatic and aliphatic hydrocarbons as well as sulfur-bearing compounds. However, because the ancient lake environment of Gale Crater was demonstrably habitable, this raises the possibility that biological activity could have contributed to the original organic input. But still, the evidence for a biological origin is insufficient to definitely determine origins. Additionally, the detection method (high temperature pyrolysis) destroys any original molecular structures that would help to determine the origin of the organics. Furthermore, post-depositional alteration, including radiation exposure and chemical diagenesis, would further degrade any original molecular structures, obscuring potential biological signals. As a result, while the presence of preserved organic matter expands the range of environments on Mars capable of supporting life, it does not definitively indicate that life was present.

When applying this case study to the criteria, it was important to clarify the system being evaluated. In the context of this study, the goal is not to determine that the detected molecules are “alive”, but rather whether they represent biological processes. The codebook answers the question of whether these organics provide enough evidence for life as described by the criteria outlined in the methods section. The compounds detected can be used as indirect evidence to assess whether the source of the detected compounds is consistent with life, rather than evaluating the organic compounds themselves as living entities.

The study scored as follows when applied to the codebook criteria outlined in the methods section: individually, the case study resulted in 0.367 for information storage, 0.000 for detectably counter-entropic, 0.000 for metabolism, 0.000 for life perpetuates, 0.500 for aging and death, 0.000 for spontaneous teleology, 0.000 for utilizes environment, and 0.222 for life creates, resulting in the final score of 13.6% confidence. To elucidate the specifics of each category, information storage scored all its points under the external storage primary code, fulfilling the distinguishable states, stability for persisting beyond the system, and readability criteria, since mass spectrometry enables humans to interpret mass-to-charge ratios. For metabolism, points are given for tertiary codes of produces waste, catabolism, and selection; failing to fulfill all secondary codes, only meeting the criteria requirement for harnessing free energy and regulation. For aging and death, the values score a point for visible deterioration due to signs of superheating and die once superheated under pyrolysis. Life can create, received all its points, in the under the producing byproducts category. Additionally, a point was granted for being able to produce new life with outside help, due to the sulfurization process. For categories, life is counter-entropic, spontaneous teleology, and utilities environment, 0 points were given due to a lack of relevant information.

Compared to the other case studies, the compounds detected by Eigenbrode et al. (2018) [27] display a nonzero confidence score when classified under the assumption that the system in Gale Crater that generated the organics was once living and producing byproducts. While not a definitive answer to life, the possibility cannot be disregarded. Unfortunately, when the concentration and complexity of the molecules are compared with those of inorganic sources, the results are indistinguishable. The codebook is structured to classify how confident one can be when looking at a system; however, in this case study, it analyzes biosignatures of potential past life. More conclusive evidence, such as non-random molecular distribution to determine preference for carbon chain length or predominance in structural formation, and isotope analysis of carbon and sulfur to indicate metabolic bias. Based on this analysis, we conclude, with a confidence of 13.6%, that these compounds detected in the lacustrine mudstones in Gale Crater were alive.

3.5 Phosphine on Venus

In the article by Greaves et al. (2020) [28], the authors report the apparent detection of phosphine gas in the cloud layers of Venus, using observations from the James Clerk Maxwell Telescope (JCMT) and follow-up measurements from the Atacama Large Millimeter/submillimeter Array (ALMA). This was considered a potential biosignature based on data we have on Earth that shows this gas is primarily produced by biological processes. Through careful tests, they ruled out possibilities of contamination from other molecules like sulfur dioxide, confirming their detection appears in multiple data-processing methods.

The suggested abiotic processes that could be occurring on Venus to produce these signatures were photochemistry, lightning, volcanic activity, or even possible meteoritic delivery. All of these seemed to fall short by several orders of magnitude, along with the fact that phosphine gas should be rapidly destroyed by oxidation processes occurring in Venus's atmosphere. This led the authors to conclude that there must be something continuously producing the phosphine in the cloud deck. They state that although this is not evidence for life, future observations are needed to fill in the gaps in our understanding of Venus's atmospheric chemistry.

When reading the paper discussing the possible detection of phosphine on Venus, we attempted to apply the criteria in our code book to this supposed detection. I do not believe that our code book was designed to assess biosignatures, which is the main issue here. Our code book was designed based on the assumption that we have already detected something and are now questioning whether or not it is alive. To be honest, I think it makes more sense to agree with the fact that we know what life looks like when we see it. After coming to this realization, a more realistic approach to actually finding life in the universe would be to develop a similar code book that assesses the validity of biosignatures, since that is all we can really "detect" with current technology. That being said, this list of criteria in the code book that we have developed would be useful in the event that we detect a space-borne virus, and the public (along with the scientific community) would probably debate whether or not this could be classified as "finding extraterrestrial life". This code book, unfortunately, did not end up helping us necessarily find life in the universe, but instead it played out as more of a thought experiment about how we define when something is alive or not. Future developments in this research project would hopefully progress toward the development of a code book with criteria to assess the level of potential for finding life based on biosignatures and their detection methods.

3.6 Jezero Crater Organics

In a 2023 study, researchers reviewing data from NASA's Perseverance Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC) instrument discovered evidence for the presence of organic molecules in the form of cyclic and polycyclic aromatic molecules within the mafic rock of the Jezero crater floor [29]. Such molecules are known to form abiotically in aqueous environments, hinting at the possibility of Mars possessing a wetter environment in the past. It can also be interpreted that Mars might have possessed a complex organic geochemical cycle in the past, where key building blocks for life might have been present for extended periods of time [29]. This might not, in itself, be a direct detection of a biosignature on Mars, but it does hint at a past environment that life could have arisen in. However, further research has shown that the source of UV fluorescence detected by SHERLOC might also be explained by inorganic sources such as anhydrite sulfate minerals containing cerium (Ce^{3+}) impurities, which have been shown in lab experiments to undergo fluorescence within the same wavelength range as the ones observed by the SHERLOC instrument [30]. It is likely that we will not be able to say for certain which interpretation is correct until samples can be brought back to Earth to undergo further lab testing.

First Evaluator:

Taking this information at face value, it is very poor evidence for the existence of life. Bearing in mind the fact that the readings could be completely inorganic in nature, the presence of organic molecules in Jezero Crater can still be explained by known abiotic factors. Arguing generously that these organic molecules have biologic origins on Mars, they still fail the majority of the criteria within our codebook, as they simply do not provide enough evidence to suggest its hypothetical biotic origins. For "Metabolism," these organic molecules would only indicate subtle evidence for harnessing environmental free energy due to the formation of these carbon rings being thermodynamically unfavorable unless catalyzed by some biological process, assuming the extremely generous case that life did produce these molecules and not some other abiotic source. An argument could also be made that some form of regulation in the form of structure would have needed to be present in order to facilitate the production of these molecules, but despite this, the metabolism test also ultimately fails because these molecules possess no evidence supporting the existence of the hypothetical organism's ability to experience stimuli (0 for metabolism). "Counter-Entropic" possesses a similar story. This life form would likely need specific and replicable pathways to produce these molecules, but their existence provides no evidence of how these reactions were coupled within the organism for environmental implementation (0 for counter-entropic). This is the same case across the board with most of the other criteria. The existence of these molecules provides no information about this hypothetical life-form's ability to perpetuate, die, partake in spontaneous teleology, and utilize/adapt to its environment (all scoring 0). The only codes that scored a non-zero number were "Life Creates" and "Information Storage". These molecules would technically be a byproduct of this hypothetical organism, and therefore scores a 1 for the "produce byproducts" category within the "Creates" category, resulting in a final score of 0.0556, representing a very low confidence of a living system being present. For "Information Storage," these molecules provide minimal evidence for some form of storage system that possesses some sign of readability as the hypothetical organism likely requires some form of information that needs to be read to initiate the production of said molecules. However, they provided no further evidence for the presence of an information storage system that possesses distinguishable states, is writable, stable, and capable of propagation (final score of 0.0667). This equated to an overall final score of 0.0153 (1.53% confidence), indicating a very low confidence that the hypothetical organism being observed is actually alive based solely on the production of these molecules.

The obvious take away from this is that the code book, as it currently stands, requires a lot more information than just a simple biosignature in order to identify whether something is alive. The ability to directly observe and interact with a system in question is essential in order to properly use this codebook to determine how confident we are that it possesses "life-like" traits. Even when we were being super generous and stated that the molecules were produced by a hypothetical life form that we were capable of observing directly, they alone still did not provide nearly enough evidence to support that the life form making them was in fact alive.

Second Evaluator:

The 2023 paper "Diverse organic-mineral associations in Jezero crater, Mars", authored by Sharma et al., [29] presented the discovery of potential organics through the use of the Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC) instrument on the Curiosity Rover. Across ten targets on the floor of Jezero Crater, the paper found fluorescent and Raman spectroscopy results that could be attributed to biological origins with the acknowledgment that such claims would require further examination of the samples on Earth to confirm.

The main issue with the claim is that there is the potential for these organics to have abiotic origins. The most convincing counter-point is that the fluorescence results of the Group 1 is consistent with naturally occurring Ce3+ [31], as an equivalent doublet result has been seen with "UV luminescence (DUV excitation) of anhydrite: Ce^{3+}" [33], with even sub-10 μg/g Ce level having a comparable result [33]. It is the conclusion of [33] that, even with the mitigation of limitations and a more concentrated approach, a terrestrial analysis is required to gain full insight into the organics at Jezero Crater.

Applying the codebook to something that is a debated biomarker rather than something that is debated to be alive requires a suspension of disbelief as the code is intended to diagnose whether something is alive and not whether something of created by life, and therefore I am applying the codebook to the Jezero Crater Organics as if it were highly likely to be a byproduct of life. The result of this application is a confidence of 1.53% that the Jezero Crater Organics indicates that something was alive to create it. Codes A and H each resulted in nonzero results as the interpretation of it being an organic likely indicates the readability of an information system to create it creating a 0.0667 out of 1 for Code A, and the organics being considered byproducts of life creates a result of 0.0556. The rest of the Codes resulted in 0s due to how little information was available to answer the secondary and tertiary codes properly. These summed together, divided by 8, and multiplied by 100% results in 1.53%.

3.7 DMS/DMDS Detection on K2-18b

Recent analyses of the atmospheric spectra of the exoplanet K2-18b by Madhusudhan et al. (2025) [34] have shown the possible presence of DMS and/or DMDS gases. Since marine life primarily produces DMS on Earth, this is a rational indicator to the potential for life on K2-18b, which may also be producing DMS. While there are *some* abiotic sources that can create DMS and DMDS, Madhusudhan et al. (2025) [34] argue that there are no known sources for the amount observed other than life. As such, this is considered to be a possible sign of life.

Due to the limited information available in this case study - at least of the type(s) we look for with these diagnostic criteria - some assumptions must be made when applying the criteria. Namely; the detection is real - the model that shows such high levels of DMS/DMDS is accurate, and second; there are no geological or astronomical sources that can result in the high amounts of DMS/DMDS detected.

After running this case study through the codebook; we get a final averaged score of 0.0278. Which can be interpreted as us being 2.7% confident that this case study shows the presence of life. Breaking it down further, this system receives a score of 0 in 7 out of 8 criteria: Information Storage, Counter-Entropy, Metabolism, Capability to Die, Spontaneous Teleology, Perpetuation, and Adaptation. It did check a few boxes for Metabolism, and Perpetuation, but scored extremely low and failed one or more secondary codes - leading to the scores of 0 - so it only received a score of 0.222 for Creation. The score for Creation was reached by interpreting the DMS detection as a biosignature that maintains a detectable concentration. As DMS is primarily removed and chemically broken down from photochemical oxidation, this implies that a system of some kind is responsible for maintaining this stable concentration [34].

This low score is primarily due to the amount of unknown information, which was marked as 0 in the individual codes. There is also further research needed to determine whether DMS detection indicates a robust biomarker, or a marker of metabolic potential. If DMS is dependent on the level of CO2, then DMS abundance is also dependent on the oxidative capacity of the environment. Therefore, organosulfur gases like DMS can have abiotic production pathways from planetary organic haze chemistry, showing a further necessity of experimentation considering broader exoplanet atmospheric conditions [35].

3.8 Carbon-bearing Molecules in a Possible Hycean Atmosphere (2023)

This paper by Madhusudhan et al. (2023) studies K2-18 b [36], which is an exoplanet that may be a Hycean exoplanet, having a hydrogen-rich atmosphere above possible global oceans. These findings come from JWST transmission spectroscopy, detecting evidence of methane and carbon dioxide. This study also found no evidence of ammonia, which waves in support of the idea that there could be an ocean beneath the thin H2-rich atmosphere. Biologically, the most interesting result comes from the possible detection of dimethyl sulfide (DMS), which is important because DMS on Earth is commonly connected to marine biological activity. However, the DMS signal is only tentative, and these results should be taken as the sign of a possibility that life could exist on K2-18 b.

When applying the tertiary codebook, this particular case scored low due to the evidence being indirect and only on atmospheric molecules. Information storage, this case scored a 0.00, because there was no genetic material or biological code that was found. Metabolism scored a 0.00. Though it is possible that DMS could be the byproduct of biology, there is no direct metabolic pathway observed nor any evidence for the presence of stimuli. Aging and death scored a 0.00, again because there was no physical biological evidence detected; therefore, there was no evidence of growth, deterioration, or death. Detectably counter-entropic got a score of 0.00, because the chemistry observed is unusual and could be potentially organized, but there is still no confirmation that it is being maintained by a living system. Creates scored a 0.0556, because of the possible DMS may be considered a biosignature byproduct, still there is nothing more complex that was observed. Utilized environment got a score of 0.33 as well, because having a biological source of DMS can employ environmental use, but it continues to remain uncertain. The overall score that this case scored from the tertiary code is 0.0482, showing that the presence of DMS in K2-18 b is not a strong indicator of life for astrobiology, and that we do not have enough strong evidence to believe that life is there.

4. LIMITATIONS AND NEXT STEPS

As the diagnostic criteria system we developed is a prototype and a living document, it is necessarily limited. With more time and future research, many improvements could be made. Both the creation of new criteria that may be helpful, and the paring down of existing ones to eliminate overlap would be among the largest changes. Relatedly, the current process does not well handle traits that are strongly indicative of life's presence when they are present, but are not found in all life — such as sapience.

Additionally, it would also be possible to weigh each criterion based on their importance to life. This could incorporate conditional statements to weigh certain criteria more strongly depending on what exactly is observed.

Another improvement that could be made in the future would be a way of measuring uncertainty. This uncertainty comes from the fact that in none of our case studies, do we know everything about the system - we often only know one or two things, and so the unknowns are marked as a 0, deflating the confidence score. This uncertainty measurement would likely be in the form of the score, plus the difference between the maximum score if all information was known and the actual score:

$$S + (S_{max} - S) \quad (5)$$

In other words, currently both a "no" and a "don't know" are scored the same. Ideally, this would be improved such that we can tell the difference between a low score because it does not meet many criteria, and a low score because there is very little information available.

Delineating between "no" and "don't know" cases could also be achieved through the establishment of a counterfactual criteria set that would act as negative indicators of life in a case entity. This counterfactual set would produce a higher resolution dimensionality to life criteria assessment as criteria categories might sum in a negative value. To reflect these “disqualifications” of life, an adjusted confidence interval would be introduced to account for negative values; this adjusted range would include -1 as a value describing maximal confidence that a case entity is *not* alive, 0 describing true uncertainty and ambiguity in a case entity, and 1 remaining the maximal confidence of life in a case entity.

With more time, we would also like the individual codes to be better structured for use in astrobiology case studies — where we can not observe the system itself, and only its detectable output. We would also like to look into more guidelines when applying the criteria, in order to decrease the effect of user bias. Another way to reduce bias would be to improve the language of the codes, so that there is less room for misinterpretation.

A large limitation with the application of the code to many of the case studies is that the code was not designed to assess the likelihood of a potential biomarker being an actual biomarker. This runs into the issue of not having many case studies that would properly test the effectiveness of the codebook outside of things we know to be alive or not on Earth, with the Viking Lander Labeled Release Experiment being the best test of the codebook. Perhaps the rigorous testing of the various types of viruses on Earth would reveal how well our codebook diagnoses life.

5. CONCLUSION

The calculated confidence levels established from our code of diagnostic criteria have effectively demonstrated a useful system to define life in the astrobiological context. We have shown that by applying our diagnostic criteria to eight possible life detection events, and evaluating their confidence level, that these case studies can be assessed to a measurable indication of life.

Although, due to the limited amount of evidence for confirmed planetary and exoplanetary biomarkers, the eight possible life detection events we evaluated using our diagnostic criteria have collectively scored a low confidence level for a detection of life. We determined the Viking Lander Labeled Release Experiment to have the highest confidence level of life detection, at a value of 22.7%, within the eight events we examined. Given the fact that the low scores are primarily caused by unknowns, we find that most potential astrobiological life detections do not provide enough information to determine if life is present — even if the chemical or physical detections are confirmed to be accurate and not caused by instrument error or analysis methodology.

Considering the possibility of user bias towards the application of our codebook criteria to detectable biosignatures, we propose further clarification to the diagnostic criteria to reduce bias and misinterpretation. We also propose that further astrobiological testing and research into these eight events and more detectable biosignatures, will contribute to diagnosing life by using our codebook criteria and any necessary modifications the codebook requires. Overall, while our criteria are limited and in need of refinement in their current form, they still indicate the importance of simply having more information before we can say whether a given phenomenon is strong evidence of the presence of life.

APPENDIX

APPENDIX A - Diagnosing Life Codebook

CODE	Secondary Code	Definition
A - Life must have an information storage system	Distinguishable states	The amount of information states the system possess
		The states can be analyzed and separated
		States can be changed without impacting other states
	Stability	The storage lasts beyond the system
		The states are prolonged by the system's use of energy
		The states cease to exist once the system degrades
		The state exists outside of the system
	Readability	A reader can decode the storage states
		The decoder exists within the system itself
		The system generated the reader and storage state together
	Writability	Once the system is formed, the writer begins to write the states
		The system can encode new information
	Propagation	The information can be replicated to an offspring or stored in another system
		The system propagates itself
		When replicating, the information retains its core functions
B - Life is detectably counter entropic	Reactions are specific and replicable	Reaction occurs on regular intervals
		Products and reactants are always the same between intervals
		Intermediates, mechanism of reaction are always the same between intervals
	Coupled, overall low entropy reactions occur in systems that are integrated within their environment	Reactions happen in a cause-and-effect manner
		Prompted reactions of interest occur independently within an enclosed system
		Prompted reactions of interest interact with the surrounding environment, exchanging energy/matter
C - METABOLISM	Harnesses Environmental Free Energy	produces waste as an unavoidable product produced by the action of harnessing free energy (a system must always produce entropy when harnessing energy for work)
		breaks down complex species into less complex forms
		builds complex species from less complex species
		In order for energy to be used to do work, the laws of thermodynamics state that energy must flow from high to low concentration. life generates a space of high energy concentration and uses its surrounding environment as the final energy sink
		Life can maintain a system whereby it moves species from areas of low concentration to high concentration

	Regulation	life consumes and produces only a select few species relative to the number of possible species the environment can produce with the same building blocks
		Ability to turn processes on and off
		The system utilizes free energy through a sustainable pathway which encompasses more than one phase/step
	Stimuli	The system is likely to show preference for areas/circumstances where it can most effectively harness environmental free energy
		The system can change how it interacts with its environment in response to changing environmental factors
D - Life perpetuates itself	Thermodynamic difference	remains more complex than environmental equilibrium beyond what thermodynamics would allow assuming no active regulatory processes
	Longevity	Life of this specific species is feasibly continuous for a defined period of time, showing continuation across evolution of surrounding systems.
		Life of this species can heal on a cellular level, i.e. recover from sickness or injuries
		Life of this species can increase health of the organism, i.e. through working out, medication, supplementation
	Reproduction	Asexual reproductions
		Sexual reproduction, new evolution and individual, NOT an exact replica
E - Life should be capable of aging/dying	Ages	continues to increase in size/develop over time
		shows physical signs of wear or aging physically
	Dies - Molecular activity irreversibly ceases	Molecular failure can occur without the possibility of repair (even if the structure remains intact).
F - Spontaneous teleology	maximum action principle	lack of equilibrium with environment
		active selection of endpoints
	utilization	receive advantages
		receive harm
		can exploit resources
	goals	aims to maintain system
		aims to increase quantity of system
		aims to progress and change existing system
	autonomous	decisions governing functions
	self-interest	ability to sustain system
		awareness of system
G - Life utilizes environment specifically for its benefit and in its favor	Adapts to it environment, but doesn't necessarily use it or uses it inefficently	Cyanobacteria - flourishes by makes oxygen, but eventually made their environment toxic to themselves; Camouflage - adapts but is a passive adaptation; Extremophiles - can survive extreme conditions, but ill-suited outside them; Viruses - the host is their environment and can't continue without it

	Maximising use of resources and minimizing waste; sustainable, closed loop systems	Nest builders, burrowing, foraging, decomposition, symbiosis, Goffin's Cockatoo, recycling
	utilizing the environment for the goal of changing it, cultivation	Mining, harnessing electricity, farming, beaver dams, Social utilization - domestication, terraforming, leaf cutter ants

H - Life can create				Points Value:
	1 - Can create byproducts	2a - With outside help/interactions	Biosignatures are a chemical signature we can observe	1
		2b - On its own		2
	2 - Can create new life	3a - With outside help/interactions		1
		3b - On its own	i.e. Asexual Reproduction	2
		3c - As a group	i.e. Sexual reproduction	2
	3 - Can create objects	4a - On its own		3
		4b - As a group	Over generations, building on past creations	4
		4c - With outside help/interactions with other "species"		5

APPENDIX B - Key Terms

1. **Species, System, Phenomenon, Case Entity:** These terms are used interchangeably to refer to what the diagnostic criteria are being applied to. As these criteria are intended to be applied in an astrobiological sense, where you are often observing only one or two chemical or physical signatures, these terms serve as a reminder that the criteria are being applied to the hypothetical system that created those signatures, not, say, the chemical itself.
2. **Confidence:** This is our measure for how likely it is for a given phenomena to be alive or indicate the presence of life.
3. **Code, Criteria/Criterion:** These terms are used interchangeably to refer to the subdivisions of this diagnostic system. In general, Criteria/Criterion refers to the top level subdivision, e.g. Information Storage, while Code can refer to the same or smaller subdivisions - typically referred to as secondary codes and tertiary codes.

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