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d measurements must be undertaken to different sites to ensure all reasonable target areas have been explored.

**Table 2**. Possible sources of organic carbon that need to be distinguished in Martian samples.

|  |  |
| --- | --- |
| **Source of Carbon** | **Carbon compounds. examples/comments** |
| Abiotic molecules from meteoritic / cometary influx | Amino acids, purines and pyrimidines, polycyclic aromatic hydrocarbons, chain hydrocarbons, fatty acids, sugars and sugar derivatives. |
| Prebiotic/abiotic molecules from synthesis reaction process on Mars | Amino acids, purines and pyrimidines, polycyclic aromatic hydrocarbons, chain hydrocarbons, fatty acids, sugars and sugar derivatives. |
| Terrestrial contaminating organics | Condensation products derived from rocket exhaust, lubricants, plasticizers, atmospheric contaminants |
| Terrestrial contaminating organisms | Whole cells, cell components (LPS, DNA, proteins, cytochromes) found on AFL itself. |
| Terrestrial like organisms – from Earth | Organisms not present on the craft measuring them, but had been previously transferred from Earth by either meteorite impact or contamination of previous spacecraft. Target molecules could include individual genes, membrane constituents, specific enzymes, and co-enzymes that would be expected to be over expressed or adapted in Martian conditions |
| Terrestrial-like organisms – evolved on Mars | Organisms that utilize terrestrial like biochemistries and have evolved on Mars Target molecules could include individual genes, membrane constituents, specific enzymes, and co-enzymes that would be expected to be over expressed or adapted in Martian conditions or organisms using metabolisms that would not be present on a space craft contaminant such as methanogens, psychrophiles endolithic survival mechanisms. |
| Non-terrestrial-like organisms | Utilizes an array of molecules for information storage, information transfer, compartmentalization and enzymatic activity that differ from those used by extant terrestrial life. Examples would be the use of novel amino acids and nucleotides or the use of novel nitrogen utilization strategies. |
| Fossil biomarkers | Detection of established terrestrial fossil biomarkers such as hopanes, archaeal lipids and steranes, for the detection of the diagenetic remains of terrestrial based life. Characterization of potential breakdown products that can be reasonably extrapolated from the detection of molecules comprising an extant Martian life form. Detection of the diagenesis products of extinct Martian organism based on carbon compositions consistent with biological fractionation of a narrow range of abiotic precursors. |

#### 5.2.2.1 What techniques have been used to detect and characterize terrestrial and meteoritic biosignatures?

1. Morphological observation using microscopic tools (Light, SEM, TEM, AFM, Fluorescence). The controversy mentioned earlier regarding the oldest fossils on Earth illustrate that it is difficult using all available analytical tools in a laboratory to unambiguously determine if something is truly of biological origin. Recognizing a fossil using the criterion of shape alone poses some challenges, particularly without actually being on the surface of Mars and knowing *a priori* whether it has a fossil record. In contrast, observing movement in extant life is easy. However, not all extant life moves, especially microbes, therefore making it difficult to determine if it is alive by shape alone. Interdisciplinary multi-instrument approaches have been shown to be effective for studies on deep subsurface ecosystems on Earth (e.g., Fisk et al., 2003; Steele et al., 2002; Toporski et al., 2002; Steele).

2. Biochemical analyses. A range of analyses based on either pure chemical or biochemical methods have proven to be useful on Earth in determining if a sample is of biological origin. However, in difficult cases it has usually taken several different methods of analyses to determine if a sample is unequivocally of biological origin. Carbon isotopes have successfully been correlated with individual Proterozoic microfossils (House et al., 2000) and FT-RAMAN spectra were obtained on presumed Proterozoic microfossils (Schopf et al., 2002). Furthermore, fossil and modern bacterial biofilms have been classified using a combination of bulk and spatially resolved measurements including XPS, EDX, XRD, Time of Flight – Secondary Ion Mass Spectroscopy (ToF-SIMS), pyrolysis GCMS, GCMS, GC-IRMS confocal laser microscopy and Raman and infrared microspectroscopy (Steele et al., 2001; Toporski, 2001; Toporski 2002; Toporski 2004, Hall-Stoodley et al, 2004; Benning et al 2004). Only the combination of a multiple-set of instruments lead to a unequivocal determination of the specific characteristics of biofilms.

#### 5.2.2.2 What are the challenges for AFL in the search for biosignatures on Mars?

1. Tested Technologies. Of the techniques listed in table 1 those that have been shown to be successful during space missions include: gas chromatography, mass spectrometry, simple thermal analysis, Mossbauer and some types of interactive chemical techniques (e.g., the Viking biology experiments (see Mancinelli 1998 for review).

For Mars applications, it is necessary for the detector to be sensitive to the picogram level and capable of responding to a broad variety of compounds, i.e., have universal response. A flight proven detector that is both universal and sufficiently sensitive is the metastable ionization detector. The primary disadvantage of gas chromatography is the small margin of error associated with the column retention times for definitive identification of compounds, which can lead to mis-identification of compounds with similar retention times. This disadvantage should be minimized by use of multiple columns with different separation capabilities (i.e., different column coatings or packings) and calibration standards. A GC/MS has been used successfully on space missions, including the Viking mission The disadvantages are that the MS cannot be simultaneously tuned to be sensitive for the analysis of low and high molecular weight substances at the same time, and it is a bulky and heavy instrument*.* Various types of analytical instruments equipped with different pyrolytic devices have been used during space missions. These ranged from simple pyrolysis (combustion) to step-wise heating of samples and measuring the power input and temperature. Step-wise heating is usually followed by collecting any volatiles evolved from the sample during heating, and identifying and quantifying them by GC, or GC/MS. For example, heating samples of soil from earth in a step-wise fashion would first volatilize adsorbed water and gases (e.g., CO2, and lower molecular weight organic compounds) at the lower temperatures. At higher temperatures, water from mineral hydration, CO2 from carbonate decomposition, and volatiles from pyrolysis of higher molecular weight organics would be released. Although this technique allows one to analyze the evolved gases, it does not yield any direct information regarding the nature of the sample (e.g. clays vs. hydrated silicates). Mossbauer spectroscopy provides information on the valence state of specific elements (i.e., Fe, Sn, Sb, Ru, and Au), how these elements are combined in the structure of a compound, and the magnetic properties of the sample. Mossbauer spectroscopy can provide information about H2O only if it is associated with the elements Fe, Sn, Sb, Ru, or Au. This again is an area where micro total analytical systems and micromachining may allow significant weight and energy savings.

2. Non-tested technologies. Scanning electron microscopy-energy dispersive X-ray spectrometry (SEM-EDS), which maps electron intensities for identification of elements with atomic numbers greater than sodium, although windowless detection allows all elements heavier than boron to be detected. Electron spectroscopy for chemical analyses (ESCA) quantitatively determines the valence states and bonding energies of most naturally occurring elements (excluding hydrogen and helium). This technique is limited to analysis of the top 1-10 monolayers of the sample. X-ray diffraction (XRD) analysis directly and nondestructively probes atomic scale structural correlations of mineral samples yielding sample mineralogy along with information about the presence of H2O. X-ray fluorescence (XRF) analysis non-destructively provides information on the elemental composition of a sample for elements having atomic numbers greater than that of boron. However, no information is given about how those elements are combined in the sample. Rutherford backscattering spectrometry (RBS) maps the elemental composition and distribution measured on sample surfaces (the top 0.5 – 3 microns). Elements that can be analyzed by this non-destructive technique range from Li to U. Secondary ion mass spectrometry (SIMS) analysis has a very high sensitivity and can identify all elements including hydrogen and deuterium. A mass spectrometer (MS) provides information on elemental and molecular composition, including that of H2O, and the isotopic abundances found in a sample. Differential scanning calorimetry (DSC), in which the amount of heat required to maintain isothermal conditions between the sample and an inert reference placed in a continuously heating oven, is recorded, and the enthalpy provided directly. Sample identification is made by examination of the patterns of exotherms and endotherms along a temperature scale. The DSC provides quantitative data to ~700°C. For temperatures >700°C the signal-to-noise ratio becomes too great. Differential thermal analysis (DTA) is similar to DSC in that the sample and an inert reference are heated at the same rate, but to ~1200°C. The temperature of the sample and reference are monitored simultaneously. It differs from DSC in that when endothermic and exothermic events occur in the sample, no attempt is made to keep the sample and reference isothermal to each other. In DTA, the temperature difference between the sample and the reference is recorded as a function of oven temperature and provide the information for sample identification. The thermogram obtained from a DTA or DSC analysis provides information on the mineralogy and chemical composition of the sample. Where the DTA or DSC is coupled to a gas chromatograph (GC), the GC collects and analyzes the volatiles (including H2O) evolved from the sample as it is heated.

Specifically for extant life detection interactive chemical methods were performed as part of the Viking mission. This approach is fraught with problems. It assumes prior knowledge of Martian organism metabolism. Using these culturing methods only detect 1-2% of the microbes in earth soil can be detected. A distribution mass peaks obtained by a mass spectrometer of alkanes showing a decrease in concentration with increasing carbon number would indicate abiotic processes. Similarly a predominance of biogenic amino acids with an excess of the L isomer would indicate extant or recently extinct life. Whereas, a suite of racemized biogenic amino acids may indicate fossil life. Detection of hopanes by Time of Flight Mass Spectrometry may also be indicative of life. Field ATP luminometry measurements of the cryptoendolithic communities may provides a rapid method of detecting relative amounts of metabolic turnover in microbial communities. None of these techniques would provide definitive evidence of life during the MSL mission. Clearly, multiple approaches need to be done on samples to determine if they contain viable extant organisms. For example, if organic mass gas chromatography spectrometry analyses combined with deep UV florescence, SEM and RAMAN all point toward life, then there is a high probability that the sample may contain life.

## 5.3 Preservation Potential

A biosignature preservation model, guided by data from AFL, will be critical to long term Martian life detection strategy. That is to say that AFL in detecting carbon chemistry in various sites of possible habitability (see definition) can indicate whether such niche areas could preserve clues of Martian life. This must be modeled by suitable experimentation in laboratories before suitable interpretation of any data can be undertaken. We still do not know the exact composition of the mysterious Martian oxidant postulated in the Viking experiments.

Long-range Astrobiological exploration of Mars will require an understanding of the preservation potential of biosignatures. This is an important part of the scientific logic of going from possible biosignature to confirmed biosignature.

Lessons from Earth

•Life processes produce a range of biosignatures, and geological processes progressively alter and ultimately destroy them.

•Understanding the potential for preservation has been a key part of biosignature interpretation.

Application to Mars

•We don’t know the biosignatures of Martian life forms (if they exist).

•However, with appropriate data, it should be possible to postulate a preservation model relating biosignatures as we understand them on Earth to various Martian geologic environments. This model will likely have important predictive value in guiding future search strategy. Models predict that biomolecules and organisms can survive in simulated conditions such models need refinement and to address diagenetic processes in predicted conditions (Scheurger et al., 2003).

# 6.0Precursor Discoveries

Relevant data may already be available but two major classes of discovery would be of essential relevance to AFL mission planning:

MRO

•Sending AFL to a hydrothermal site is impossible with present knowledge, because none are known. However, the CRISM spectrometer on MRO is very powerful, and it has potential to discover the mineralogic expression of hydrothermal zones.

Phoenix

•Phoenix will be the first lander designed to acquire and analyze ice-bearing samples.

•It will collect data of relevance to each of the three primary components of habitability (water, carbon, energy), and thus is capable of returning a result which significantly improves or reduces our interest in sending AFL to an ice-related site.

Table 3 A summary of types and amounts of biomolecules present in a single bacterial cell and compared to known preservation potential for such molecules.

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **% Total Weight (or mass C x10-13 g)** | **Number of Types** | **Preservation** |
| **Water** | 70 (NA) | 1 | Unknown in Organic and mineral phases |
| **Proteins** | 15 | ~2000 | 1000’s without protection by a mineral matrix. ~45Ma with protection?. |
| **Nucleic Acids** |  |  |  |
| DNA | 1 | 2+ | Oldest ? ~350,000 |
| RNA | 6 | (see below) | Days – Months (studies on longevity of RNA other than in clinical settings have not been performed. |
| *rRNA* | 5.5 | 3 | Days – Months |
| *tRNA* | 0.1 | ~32 | Days – Months |
| *mRNA* | 0.3 | 1000’s | Days – Months |
| *Non coding RNA* | 0.1 | 1000’s | Days – Months |
| **Polysaccharides** | ~1 | Uncounted | Chitin - 25Ma. Exopolymer sheaths ~2Ga |
| **Lipids** | 2 | ~50 | Cell wall components - Hopanes 2.7Ga |
| **Amino acids** | 0.4 | ~100 (20 main ones) | As protein diagenesis – Ma.  Chiral signal in fossils lost after ~ 1 Ma. |
| **Sugars** | ~3 | ~200 | Days to weeks (see polysaccharides) |
| **Other small organics** | 0.2 | ~200 | Porphyrins ~ 2 Ga |
| **Inorganic species (C, H, N, O, Fe, P, S etc).** | 1 (~100% dry weight) | ~20 – 30 (including inorganic complexes) | Isotopes may preserved for ? 3.5 Ga for C.  Research is continuing to define other isotope systematics for preservation of a biogenic signature. |
| **Diagenetic Macromolecular material** | Total cell breakdown products (100% dry weight of cells) | Kerrogens (4 types)  Melanoidins (100’s) | Kerrogens – ? 3.5Ga for biogenic (Type 1-3). Type 4 indicative of meteoritic input.  Melanoidins conbination of sugar and proteins, ~50 Ma. |

? – debate over the data. Total mass of the organic inventory is based on the assumption that most terrestrial prokaryotes contain approximately 10-13 g of carbon per cell.

# 7.0Mission Site Selection

Four subgroups were founded to begin to address the need for AFL to respond to the discoveries and requirements for as yet to be determined site. Through this process a core mission concept was arrived at and presented to the engineers for costing.

There are four obvious general types of site in which the overall scientific goal of AFL (major advance in A/B) can be pursued:

•The sedimentary record.

•Fossil (inactive) hydrothermal systems

•Sites with ice

•Sites where it may be possible to sample liquid water

We do not have enough information as of this writing to know how these four options would be prioritized by a future SDT. Future discoveries could have a major effect on planning. At the time of writing this document all of the above sites may be postulated to currently exist on Mars. The sedimentary record has been explored by at Gusev and Meridiani by Spirit and Opportunity respectively (Squyres et al., 2004; Grant et al., 2004; Morris et al., 2004; Kerr 2004c (commentary); Arvidson et al., 2004; Bertelsen et al., 2004; Herkenhoff et al., 2004; Gellert et al., 2004). Fossil (slightly active) hydrothermal systems may be concluded from initial papers outlining the concurrence of water vapour, shallow ground ice and methane at Arabia Terra, Elysium Planum and Arcadia Memnoma, (See Kerr 2004a,b and c for commentary). Sites with ice and the obvious poles or shallow “dirty” ice sites such as Phoenix proposes to explore. Sites with possible hydrothermal activity represent a chance to sample liquid water, although this may be at some distance below the surface. To remain flexible to current and future discoveries we

Figure 3 Shows the antecedent discoveries that will impact and guide the choice of sites and final payload of the AFL mission

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## 7.1 Sediments

Here we present a mission concept with the overall goal of finding evidence for past or present life in the Martian stratigraphic record in an environment that is highly likely to have formed from the subaqueous deposition of sediment in a shallow marine or lacustrine environment such as exposed in craters at both the Spirit and Opportunity sites (Squyres et al., 2004; commentary by Kerr 2004c).

Objectives

Specific supporting objectives that support this goal are to:

Assess spatially resolved changes in mineralogy with depth on a scale consistent with the depth of individual strata.

Determine the abundance and nature of organic chemicals at the same scales as above.

Seek information regarding water cycling from the strata, eg. is there free or bound water in any of the layers? Ice? Hydrous mineral phases?

Confirm the depositional environment.

Determine provenance of the sediment

Assess the fossil preservation potential of the environment. Factors which might be considered are temperature, rock type, local weather, UV flux, depositional regime as suggested by sedimentary structures

Approach

Central to this mission is the selection of a landing site that possesses multiple outcrops of layered sedimentary rock. We would use remote sensing methods that possess sufficient spatial resolution to resolve individual layers to acquire information from several outcrops. Subsequently, a rover would visit at least one 3D outcrop of layered sedimentary rock, measuring variation in chemistry, mineralogy and texture of the strata for at least 100 meters along the strike and ten meters in the dip of the outcrop. Subsurface penetration would be an important feature of this mission for the acquisition of subsurface samples that are from depths great enough to extend beyond the level of surface oxidation. This may mean accessing a depth of one meter in a horizontal area, though it would be desirable to penetrate the exposed bedding along the slope of an outcrop in a larger feature such as the wall of a crater. Examining the subsurface of such beds would only require a relatively shallow penetration (perhaps a few centimeters), and we would then have access to the primary sediment without having to go through the more recent Aeolian deposits.

Required measurements for meeting the scientific objectives must be conducted at multiple spatial scales, and we recommend three suites of instruments that can provide integrated measurements *a la* the remote sensing, non-contact/contact and analytical suite designations originally suggested by the MEPAG PSIG for the MSL mission. Both spectroscopy and imaging will be key to an integrated science package, and we assume technical progress in science autonomy before the launch of AFL that optimizes science operations on the Martian surface.

There are several engineering /science trade issues associated with taking a large number of measurements from a large outcrop in three dimensions. Some of them are:

“Go to” mobility is required. The degree of mobility will be complementary to the degree of precision of the landing.

The ability to land in a terrain which is rougher than previous targets would be valuable. A priority should be given to precision targeting and hazard tolerance.

Fresh material should be exposed with a RAT or its descendent.

Surface penetration is also required to a level below any weathering layers, a few cm to perhaps a meter.

Sample acquisition and some processing, at least to the level of crushing will be required.

There will be a requirement for positioning—perhaps a laser range finder.

Autonomy should be plentiful—not just for the rover, but for some of the scientific operations in order to maximize efficient use of resources.

Landing Site Selection

One of the primary assumptions of this mission concept is that we will have advanced in our ability to assess habitability for a range of potential landing sites by the missions that are to precede AFL. For example, recent inferences made regarding the environment of deposition for the MER B landing site, Meridiani Planum would suggest that it is an excellent candidate site for an astrobiology follow-up mission. However, as of the time of this writing, there are few exposed examples of the cross-bedded rock from which the shallow marine inferences were drawn at that site. Much of the Martian surface will be mapped in exquisite detail by the time the AFL mission site selection is made, and there are likely to be other candidate target areas that demonstrate appropriate geomorphological and mineralogical character to suggest deposition in a standing body of water. For example Northeast Holden crater, may be a good candidate; geomorphological evidence strongly suggests classical deltaic deposition (Bhattacharya, in prep):

Figure 4 Holden crater



## 7.2 Hydrothermal

Science theme: Assess past Martian Astrobiology in an inactive hydrothermal system.

The apparent harsh climate at the surface of Mars suggests that, should life exist on Mars, the most likely energy source would be subsurface / chemosynthetic rather than surface / photosynthetic. Hydrothermal systems are attractive sites for Astrobiological exploration because they contain all of the requisites for the origin and maintenance of a biosphere and the subsequent preservation of its biosignatures. In such systems, water is typically present in the liquid state in a near-surface environment. Both thermal and chemical energy are made available for use by chemosynthetic organisms as a result of water-rock interactions. Common reactions between mafic/ultramafic minerals, water and volcanic gases such as CO2 lead to the formation of reduced carbon compounds that could have been the building blocks of early life. Secondary mineralization of hydrothermal deposits by carbonate, silica, and other hydrothermal precipitates can preserve evidence of prebiotic carbon chemistry as well as evidence of life. Finally, while the bulk of a hydrothermal system is quite likely to be beyond detection in the subsurface, surface expressions of such systems should be morphologically and mineralogically identifiable from space. However, even when surface expressions of hydrothermal systems are missing or cryptic, impact gardening, mass wasting and simple erosion by wind or water will dissect and expose such systems over geologic time. The detection of the correlation between the concurrence of water vapour, shallow ground ice and methane at Arabia Terra, Elysium Planum and Arcadia Memnoma, may indicate such a system exists in these areas (See Kerr 2004a,b and c for commentary).

**Finding hydrothermal areas:**

At present, we know of no *bona fide* hydrothermal zones or regions on Mars. However, the apparent association of fluvial features with volcanic terrains in many places on Mars suggests that such areas must be common. One can deduce from the young crystallization age of most Martian meteorites (which appear to post-date major fluvial/lacustrine features on the planet) that volcanism and (presumed) associated hydrothermal activity persisted throughout Mars history. Indeed, a number of Mars meteorites (including the famous meteorite ALH84001) contain carbonates or minor hydrous phases suggestive of a hydrothermal setting (Treiman et al 2002).

Clues to the presence of fossil (inactive) hydrothermal zones include morphological, mineralogical and chemical features. A morphological feature could consist, for example, of a spring mound (positive topographic feature) associated with evidence of water flow. A mineralogical feature could consist of surface deposits of carbonates, silica, etc. Global surveys of hydrogen in the near-subsurface, discussed largely in the context of near-surface water, could in some cases represent hydrated mineral phases associated with hydrothermal features.

Future missions will provide clues, perhaps even compelling evidence of past hydrothermal activity. The Mars Reconnaissance Orbiter will have a high-resolution camera from which morphological data will be obtained. CRISM will provide high resolution chemical or mineralogical maps of surface features. Orbital or landed neutron detectors and radar sounding devices could provide maps of near-surface water over large areas of the Mars surface. The ’07 Phoenix Scout mission, as well as Mars ’09 MSL will provide *in-situ* information on both morphology and mineralogy at the sub-meter to sub-millimeter scale.

Five possible landing site hydrothermal geologic settings are envisioned:

Point source hydrothermal zones (igneous-driven convection systems).

Point source hydrothermal zones are well known on the Earth – as for example those present in Yellowstone National Park (a continental-type environment) (e.g., Walter and Des Marais, 1993) or at the mid-ocean ridges (oceanic-type “black smokers”) (e.g., Kelly et al., 2001). These features should be identifiable by their morphology and their mineralogy/chemistry (Farmer, 1998). High-resolution mineralogical data should allow the identification of systems such as these, which may vary in size from kilometers (Grand Prismatic hot spring, the largest hot spring on Earth, is ~1 km in size) to meters in size. Mineralogical signatures of these systems range from monomineralic deposits (silica, carbonate, sulfide, oxide) to polymineralic assemblages. In general, the areal extent of hot springs, which are the surficial expression of point-source hydrothermal zones, are dwarfed when compared to the volume of hydrothermally altered rock in which chemosynthetic life could live in the subsurface (Cady et al., 1997). As a result, even without a large surface expression of hydrothermal activity, one could search for hydrothermal alteration minerals similar to those found around ore deposits on Earth (Horn, 1996). Surface and near-surface deposits of hydrothermal systems will contain a variety of alteration minerals that vary as a function of the underlying mineralogy of the system (e.g., oxides, carbonates, sulfates, hydrated minerals, etc).

Impact-generated hydrothermal systems (craters).

Newsom et al. (2001) reviewed many of the key concepts that support a search strategy for life on Mars in aqueous and hydrothermal deposits associated with Martian impact craters. For example, impact craters on Earth (e.g., the Sudbury impact crater, 1.85 Ga ; ~250 km diameter in Sudbury, Ontario) contain extensive evidence of post-impact hydrothermal activity. Impact melt and uplifted basement heat sources could sustain hydrothermal activity and keep crater lakes from freezing for thousands of years, even under cold climatic conditions (Newsom et al., 1996). Post-impact fluids could result from dewatering of deeply buried hydrated materials, and the breach of local aquifers or regional cryospheres. The lifetimes of impact-generated hydrothermal systems depend on the size and cooling rate of the heat source, the permeability and depth of the disturbed zone, the presence of deeply buried water or hydrated materials, and the rate of burial of the impact melt (e.g., Newsom et al., 2001). The lifetime of hydrothermal systems, which is perhaps long enough to create or sustain a biota, has been estimated as 104 – 105 years for terrestrial craters 100 km in diameter, and up to 106 years for 180-km diameter craters. Impact-generated hydrothermal zones may be quite common in areas of subsurface water or permafrost, such as those areas present in the high latitudes. The surface manifestation of such a system could be mineralogical or morphological, but would be co-located with an identifiable impact structure from which it was generated.

Serpentinizing terranes.

The single most widespread environment of *chemical* disequilibrium on present-day Earth is the oceanic crust (Deming and Baross, 1995; McCollom and Shock, 1997). The composition of the modern lower crust and upper mantle of the Earth is essentially the same as that of the early Earth and Mars (Nisbet, 1987; Longhi et al., 1992), and the early histories of these two planets are similar. It follows that an understanding of these zones of chemical disequilibria on Earth would be of great value in devising a search strategy for similar regions on Mars.

In addition to being potential sites for the genesis of life, hydrothermal systems associated with serpentinization are also excellent candidate sites for the study of prebiotic biogeochemistry. On Earth there is abundant evidence for the formation of abiotic organic compounds along the modern mid-ocean ridge system where it has been linked to serpentinization (H2 source) and hydrothermal activity (Rona et al., 1992; Bougault et al., 1993; Charlou and Donval, 1993; Holm and Charlou, 2001; Schroeder et al., 2002; Kelley and Fruh-Green, 1991; 2001). Serpentinization has also been linked to hydrogen and methane generation onshore in association with ophiolites (Neal and Stanger, 1983; Abrajano et al., 1988). This may also be an explanation of the observations of methane in the Martian atmosphere (Kerr 2004a,b)

An excellent example of subsurface life on Earth is associated with the “Lost City hydrothermal complex” located in an off-axis area of the mid-Atlantic ridge hydrothermal system (Kelley, et al. 2001). Similar sites have been described elsewhere (Chapelle et al, 2002; Stevens and McKinley, 1995; Mottl et al., 2003). In locations such as this, ultramafic rocks from the oceanic crust react with water to form secondary minerals such as serpentine. The process is exothermic, and yields a volume increase of nearly 60%. This type of hydrothermal activity is distinct from all others in that no external source of heat is required (the heat generated by the reaction is sufficient to initiate or perpetuate the system), and the volume increase produced by the reaction results in a self-perpetuating system in which cracks formed in freshly altered material create pathways for water to react with fresh ultramafic rock. The process of serpentinization, through which olivine and pyroxene are altered into serpentine minerals, can be generally described as:

olivine + H2O = serpentine + brucite + magnetite + H2(1)

and

olivine + pyroxene + H2O = serpentine.(2)

Reaction (1) could provide a biological energy source through the production of H2, the basis for many chemoautotrophic biochemical processes, including methanogenesis (CO2 + 4H2 = CH4 + 2H2O).

The serpentinization process should be relevant to present-day Mars, which lacks plate tectonic processes, and even to an ancient Mars that never developed standing oceans or large-scale plate tectonics. The apparent widespread distribution of olivine-rich basalts at the surface of Mars as well as reported outcrops of olivine on the Mars surface (Hoefen et al., 2003) suggest that interactions of ultramafic rocks with water might have been commonplace in the past.

4.Meridiani type areas – hematite or water-associated mineralogy.

Prior to the MER missions, remote and spectroscopic images of Sinus Meridiani suggested an ancient (~4 Ga,) wind-eroded subarial or subaqueous sedimentary comprised of 10-15% hematite. As reviewed by Christensen et al. (2000), five possible mechanisms that involve water could explain the formation of the hematite deposit at Sinus Meridiani: (1) direct precipitation from standing, oxygenated Fe-rich water; (2) precipitation from Fe-rich hydrothermal fluids; (3) low-temperature dissolution and precipitation through mobile ground-water leaching; (4) surface weathering and coatings; and (5) thermal oxidation of magnetite-rich lavas. Allen et al., (2001) discussed, on the basis of terrestrial examples, the possibility that a Martian hematite deposit could be associated with microbial mediation and discussed: (1) four possible mechanisms for producing banded iron formations; (2) the accumulation of iron oxides in hydrothermal deposits; (3) formation mechanisms for iron-rich laterite and ferricrete soils; and (4) the association of bacteria that can oxidize ferrous to ferric iron at neutral pH in rock varnish. It is clear from the recent discovery of buried and exhumed hematite concretions and impact ejected hematite-rich rock near the MER landing site that the area exposed to iron-rich fluid is quite extensive, and much remains to be learned about its origin (Squyres et al., 2004, Kerr 2004c commentary). Such sites are important not only for elucidating the history of water on Mars but also because aqueous mineral precipitates could preserve evidence of an early biota, prebiotic chemistry, or exogenous delivery of organics to the planetary surface during the heavy bombardment period.

Sub-ice Volcanos

A distinctive source of hydrothermal fluids and water-rock interaction is volcanic eruptions into ice or icy regolith. These eruptions necessarily involve heat, liquid water, and reactive rock (fresh lava), on which a biota could thrive. Evidence of “catastrophic outflows” of water from beneath polar caps is reminiscent of similar environments in Iceland and elsewhere, where sub-ice volcanism might create habitats for life. Evidence of habitable under-ice environments might reside within frozen outflows that extend outward from the margins of the polar caps.

The advantages of seeking sub-ice volcanos on Mars are: [1] Volcanos, ground ice, and surface ice are known to be present, and [2] Sub-ice volcanos produce distinctive landforms, easily recognized from orbital imagery. Point eruptions beneath ice produce a characteristic landform, a tuya – a sharply bounded mesa, capped by lava flows, and commonly with volcanic cones and flows visible on its top (Allen, 1979; Hodges and Moore, 1979). Fissure eruptions beneath ice produce distinctive, parallel Moberg ridges (Allen, 1979). Many hills in Mars’ northern plains resemble tuyas, at least in Viking imagery (Allen, 1979; Hodges and Moore, 1979), and the Valles Marineris interior deposits have been similarly interpreted as tuyas (Chapman et al., 2003).

## 7.3 Ice

Science Theme: Assess the potential for Habitation in Icey samples

All life on the Earth is constructed from 2 major ingredients: Water and organic carbon. One of the basic investigation AFL will perform is the identification and inventory of organic carbon species on the Martian surface. The understanding of the nature and chemistry of carbon on Mars can help elucidate astrobiology principals and help us understand the potential of Mars as an enclave of life. The other key ingredient of life, water, has been shown to be present in the polar caps as well as mixed in the regolith at higher latitudes. Therefore a search strategy including exploring a sites that contains a significant amount of H2O (i.e. follow the water) is a possible mission scenario for AFL.

Orbital data has indicated that there exists sub surface water ice in large quantities, as well as making up the majority of the northern polar caps. Mars Odyssey has detected large amounts of subsurface Hydrogen, especially accessible in the northern plans indicating that there exists a reservoir of subsurface H2O (Feldman et al. 2002, Anfimov et al. 2002). This water has been systematically moved from the low latitudes where geologic features indicate there was water present at one time and redistributed in the higher latitudes region (Mellon and Jakosky 1995, Crisp et al. 2000).These permafrost like regions constitute a mixture of regolith and H2O that is accessible in the upper few meters and is accessible by a rover. The current orbit Mars Express orbiter will be deploying the MARSIS orbital radar to better map the subsurface water distribution, and the up coming SHARAD instrument on the Mars Resonance Orbiter, will be able to produce maps of subsurface water to a better resolution and sensitivity then is possible from the Odyssey data. This mapping of the subsurface H2O will enable a determination of the accessibility from a rover type platform, and hence its likelihood of exploration by AFL.

While the current temperature and pressure conditions on Mars does not allow for stable liquid water on the surface, it potentially can exist in a meta stable state in some specific environments (Hecht 2002). Additionally, it has exited in the geologic past when Mars possessed different orbital and atmospheric conditions which allowed liquid water in at least transient states (Malin and Edgett 2003). This can be demonstrated by numerous geomorphoicial features, photographed from orbit, which were created by large amounts of liquid water as well recent evidence found by the MER rovers of evaporative deposits from standing water (Squyres et al. 2004). If life formed on Mars it may still exist in an environment where it has access to H2O and energy to sustain itself. If life never started, discovering the differences between Mars and Earth is vital for the determination of how prevalent life is in the universe. Visiting a site with ice can help us understand both possibilities.

Life also has the ability to exist in terrestrial environments where the temperature is below 0°C for a vast majority of the time. These organisms can exists in environments where only occasionally does the temperature rise above freezing, (Nienow, et al. 1988; Friedmann, et al. 1993), in regions where it reduces the freezing point of water by existing in either brine solutions or excreting chemicals to lower the freezing points of the water (Junge, et al. 2004) and by potentially becoming dormant only to repaired itself in intervening thaw periods (Thomas, et al. 2000; Bakermans, et al. 2003; Gilichinsky, et al. 2003). These vastly different terrestrial settings all have analogies on present day Mars which makes them interesting targets for Astrobiology in situ science.

Finally, there is the exciting possibility that a preceding Mars lander mission making a compelling discovery and having AFL return to that same location. By visiting the same site that a previous mission has explored, at least some of the preliminary reconnaissance of that region, can be accomplished. For example, the Phoenix 2007 scout lander will be performing investigations of the chemical compositions of the soil including bulk constituents and mineralogy (TEGA with MS) and astrobiologically important characteristics (MECA) such as Redox potential, pH, and trace metal content, among others, in a region of the Northern permafrost regions. If compelling science discovery is made at this landing site, a follow up mission will be able to expand upon the discoveries. This can be thought of as being analogous to the early practice of planetary flybys followed by orbiters, and then eventually a lander or two. There are also possible discovery driven missions in response to MSL in 2009, and a scout mission in 2011 which an 2018 AFL can capitalize on.

*Proposed Landing Site Geologic Setting*

Recent orbital data from Mars Odyssey has located potential water ice that can be accessible to a rover with access to the near subsurface (up to 2 meters) (Boynton, et al. 2002; Mitrofanov, et al. 2002) in vastly different geological settings of high latitudes. We have identified several of those sites as potential sites for exploration by the AFL to include but not be limited to:

Northern Polar Caps

Northern Polar Layered Deposits

Northern Permafrost regions

Site with recent evidence of ground melt

The northern and southern polar caps are different both in composition and geologic setting (Thomas, et al. 2000). This includes the age of the deposits in which the southern cap can be 2 orders of magnitude older then the northern one (Herkenhoff 2000; Thomas, et al. 2000) The northern polar caps offer a better target for AFL exploration then the southern cap due to H2O (Vs CO2) and geological formations including layered deposits which can have a record of part geologic and climatologic activity (Thomas, et al. 2000). These polar layered deposits can be created by Aeolian processes which can strip material from the base of the scarp. A mission to the polar caps would obtain and analyze ice cores for remnants of biological activity. Orbital data indicates that recent activity Martian gullies has taken place, and that this can be a result accompanied by submission and ablation (Howard 2000; Edgett, et al. 2003) of ground melt (Malin and Edgett 2000). This indicates that there is some cycling of material in the near surface ground which has potentially huge astrobiology relevance.

*Proposed science objectives and requirements*

The science objectives for the mission to an ice rich environment include the search for both extinct and extant traces of life. Due to the different types of sites that can be visited, these science investigations require different payload accommodations which would need to be made when the instruments are selected to fully maximize the science return for the AFL. The universal science objectives for any exploration of ice rich environments include:

Detect the geo-chemical remains of extinct life.

Determine the potential for extant life in an environment where H2O is present.

Detect of dormant organisms in an environment which can periodically contain liquid water.

Determine if extant life is in contact with the Martian atmosphere elsewhere on the Martian surface.

Understanding the long term climate and geological evolution to determine if Mars could have been habitable in the past.

One underling theme of astrobiology is the differences in planetary evolution and how that relates to habitability of planets. If Venus, Earth and Mars all formed in the “Habitable zone” of the sun why is Earth the only one to be teaming with life? An AFL mission to high northern latitudes can help elucidate this concept, by helping to understand both geologic and climate changes on Mars over it’s history.

Ice exists on Mars in vastly different geologic settings and therefore there are several major differences in the science requirements both with respect to ice bearing regions as well as other Martian regions (i.e. sedimentary and hydrothermal environments). Here we will discuss science requirements that span the different geologic settings, above and beyond what the core AFL science requirements. As mentioned previously life can exist in these locations by either becoming dormant until conditions exist where the temperature is above freezing point of water, or by creating pockets of liquid water by lowering the freezing point of water. Determining if an acquired sample contains liquid water requires the collection of sample without raising the temperature above the local melting point of water (keeping in mind that the concentration of brines in the sample can dramatically lower the melting point below 0°C). The determination of liquid water in a sample is not necessarily a measurement of life, because liquid water can exist in meta stable state in some Martian environments without being associated with life (Hecht 2002). However, samples containing liquid water would be a priority target to be analyzed by the analytical laboratory instruments. In the Northern polar layered deposits the measurement of strata of layered terrain to see potential differences in layering and effects due to Aeolian processes. This would require imaging at several spatial scales.

A determination of the yearly cycling of CO2 and H2O will not only lead to a better understanding both current and past atmospheric dynamics (Clifford, Crisp et al. 2000) it can potentially identify if a biosphere is in contact with the surface elsewhere on the surface. Recent discovery of methane in the atmosphere from both ground based observations and from the Planetary Fourier Spectrometer (PFS) onboard the ESA’s Mars Express, although most likely not from biologic process, demonstrates that a better understanding of atmospheric process are needed (Kerr 2004a,b). If biology is in contact with the atmosphere, this maybe detectable from orbit (i.e the recent measurements of methane) but whether life produced these gases can only be ascertained by painstaking surface measurements.

*Science Trades*

Because potential ice missions have different geologic regions there are several science trades that can be made so as to maximize the science return of the mission. The first science trade that can be made is the level of mobility requirement. For missions to the permafrost regions and on the polar caps potentially require very little mobility (only 10’s to 100’s of meters) depending on high resolution orbital mapping by Mars express and Mars Reconnaissance Orbiter. Current orbital data on those scales indicate not much difference in geologic setting over km distances. Therefore large surface mobility could be not as scientifically important as it is for other regions. There would be, however, a need for greater subsurface access including drilling well below 1 meter to increased ice concentrations. Therefore a potential trade of horizontal distances vs. depth, would need to be made

On the other end of mobility spectra is the recent ground water site which can require large “goto” capability of at least the level of the landing precessions if of a landing ellipse can be placed near that site. This may require mobility in the 10’s of km, similar to what would be required in the sedimentary region.

The nature of high latitude northern sites indicate that for extended missions nuclear power is most likely the only feasible alternative for mission power generation as Mars progresses through its year. However, for more equatorial missions solar power can be a feasible alternative especially given the projected longer lifetimes that the on going MER missions are demonstrating. This trade will depend on the expected duration of the mission and ground operations and ability to land at high latitudes as set forth in the science requirements.

*Site Specific Measurements and sample handing and preparation requirements*

Measurement requirements are dependent on location. The measurements that are required for ice missions resemble the instrument complement for the other missions scenarios postulated (hydrothermal, and sedimentary deposits) and the measurements requirements can be found in section 8.2. Here we discuss measurement requirements specific to ice regions.

Remote instruments

Mast based instruments must be able to do visual site reconnaissance at a level at least as well as PanCam on MER. Identifying potential targets from the distance of a daily traverse should be a requirement so that interesting samples can be targeted. Remote mineralogy of potential samples from a distance of 10 meters so that samples can be identified. The remote mineralogy instruments may have to account for ground frost when choosing a spectral range for a mast-based instrument. These requirements are virtually the same regardless of the environment AFL explores. In addition, if AFL is going to perform subsurface sample acquisitions in a high H2O environment, some subsurface reconnaissance must be done, especially if H2O varies dramatically in depth over 1 meter scales. A body-mounted detector capable of reconnaissance styled elemental abundances would also be desirable measurement if feasible and kept within the cost cap of the mission. This measurement could detect high potential astrobiological sites, as well as ground truthing orbital data. Finally, for polar cap missions, the cycling of H2O and CO2 and the interaction of those molecules from the surface to the atmosphere needs to be determined. The Martian atmospheric dynamics is not currently in equilibrium (Clifford, Crisp et al. 2000) (i.e. Aeolian processes, ablation and sublimation) Determining the atmospheric polar properties can help put a constraints on atmosphere compositions and help determine if a biosphere presently exists, as well as long term possibilities that a more favorable climate once existed. This is especially true given the recent detection of methane in the atmosphere at trace levels by both ground and orbital observations.

Contact Instrumentation:

The instrument delta between AFL ice and other AFL missions is that direct detection of liquid water present in a sample needs to be made. The Phoenix lander is attempting to make this measurement as well, and lesions learn in that mission will affect the design of this measurement. For mission to the polar cap, any contact instrument will also have to account for the ice core that is being obtained.

Sample Acquisition and Processing:

All of the hardware infrastructure referred to in this environment must be able to handle relatively large amounts of water. This includes the drills, corers, and precession sample processing and distributions stations. Water can interfere with the drilling process either by making material harder to drill into or by melting and creating a mud like material that can interfere with machinery. Drilling into this material without melting the water or using drilling fluids will need to be developed and demonstrated in both a relevant terrestrial environment and under simulated Martian conditions. Finally, for missions to the polar cap, a different sample acquisition system will need to be developed. This instrument will have to be able to melt and sublimate any CO2 or H2O while collecting impurities in the ice material.

## 7.4 Water

*Science Theme*

Assess present (*and past?*) Martian astrobiology by studying liquid water in the shallow subsurface.

*Proposed science strategies*

Drill, core, or otherwise obtain liquid water sample.

Determine concentrations of redox sensitive aqueous compounds, including O2, H2, HCO3-, NO3-, Fe2+, SO42-, H2S, NH4+.

Determine presence (if possible, concentrations) of DOC and aqueous organic monomers, including carboxylic acids, amino acids, sugars, hydrocarbons (or should be target functional groups instead?).

Determine presence (if possible, sequence or composition) of organic polymers, including proteins, lipids, nucleic acids.

Visualize microbial cells (if present) with light microscopy on stained and/or unstained cells.

Carry out microculturing on 1-3 samples using tens to hundreds of pre-designed growth media at several different temperatures.

# 8.0Core Mission Components

As discussed in sections 6 and 7, there currently are multiple possible variations on the AFL mission theme. Opinions differ as to the specifics of these variations in terms of context and priority, which may lead to revisiting the chosen site if selected. However, the AFL-SSG feels that it is possible to define an invariant core, which is common to most versions, along with a discovery-responsive and competition-responsive cap.

The proposed mission requirements to ensure the greatest scientific return for the AFL mission include:

“Go-to” mobility (ability to access a specific target).

When sites are identified from orbit that possess high astrobiological interest (see Section 6.0) the rover has to be able to access them, even if the nearest safe landing site is 10’s of km away. The rover also has to explore several different regions within a high interest site. An example of this is Holden Crater (see Section 7.1) in which what resembles an ancient river delta is clearly visible in orbital images. Exploring the specific features found there would require not only a landing ellipse directly outside the feature but the ability explore several different locations several km’s apart within the potential delta system.

+60 to –60 (seasonal polar cap) for sedimentary/hydrothermal. +45 to +85 for ice mission (See section 7.3).

Precision landing (1 km) and the ability to land in terrain that is rougher than we have targeted in the past (hazard tolerance, hazard avoidance).

In order to access more of the planet for exploration by AFL, as well as limiting costly “Go-To” traverse, having a suitable landing ellipse smaller then 10km is required. This enables access to regions like Melas Chasma, where suitable landing ellipses greater then ~5 km prove difficult to identify.

Subsurface access of 1-3 m, and multiple holes. Probably also have a need to expose / drill into material in outcrops .

Organic material on the Martian surface may be extremely scarce, primarily due to an oxidizing layer thought to exist because of UV fluxes at the surface. How far down this oxidant penetrates is not presently known or constrained, therefore shallow (<3 meters) subsurface material may be void of organic material. Accessing and analyzing this material may indicate if extant life is possible in a protected subsurface environment. However, if the surface regolith is largely made-up of unconsolidated material, organic free material may be thoroughly mixed by several billion years of global dust storms. In this scenario all organic material may have destroyed down to >3 meters, making analysis of this material a lower propriety (hence not a requirement). Subsurface access of potential bedrock and out-crops is highly desirable in any scenario where it is present.

Organic contamination: be able to collect and deliver Earth-clean samples to on-board laboratory

It is a requirement to have samples that are not contaminated by terrestrial organics to a level greater then the minimum level of detection of the astrobiology specific instruments. See report of the Organic contamination Science Steering group (Mahaffy et al., 2004).

Sample preparation including spatially controlled precision sub-sampling and liquid extractions for selected high-potential samples.

The AFL-SSG has determined that identifying the best possible sample for analysis is a primary requirement for a future AFL mission. See section 8.3 for a discussion of these requirements in more detail.

## 8.1 Payload strategy

It was determined that payload characteristics could be defined as core to any potential AFL mission concept as described in Section 7. These include:

Acquiring the right sample.

In order to maximize the probability of detecting biosignatures in a ***location with the high general habitability potential*** has to be identified. Several of the reconnaissance missions (see section 6), will be used to identify this location. In identifying the location, the ***understanding of the preservation potential*** of this location must be better understood. The Earth is inundated with biological material, where most (if not all) sites on the surface (and possibly the subsurface) should have a continual influx of biologic material. On Mars this is not the case. A location on Mars which once supported life, may not have any record of that life, due to chemical interactions, or by meteoritic impacts. Understanding how a site on Mars preserves a record of past life is essential toward acquiring the right sample. In this regard there is the need to be able to access samples with the highest probably of being astrobiologically important. This includes both ***identification of specific samples*** as well as the ***ability to acquire that sample***.

Understanding the geological, mineralogical, and chemical context of that sample

The labeled release experiment aboard Viking, released nutrients into a Martian regolith sample to determine if metabolism took place. The results of this experiment on their own can indicate that metabolism was taking place. However when taken with the GC/MS data it was generally understood that a chemical reaction was taking place within that sample due to the oxidants present in the surface material (Mancinelli 1998). A complete understanding of the relationships between geological, mineralogical and chemical characteristics of the sample is needed to determine Astrobiologically implications of analytical measurements.

Identifying the best place on the sample

Instead of introducing a core into a bulk rock crusher, in which most of the material will not be analyzed, it was determined that sampling of small features of a sample would be required. Section 8.3 describes this precision sub-sampling in more detail.

Performing at least 3 different Astrobiologically related measurements.

The detection of biosignatures on Mars would, to put it mildly, fundamentally change our perception of life else where in the universe. In order to avoid ***potential false positives***, three separate measurements would need to be preformed on a sample to confirm any one measurement. Furthermore, repeat measurements will also help to avoid ***false negatives***. Since Martian life may be very different from terrestrial life, different measurement techniques may return a positive, while others measurements may miss more subtle signs that life is present in the samples. If one or two instruments detect interesting signatures, future missions can be designed to further explore the same site for these signatures.

## 8.2 Core Measurements and Instrumentation

As stated in Section 5.2, the proposed overall scientific objective of AFL is, for at least one Martian environment of high habitability potential, to further investigate the potential for habitability, the potential presence of the chemical precursors of life, the potential for preservation of biosignatures, and possible signs of life. This is to be accomplished through measurements supporting the following (un-prioritized) detailed ***Mission Objectives***:

Within the region of Martian surface operations, identify and classify environments (past or present) with different habitability potential, and characterize their geologic context.

Quantitatively assess habitability potential:

Measure isotopic, chemical, mineralogical, and structural characteristics of samples, including the distribution and molecular complexity of carbon compounds.

Assess biologically available sources of energy, including chemical and thermal equilibria/disequilibria.

Determine the role of water (past or present) in the geological processes at the landing site.

Investigate the factors that will affect the preservation of potential signs of life (past or present) on Mars.

Investigate the possibility of prebiotic chemistry on Mars (including non-carbon chemistry).

Document any anomalous features that can be hypothesized as possible uniquely Martian biosignatures. This will constitute a set of working hypotheses, which will need refinement and further testing on Mars.

The following ***Measurement Requirements*** for the AFL Core, derived from these objectives, were specified in order to support the instrument development and selection process for AFL:

**Comprehensive Imaging** - Fully image the overall landscape and each investigation scene to assess the variety of local environments (past or present) that can be discerned from expressed surface features such as outcrops. Include both color optical stereo imaging and higher-resolution long-focal-length telescopic imaging of key areas of high interest for further investigation of habitability potential. Target range is 1 m to infinity/horizon. High magnification or high resolution imaging should be able to discern layering at the 10 cm scale from a distance of 1 km. These measurements support the decision to focus more closely on specific sites, targets, and samples. *Supports Objectives: 1*

**Definitive Mineralogy and Chemistry** - Determine mineralogical and chemical (elemental) composition at all scales of investigation: *site/scene surface reconnaissance scale* (range: infinity/horizon to meter; resolution: km to cm), *hand-sample scale* (range: meter to cm; resolution: cm to mm), and *acquired subsample scale* (bulk measurement of a few-mm subsample with high accuracy), with respectively increasing degrees of definitiveness and sensitivity. *Supports Objectives: 1, 2, 3.*

**Redox Potential** - Assess the redox potential and oxidation chemistry of the near-surface environment. This measurement details how much energy is available for an organism to use in growth and reproduction and would be required to be measured to a precession of 10 mV. *Supports Objectives: 2, 3*

**Fine-Scale Surface Analyses** - Investigate the surfaces of exposed or acquired samples at fine scales for morphological, chemical, and molecular signatures suggesting preservation of pre-biotic or biotic organic compounds. This may include directly-detected compositional markers, evidence of minerals formed in or altered by liquid water, or particular sample textures (i.e. concretions). Color optical imaging with resolution below 30 m (although for bacterial analysis in anything other than a macroscopic biofilm structure this would be inadequate) within a larger field of view should provide the context for co-focused spectroscopic tools such as UV-excitation fluorescence, laser Raman, or other fine-scale techniques to perform chemical signature detection. Spectroscopic tools must be able to analyze mm-scale regions on surface or drill core samples (e.g., through a focused excitation source or through high imaging/detector resolution). These surface measurements provide first-order astrobiological analyses and support the intelligent selection of subsamples to be processed in the analytical laboratory. *Supports Objectives: 2, 4, 5*

**Subsample Biosignature Analyses** - On selected subsamples, perform an array of high-sensitivity, mutually-confirming laboratory investigations related to astrobiology goals. *Supports Objectives: 4, 5*

The identity, abundance, and isomeric distribution of carbon compounds should be thoroughly analyzed to low detection levels (ppb or below by weight within bulk ~102 mg subsamples) and to high molecular weights (hundreds to thousands of Da) at high peak resolutions (~2000 FWHM). Measurements utilizing broadband techniques such as pyrolysis GC-MS should be configured to enable the detection of less volatile species that are particularly relevant to determining preservation of biosignatures.

The isotopic ratios of H, C, N, O, and S should be characterized with sufficient precision to enable biogenic, environmental, or meteoritic fractionation trends to be identified based on requirements determined from MSL and other measurements (sub-per-mil to % levels). Compound-specific 13C/12C ratios coupled to the analyses in (1) are highly desired. Additional isotope ratios that further characterize atmospheric loss and other environmental fractionation processes relevant to astrobiology are also desired. Analyses may also be conducted on atmospheric samples to provide a more complete understanding.

Highly sensitive tests for the presence and characteristics of specific biosignatures should be conducted on bulk subsamples or isolated downstream extraction products (e.g., phases or concentrates). Biosignatures of particular interest include molecular compounds (or abundance patterns thereof) of distinctly biological origin as known on Earth, indicators of extant metabolic processes such as disequilibrium chemistry (molecular, biogeochemical, agent response, etc.), as well as chemical and morphological traces of such compounds and processes as preserved in the mineralogical microenvironment sampled. While the specific tests to be conducted will depend on the chosen AFL landing site and previous mission results, *examples* include detection of amino and nucleic acids, lipids, and proteins (with ppt detection limits if possible); chirality of amino acids and sugars (with %-level enantiomeric excess detection sensitivity); detection of concentrations of distinct molecules or isomers of the potential abiotic inventory that may represent the use and or concentration of a fraction of the molecules available through non biological interactions and finally direct detection of microbes, cells, or their fossils.

It must be mentioned that the advent of micromachining and the concept of micrototal analysis systems (uTAS) mean that through miniaturization the payload described may be integrated into a very small space whilst retaining accuracy and possible increasing analysis times.

The above information is summarized graphically in Figure 5.

Within the proposed AFL strategy, techniques to address the above requirements are structured in “tiers” following the expected level of physical sample contact: remote/standoff; contact; and laboratory. In the remote/standoff tier, the target “sample” is a wider area and not acquired by definition. In the laboratory tier, a small sample of interest has been acquired and possibly subjected to a preliminary analysis that supported the decision to subsample and deliver it to the laboratory for further analysis. However, in the contact tier, the sample may be analyzed before or after it is acquired (or both). This is designed to allow multiple levels of “triage” for determining the appropriate course of action with a given sample. An example of a post-acquisition contact measurement is a point-by-point imaging and chemical analysis along the surface of a several-cm long core. Based on this analysis, it may be decided to grind and/or otherwise process some or all of this core for analysis in the laboratory. For a description of the suggested mapping of measurements onto instruments placed in each of these tiers, refer to Section 8.1.4.

For completeness, the connection between the *AFL measurement strategy* and the *mission objectives* may also be characterized by indicating those objectives addressed while conducting the following activities:

Acquire the right samples (primarily 1; also 3)

Understand the context (primarily 1, 2; also 3, 4)

Identify the best place on the sample (primarily 5; also 2-4)

Perform mutually confirming astrobiology measurements (primarily 5; also 2-4)

This is summarized in Figure 5.

As mentioned above the instrumentation recommended for the Astrobiology Field Laboratory is divided into three categories or tiers: 1) remote sensing instrumentation located on a deployed mast, 2) a contact instrument suite located on a robotic arm, and 3) the laboratory suite located inside the rover and/or platform and fed with a sample acquisition and distribution system. The remote sensing suite is used to provide site characterization and rover navigation targeting. The contact suite performs “triage” analyses, mimicking a field biologist/geologist. The laboratory suite performs the detailed biology, chemistry, and mineralogy experiments required to quantitatively assess samples for past or present biological potential. Sample analysis instruments are supported by sample acquisition and processing infrastructure such as an articulated corer, (cm to 1 m) a rock abrasion/polishing tool, a precision subsampling tool, and possibly a 2.5 m drill.

The remote sensing suite includes at a minimum a panoramic multi-filter camera system that is used for site characterization, rover navigation, and first-order target selection. Additional instrumentation that may also be desirable may include reconnaissance-scale chemical and mineralogical experiments, such as hyperspectral imaging, stand-off (multi-meter) laser induced breakdown spectroscopy with fluorescence and Raman detection, and thermal infrared mapping for identifying geothermal sources of heat within the near-horizon of the Martian environment.

The contact suite must provide the second order triage for sample selection. The analogy is the selection and preliminary analysis of a surface material or hand sample by a field biologist or geologist. A sample arm equipped with an articulated coring drill and a rotating abrasive tool for clearing and polishing rock surfaces is envisioned for contact arm infrastructure. The contact suite includes at a minimum a course resolution (~20 m) microscope to examine the texture and other features of rocks and fines. Sample triage on AFL will however require additional contact instrumentation that further identifies materials of high interest for subsequent precision subsampling and laboratory measurements. The complement of contact instruments will be determined by the objectives at the type of site chosen for AFL: sedimentary, hydrothermal, ice, or liquid water. Possible arm-mounted spectrometers include: near infrared reflectance, Raman, Mössbauer, APX, deep-ultraviolet fluorescence, and/or various types of laser ablation sampling spectrophotometers and direct-inlet mass spectrometers. These tools are used to probe for and characterize samples of potential biological interest that may be delivered to the laboratory analysis portion of the payload.

Figure 5. AFL Measurement Requirements

Generated

The presence and design of the laboratory portion of the AFL payload is predicated upon a high degree of flexibility with respect to sub-sampling of the acquired rock core or soil sample. Therefore, there should be a strong emphasis on an *integrated* analytical laboratory approach to fully characterize common or related sub-samples: using microscopy as the “eyes”; definitive mineralogical and chemical identification from techniques such as x-ray diffraction, x-ray fluorescence, and laser ablation; and organic chemical and stable isotopic analyses that include at a minimum instrumentation capable of similar measurements to a pyrolysis-gas chromatography-mass spectrometer. Enhanced capabilities for identification of trace pre-biotic or biochemical compounds may be provided by staining followed by fluorescence detection techniques, solvent extraction/derivatization followed by a suitable ion mobility or mass spectrometry system, and other more specific techniques that target the detection of biomarkers such as amino acids, proteins, and/or DNA such as capillary electrophoresis, use of specific probes i.e. polymer or antibody systems and chemical assays. The particular implementation of more-specific biological/chemical analyses will depend both on the results of prior missions, such that their design and interpretation is advised by a solid first-order organic chemical characterization of Martian surface samples, as well as through analog field experiments targeted at terrestrial extremophiles. Additional capabilities such as detection of enantiomeric excess (chirality), rock dating, and fine-scale chemical imaging would be strongly complementary to the laboratory suite and highly desired for AFL. Such experiments might be provided by enhancements of previously mentioned instruments or by additional instruments.

The final selection of instrumentation on AFL will be based on a careful cross-matching of measurement requirements to instrument capabilities. It is recommended that the payload resources (mass, power, cost), and thereby the mission scope, for AFL be fundamentally and primarily driven by the sample preparation and instrument needs that are required to fulfill the measurement goals, rather than vise-versa. New instrumentation techniques as well as methods to optimally integrate techniques are desired and encouraged, but these must be maintained within a reasonable cost-risk profile. This necessitates a well-funded, well-advanced instrument development and integration program with strategic oversight form cognizant AFL program members.

The core measurements of AFL has been decided upon to answer the specific questions posed in the science rationale. The high number of instruments on this mission definition is a direct response to the findings of both sedimentary and hydrothermal deposits by the Mars Exploration Rovers and the subsequent realization that samples of Astrobiological interest may be much more accessible than originally thought. This allows deep drilling to be traded off against increased number of instruments.

## 8.3 Sampling and Precision Sub sampling

According to the various mission scenarios, different types of samples will need to be obtained, i.e. from rock, ice, regolith and sedimentary samples. The design of the SHAP facility and the exact number of samples to be handled and processed will depend on which mission scenario is decided on. This number will help define the sample collection system that will have to be developed. The basic concepts and design of the facility, however, will in principle remain the same for each type of sample and each type of measurement to be performed. Four different facets of the overall process are identified:

Obtaining a sample

Precision sampling of that core

Liquid and Heat extraction of organics

Contamination concerns

Issues were discussed with respect to each of these types of environments and are discussed in more detail in the following.

### 8.3.1 Obtaining a sample

Several sample acquisition tools are suggested for integration into SHAP facility. In order to be more precise the following defining terms have been made:

Corer: A device that can obtain a core which is ~ 5 cm in length with ~ 1 cm in diameter from an outer region of a rock on the surface of Mars.

Drill: A device, which can obtain sample from inside of a rock permafrost or sediment (cm – 1m) or from a distance underneath regolith (1-3 m).

Precision sub-sampling mechanism*:* A device, which can obtain a representative sample form a larger core. This would replace the rock crusher, which crushes the entire core, and produces fines, which might not be representative of the entire core.

Scoop: A device which can collect either fragments from a RAT, or unconsolidated regolith or permafrost material from the surface.

An aspect to consider is that idly sitting and drilling for significant time spans on Mars, drilling does not seem to be an efficient use of a limit mission lifetime. However, a system that could drill while not shaking the rover or consuming major power would need to be developed. For example, if an instrument such as XRD or deep UV fluorescence can analyze rock fines and fragments during drilling, we can identify samples that might be interesting, either because they are a fundamentally different type of mineralogy, or because of interesting organic components detected in them. This will not only help in sample identification, but also in the operations profile so that overall more samples can be analyzed.

Ice:

A drill would be required capable of gathering an ice rich sample while avoiding sublimating the ice, melting it, or volatilizing any constituent molecules. The sample must be as chemically similar to the material it is collected from in order to do a proper analysis. This most likely requires nighttime drilling operations in ice rich environments with a drill cooled to a temperature lower than that of the ice to mitigate cutting, melting and drill trapping problems .

Permafrost regions:

Samples that contain a mixture of ice and other material will have to be specially treated. Terrestrial permafrost can create problems when attempting to obtain a core from it in terms of both hardness and ability to keep the sample pristine. Samples will need to be obtained from a device that can be used multiple times while not heating the sample above freezing.

Surface Rock:

The depth MSL is coring to, 5 cm, seems to be a good number for the depth inside a surface rock that should be sampled. Terrestrial organisms practicing photosynthesis inside rocks inhabit approximately the outer 2 cm of sample a rock. Sedimentary material can be identified by obtaining several cm in length cores. It would be desirable to analyze the fines from the core if coring is progressing by a pneumatic device for introducing fines into the analytical suite. In this scenario, the XRD can analyze material as it is chilled in order to identify regions of interest, since the XRD can do infinite number of samples. When an interesting sample is identified it can be further processed via the other analytical instrumentation.

Regolith:

Regolith material would be obtained by a scoop attached to the rover arm and be delivered to sample processing facility. It is worth considering that if the regolith material is unconsolidated, we may assume that it was never in contact with the atmosphere. If it was at some point in the recent past, it could mean that it would be chemically the same as the surface material, and hence not worthy of a drilling effort. Unless the rover is going to head to a site where larger amounts of near surface (to the depth of the drill) water drilling into regolith would be desirable, otherwise it most likely is not.

### 8.3.2 Sedimentary deposits:

If a landing site were selected where drilling into for sedimentary deposits is required, it would be preferable to look at the entire core length. If a complete core could not be obtained because of engineering constraints, having a borehole instruments that can be lowered into the borehole, and examining the entire length of it would be very desirable. Instruments that could be utilized to answer astrobiologically relevant questions (rather then pure geologic questions) would need to be identified. In this scenario it might be worthwhile considering a drill/corer combination, where a drill takes 5 cm cores at a time, delivers to the sample processing facility and be lowered back into the borehole and continue drilling to the desired depth. The ocean drilling industry has begun to develop instrumentation for borehole instrumentation where a geophysics package of instrumentation examines the bore hole. Further development along these lines would be invaluable to any drilling that would take place on Mars.

### 8.3.3 Precision sampling of a core

Once a core has been obtained, it would be injected it into a sample triage station. On this station we would like to look down the axis of the core with the contact suite of instruments, especially if the core was delivered intact. This way layering structures can be identified that might be indicative for a sedimentary material or any other area of interest in any type of sample. After the initial analysis, it is not necessarily desirable to process the entire core in an instrument like the rock crusher. If any regions/layers of interest have been identified in the core, these layers would be diluted/mixed with the other less-interesting layers, and thus make the analysis of the core material give results that are not necessarily indicative of a specific region. Furthermore, the rock crushing mechanisms could produce material that is not necessarily representative of the entire core, due to the crushing mechanism. Simply put, a precision sample handling system needs to be developed that is much more advanced then the rock crushers on MSL. This precision sample handling system would most likely replace the rock crushers on MSL as they are currently designed, because there most likely is not enough mass to have both type of instruments aboard.

The precision sample handling system would need to produce fines from a core that are on the order of 100 m, although finer material is always preferable. These fines would need to be produced from regions of the core that are less then 1 cm in length. Although an exact amount of samples that would be useful for each analytical instrument cannot be accurately defined without knowing what instruments have been chosen, a good working number is 100 mg.

A further developed system for AFL would allow for fines to be obtained from various parts of the core, where astrobiologically interesting signatures were observed. These fines then could be identified by the contact suite or by analysis of the fines that were collected during the coring. A possible method this material can be obtained is via a pneumatic drill, analogous to a dentist drill. This type of sample processing of a core needs to be further addressed, as it may require holding the core so that further processing can occur. If the core would be held in a fixed position, a grinder could grind parts of the core while those fines are collected. Any other material that is produced from this step could be looked at with a microscopic imager, if one is chosen as apart of the science payload, but no further analytical analysis of it would need to be made.

### 8.3.4 Ice Samples

Terrestrial organisms can maintain a layer of liquid water around them in an environment where the temperature is below the freezing value for that corresponding pressure. Therefore, one of the main science investigations with ice is to determine if there is any liquid water in a sample. In addition, volatile material present in the sample might undergo reaction and hence change its form, which might lead to incomplete analysis of the obtained sample. Because of these reasons, it seems desirable to obtain a sample without allowing the temperature to pass above conditions in which phase transition that any water (and potential brine solution) present would undergo for the ambient pressure and temperatures present. From permafrost, it is expected that a core approximately 5 cm long and 1 cm diameter would be sufficient for further studies, however, a larger core would likely allow more comprehensive analysis to be performed. With permafrost samples, the possibility should be considered to include a sample concentration device in the sample possessing suite. This concentration process would only take place after any measurement on liquid H2O on the pristine sample has been performed.

The overall strategy for a “polar” mission largely depends on the choice of landing site, i.e. whether it is permafrost or on the polar cap. If a polar region is decided upon, it is suggested that the obtained ice core is analyzed as a whole, especially if only 5 x 1 cm core are obtained. This based on the assumption that the recent ice deposits will not show much evidence of layering or zoning. However, should new data become available through *Phoenix* investigations, this approach may have to be restructured and a more capable precision sub-sampling system be integrated into this mission concept.

### 8.3.5 Liquid and Heat extraction of organics

Organic analysis has been one of the important measurements the Astrobiology Science Steering Group has identified that AFL should be able to perform. In order to identify organic material, they need to be released from the matrix material they are a part of, especially since surface organics on Mars might be very rare. Several ways to extract organics form rock samples were discussed, including using heat and solvent extractions. Each extraction technique has its advantages and disadvantages. However, it is currently not clear whether there would be enough mass to be able to perform both techniques on the same mission. Also, in an extended surface mission (~900 days), which is powered by a nuclear power source, either extraction technique would need to demonstrate that it can perform analysis on a large number of samples (exact number TBD) that such a mission would be able to collect. Different materials require different extraction strategies. For example, some liquid extractions will miss kerogen type material because of its high molecular weight and low solubility, while heat will destroy most of the more fragile biomarkers such as amino acids or hopanes, resulting in the loss of important molecular information. MSL will, most likely, have some form of heat extraction, although what this will look like will be dependent on the instrumentation that is chose for that mission.

Generally, more refractory, fossil, nonpolar compounds require organic solvent extraction. The choice of solvent depends largely on the polarity of the target compounds. Solvents commonly used are for example hexane, dichloromethane, toluoene, methanol, ethylacetate, propanol, or mixtures thereof. It is necessary to identify mixtures that have highest extraction efficiencies and at the same time covering the broadest possible polar-nonpolar stretch. Organic solvents would be needed for GC/MS sample preparation (and to some degree HPLC but this varies depending on column design and target compounds; H2O and methanol are commonly used as eluents, but could also be acetonitrile, dioxane, ethanol, isopropanol, etc.), in order to obtain molecular information from refractory compounds.

Aqueous solvents (such as super critical water) would be used for amino acid, DNA/protein extraction e.g. for microarray analyses, capillary electrophoresis, culturing experiments, flow cytometry or perhaps even PCR. Numerous proprietary and commercially available extraction kits exist using a variety of different solvent compositions, all being aqueous solutions. It would be required to identify optimized procedures and optimized solvents and/or solvent combinations in future laboratory experiments, e.g. using Mars analogue materials spiked with microbial cells and/or organic target compounds.

Other extraction technologies are currently available and need to be examined more in the context of Mars missions these techniques are not limited to but include; microwave, super critical gas (i.e CO2) ultrasonic and sublimation.

It is important to point out here that no judgment is made on which extraction technique is preferable. This is simply an attempt to identify which technique (if either) can be made a facility instrument aboard AFL, and hence have several instruments analyze the extract from the surface samples. Currently, different instrument developers focus on developing extraction techniques for their individual instruments. These techniques do not necessarily have much overlap from instruments to instrument. If consensus could be formed that a particular set of extraction mechanism is desirable (i.e. utilizing H2O at 100oC) it could necessitate a facility instrument to perform that extraction and pass the extract to different instrumentation. This would accomplish reduced mass and power requirements, as well as allow for several instruments to analyze the exact same sample.

Extraction conditions are currently being investigated utilizing different techniques solvents and temperatures. However, the extraction mechanisms need to demonstrate that they are small and repeatable. Null-results from AFL can have great meaning, but they need to be absolute and definable. The Viking GC/MS results showed no organics, but those results don’t necessarily mean there were no organics in the soil that was analyzed. The GC/MS has limits of detection that can be easily determined for single species. However recent work has demonstrated that the Viking ovens were set to a temperature that would have not released certain organics that could have been present in the soil (Glavin et al., 2001). In addition other types of organics could have been destroyed by the heat, and thus not detected. In order to determine what a possible null-result means, an end-to-end analysis would need to be carried out.

Pyrolysis heating:

The Viking landers each had ovens as part of the GC/MS system, although the ovens themselves were not able to reach the temperature necessary to detect some of the organics that could exist there. The Rosetta mission has a small oven, Phoenix has the TEGA, which has eight one-time-only use ovens attached to an EGA and a MS. MSL is intended to investigate multiple samples (24 floor, 78 goal) and has baselined a GC/MS as an instrument. If the development of a multi-use oven is not made, then it would require a ground decision as to whether or not to analyze a sample, if only a limited number of samples that can be analyzed. This would necessitate a science decision, which could delay other analysis on the surface, and limit the number of overall sites that can be visited.

Another prospect of the pyrolysis method is whether it could be designed in such a way that it is capable of concentrating signatures on a sorption trap. If so, any use of those traps will also have to be shown for the same number of samples that the rover will analyze. Also, as mentioned previously, all limits of detection should be for the entire end-to-end system, for a variety of different mineralogical samples.

Liquid extraction:

Liquid extraction is a more gentle way to extract organic molecules from rock and soil samples. One analogy to Martian surface investigations is the analysis of organics from meteoritic material. In those investigations, the organic molecules were released by either hot water extraction or by HCl extraction. Current development of novel techniques for the extraction of exophase biomarkers needs to continue, as does the determining the most efficient solvent extraction parameters. Should a sample be analyzed for its indigenous water content, it might require using another, yet to be determined, technique. In addition, different solvents can extract different types of molecules, water, as it approaches the critical point, becomes a good organic solvent. Clearly, more science groundwork has to be carried out to obtain comprehensive information to allow the best possible choice of solvents to be used. Other solvents that are used in the laboratory include HCl and other acids. These acids perform a more complete digestion of the matrix material, and increase efficiency of extraction, but are harder to handle because of their corrosive nature. With any solvent that is chosen for this step it should be noted that it would be able to concentrate the material to ensure a better signal to noise level.

It is currently unclear whether the liquid sample handling system needs to be completely reusable or whether one-time only use should be the preferred option. This information will become available as experimentation and technology development continues. The only stipulation that needs to be made is that the extraction technique minimizes mass and power resources.

Finally, there are other measurements that can be made during the extraction phase, which would not be possible during pyrolysis heating. These include pH, Redox potential of the material, etc. All of these measurements can help elucidate habitability issues and are an extra measurement that can be made, and if the liquid extraction step is a facility instrument should be made.

### 8.3.6 Contamination concerns

There are two issues that need to be addressed from for contamination concerns:

Contamination issues from organisms brought from Earth

Cross sample contamination

The issue of terrestrial contamination being detected and identified, as material present on a Martian sample is, by far, the main concern. Several different mechanisms can help reduce the possibility of this.

A sterile sample can be brought from Earth and run through the system for the first analysis to show that the end-to-end system is clean and contamination free. If this step produced positive results, it will show that the sample system was not clean and would have to be cleaned e.g. by flushing with a sterile material blank. After the initial sterile material is analyzed, surface dust could be analyzed next. This material is most likely sterile due to UV irradiation and is most likely homogenous across the planet. After the analysis of such sample through the entire system, this material can be used as a negative check for the entire system. If a sample is later found to have the signatures of life, analyzing another soil sample can perform a negative response check of those results, which will further validate the biosignatures that might have been identified.

The other form of contamination is sample to sample. While it should be noted that a general cleaning between samples should be performed, reducing the cross sample contamination should not be a major power and mass drain, which could be better used in other systems.

## 8.4. Time resolved Measurements

For some versions of AFL, time-separated repeat measurements (to observe changes) will be valuable, and these were strongly advocated by some members of the SSG. Given current understanding of Mars, we do not know enough to design the time gap that would be needed in such an experiment (minutes?, hours?, days?, months?), or the fidelity to which the subsequent experiment(s) needs to duplicate the conditions of the first in order to provide a meaningful hypothesis test. The AFL SSG takes the position that time-separated repeat measurements are not essential to all versions of AFL.  Thus, this should not be a part of the common overall mission scientific objectives. The AFL SSG recommends that the capability to do at least some time-separated repeat measurements be a general functionality of the surface science system, and that the decision on how and when to use it be deferred to the competitive process***.***

# 9.0Engineering analysis of AFL core

Based on input from the AFL SSG, a preliminary engineering design concept was defined so that basic mission parameters (such as mass, cost and power generation systems) could be developed. This was done so that technology developments that will be required to undertake the mission could be identified and pursued. This design concept was based upon the AFL SSG core mission requirement and included possible investigation of sedimentary, hydrothermal and liquid water regions. Other investigation (namely to ice covered and sub-surface ice regions) may require a different architecture and hence have a different mass, cost, and power generation systems. The mission architecture was defined by taking into account the measurement objectives, payload infrastructure rover mobility requirements and lander capabilities (Section 8). Given all these requirements and capabilities, a core AFL mission was developed.

The mission studied included 2 instruments for remote sensing placed on the main mast, 2 contact instruments located on an instrument deployment device (IDD), and 6 analytical laboratory instruments capable of analyzing samples obtained from the Martian surface for a total of 10 instruments. The analytical instruments, as well as the sample acquisition and processing infrastructure, will be able to process 25-75 physical samples (rock, regolith, and ice) for detailed analyses by both pyrloysis and wet chemistry instrumentation. Landers, Entry Descent Landing (EDL), cruise launcher, were defined in such a way to meet the mission requirements and so that costing the rover and mission could be done. In order to accomplish this, a list of generic instruments were identified so that parameters such as cost, mass, volume, and power requirements could be included in the engineering design concept. No attempt was made to identify and place individual instruments on the strawman payload (used to assess cost only) and where several instrument from different developers were identified, average mass power and volumes were used.

The engineering design concept assumed a launch in either 2013 or 2018 with a Technology Readiness Level (TRL) of 6 for instruments and subsystem technologies that would have to be reached by 2009 and 2014 respectively. Functional redundancy was required on all subsystems except for the science payload, and this included the sample acquisition and processing infrastructure. Landing site availability for the AFL SSG included access to the Martian surface between: +85 to –60 so that access to both ice regions as well as a wide variety of potential Sedimentary and hydrothermal regions can also be investigated. Landing altitudes of 2.5 km or less relative to the MOLA geoid should be reached within a 10x10 km (3-sigma) landing dispersion ellipse assumed for landing. Because AFL will be assumed to be a mission to a specific site of high scientific interest, rover was designed with “Go-to” mobility capabilities of 10-15 km (linear traverse range) so most astrobiology interesting sites could be reached and explored. For data transmission between Earth and Mars, either MTO or the second generation Mars Telecom Orbiter (MTO) was assumed to be available for Mars to Earth telecom greatly increasing the amount of data that could be acquired on the mission. The collected data would be passed to Earth via 0.3 m HGA for 1024 kbps link via MTO. This design allowed for a data intensive 1-3 GBits of daily science data generation. X-Band from rover direct to Earth (DTE) would be used for back-up purposes only. Finally the main power system of the mission was assumed to be a Radioisotopic Thermal Generator (RTG) system, although solar power could also be utilized for missions that are more equatorial, and potentially shorter in duration (depending on final MER mission power results). The power systems was sized to be able to provide sufficient power with reserves for “worst case” extreme drive Sols (large rocks and slopes) and for analytical laboratory days. Based on this analysis a 4 Brick Small RPS system capable of producing 50We, or 1200WeHRS per sol in combination with a 2 x 8 Ahr-Li-Ion battery system was chosen. Because of the inefficiencies in power generation from an RPS system waste heat has to be dissipated. Therefore, A passive thermal loop system driven by the 1000Wt energy from the RTG system, in combination with electrical heaters, thermal switches, and radiators was designed for the rover for keeping the Warm Electronic Box (WEB), external actuators, and instruments at acceptable temperatures ranges. The passive thermal system on the rover would in combination also be used for dissipating energy from the RTG system the during EDL and cruise stages.

To generate the required science and analyze 25-75 samples, accommodate the selected science payload strawman and provide sufficient power, data storage, data rate, and telecom to an MTO type orbiter, the rover itself would have a mass of ~550 kg (30% reserve included). Of this ~110 kg (~20% of rover mass) would constitute the science payload (once again, depending on the exact parameters of the instruments selected through AO). Bringing such a rover to the Martian surface would require a launched mass of 2456 kg, which would demand an Atlas V521 or a Delta IV 4040 launcher. Assuming, a MER cruise stage, Viking style EDL system with a live lander, this would give an injected mass at Mars of 2174 kg, and require a 4.57 m aeroshell and two chutes during descent.

The rover assumed in this study shares heritage with MER however, final design characteristics for the 2009 MSL mission will influence this decision. The rover includes a mast for the remote sensing instrument, an IDD for the contact instruments and sample acquisition, a detailed sample handling system and an analytical laboratory suite of instruments. The six rover wheels were increased in size to 35 cm (diameter) to negotiate larger rocks and extensive Go-To requirements (as discussed below). Each wheel includes a brushless actuator, which would draw 16-25 W per wheel, and a total of 100-150W for all wheels during traverse depending on surface characteristic of the site (i.e. slop, rock distribution, surface material etc.).

The result of the costing exercise resulted in a 2013 mission cost of $ 1.55 Billion (in RY dollars) and 2018 mission cost of $ 1.78 Billion (in RY dollars). This includes ~ 200 million for instruments and infrastructure and ~ 500 million for all the rover subsystems. These numbers should be adjusted as the design for MSL becomes more set. Savings for things like built-to-print hardware and heritage in the EDL and avionics systems may result in mission savings.

In order to meet the mobility requirements for AFL, the mass of the rover and the potential investigation site are taken into account. One requirement for AFL is to investigate a site(s) that are most likely to have high astrobiology interest. This requirement can mean traverses of up to 10’s of km depending on landing ellipse constraints such that the rover design for longer traverses in Mars terrains must be taken into consideration. In addition, the AFL payload will be much bigger than MER with a scaled rover and hence the wheel contact area has to grow from the 25 cm wheel diameter on MER to accommodate low surface pressure for minimizing wheel sinkage. There are some basic assumptions we can make based upon Mars geology and the proposed investigation sites, such that the mission requirements (see section 8.0) can be accomplished and \ a reasonable preliminary design can be created from which approximate mission costs can be estimated. It needs to be pointed out here that this preliminary analysis is by no means a complete engineering analysis, but it is designed to show approximate system requirements for planning of total mission costs as well as mobility potential for site selection. Finally, with this analysis a decision on the level of required precision (or pinpoint) landing can be made so that investment in technology development for AFL can be carefully planned.

Figure 6. A schematic diagram of how AFL may look



Technology development to fulfill science and engineering goals is summarized in Table 6.

can be seen from Table 6 significant development of critical enabling technology should begin as soon as possible, especially for 2013 launch.

As with all other JPL Rovers, AFL’s drive train subsystem was assumed to be a 6-wheel design. Each wheel has two motors: one turning the wheel, the other steering the wheel. All motors are brushless and 2, 4 or 6 wheels can be driven at a time depending on the terrain. Each wheel consumes approximately 8 W in stand-by mode and about 18 W when driving, making the drive train subsystem the largest power consumer (when operating) on AFL. Additionally, a maximum slope tolerance 30o is assumed due to both current design configurations and projected technology advancement. We have assumed that the technology for continuous drive and autonomous hazard avoidance will be developed and eventually will undergo flight qualification so it can be utilized on AFL. The wheel diameter to be chosen will be large enough to avoid typical Martian hazards (i.e. surface rocks) so that linear odometer distance can be maximized while being small enough to minimize mass and power (which is related to wheel size).

Table 6. Summary of necessary technology for AFL, in particular highlighting instrument development in critical areas as defined by the AFL team. This is not to exclude established technologies from development but merely highlights other critical technologies that should be further developed.

# 10.0Planetary Protection

The different variants of AFL may end up in any of three Planetary Protection classifications.

Category IVb is applied to missions that investigate extant Martian life forms. This may include AFL-Liquid Water and AFL-Ice (depending on the instruments).

Category IVc is applied to missions that access Mars “special regions”. This would include AFL-Liquid Water, AFL-Ice, and perhaps other AFL versions, depending on landing site.

Category IVa is applied to landed missions other than the above. This could apply to AFL-Sedimentary and AFL-Hydrothermal (depending on landing site).

To achieve maximum flexibility, mission engineering should be planned assuming IVb, and de-scoping, if appropriate, can take place from there. The four variants of AFL will have very different implications for Planetary Protection and therefore must be reviewed on a case by case basis.

It is noted that many developing technologies are available for contamination monitoring, decontamination and space craft cleanliness issues. These technologies should be vigorously pursued. In particular the following;

Low temperature sterilization techniques such as microwave plasma and other plasma ashing techniques,

Radiation sterilization technologies for whole space craft as well as ‘hot-spot’ removal.

Real time non culture based systems for monitoring amount and types of bioburden.

Providing of a suitable mineralogical bio and organic clean sample blank for proofing critical sample handling pathways and

It should be noted that several of the analytical techniques mentioned in the AFL instrument section cannot undergo heat sterilization. Protocols that either ensure that instruments are delivered cleaned to the level of the space craft and integrated to the craft after heat sterilization, for the use of cooling loops to keep critical instruments cool during sterilization (obviously the previous point would apply here) or alternatives to heat sterilization must be put in place for these technologies to fly.

# 11.0Relationship between AFL and MSL

AFL will depend on the following heritage from MSL.

Precision landing using a novel (non airbag) landing system

The use of RTG technology

The use of remote, contact and analytical suites of instruments

Crude sample processing to be used but improved on AFL

2.AFL will differ from MSL in the following essential respects:

Advanced sample preparation system.

Precision sub-sampling is an advanced sample management step that will allow a scientific focus on meso- to micro-scale discoveries of enhanced astrobiological interest. This will allow a much higher capacity to investigate specific anomalous features.

Liquid extraction. For advanced studies of carbon chemistry, more efficient sample extraction (and instrument delivery) methods are needed.

Better and miniaturized organic molecule and life-detection related instruments.

Greater interplay between

Precision landing, hazard tolerance/avoidance, go-to mobility.

–Will give us the ability to follow-up on specific discoveries, including in “interesting” terrain.

# 12.0The Future of AFL

It is suggested that the SSG reconvene at a later date to

Respond to discovery to hone mission concepts for site selection

Review sample handling and instrumentation choices and feed-forward to a possible sample return mission

Respond to shifting of the AFL timeline from 2013 to 2018, this would include revisiting the instrument choices based on comments from the SSG as to the use of instruments currently in development but of such a low TRL that it could not feature in the 2013 timeframe example include high vacuum and high voltage instrumentation such as electron microscopy, or photoelectron spectroscopy.

In the past, there was competition between in-situ and sample return mission concepts and there was a question as to whether the AFL was to fly before MSR or after. The current schedule envisions an AFL flight as early as 2016 and an MSR some time after 2020.

The advantages of flying in-situ missions first are that they are relatively low cost compared to MSR (although the costly infrastructure put in place for an initial MSR would not be needed for follow up missions) and there are no issues of sample degradation, sample amount, sterilization, quarantine or ‘off nominal’ delivery to earth.

In addition, the strength of in-situ missions is their ability to assess multiple samples over a spatially diverse area without degradation of the samples. AFL will aid in the identification of sample types for future return missions. This may even include aiding sample caching for a future MSR mission, although that would necessitate a further assessment of precision landing of an MSR mission.

A point to remember is that if / once detected life on Mars should be characterized in its entirety for similarity to earth life, evolution and biochemistry (if viable). Therefore both AFL and MSR must be considered necessary tools to be used at the right time to answer science questions within the foreseeable realms of technology..

Several aspects of both the sample handling capabilities for AFL and the choice of instrumentation will allow the further development of robotic tools to explore elsewhere in the solar system e.g. Europa. This instrumentation although initially geared for the detection of life would upon the successful accomplishment of this task be needed to be further developed to characterize that life in whatever form. It will not be enough to ask was/is there life there, the next logical step is how did it arise, how is it different from earth life and why? It is only by taking this step will we able to understand truly the processes of abiotic / prebiotic / biotic chemistry in the solar system.

Note, the bulk of this work and the draft white paper was completed by September 2004. There have been unavoidable delays to its publication. In the meantime thinking about AFL has progressed. This document reflects the thinking in September 2004. Whilst engineering and programmatic changes have occurred since then, the strength of this document lies in the science definition for the mission.

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| **Table 8- Techniques Suggested for AFL by SSG Members** | | | | | | | | | | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | Measurement Tier | | | Example Target/Sample Information | | |  | Example Technique Characteristics (instrument implementation) where appropriate | | | | | | | |  | Discovery/Follow-up per Table 7 | | | |  |  |  |
| Technique | Data/ Signatures Sought | Mmnt Reqts Addessed (Section 7) | Remote Sensing/Standoff | Contact or Close Range | Analytical Lab | Physical Form (Solid, Gas, Liquid) | Example Origin/Host Material | Processing Required/ (Desired) | Sample Mass/ Volume | Distance to Target | Size of Area Probed/ FOV | Target Feature Scale | Selectivity | Detection Limits | Resolution | Precision | Mass Range | Other | Recent Surface Water | Hydrous Mineral Phases | Organic Molecules | Sedimen. Structures | Sedimen. Rocks | Evidence for Fossil Life | Microbes |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| stereo optical imaging | identify targets, evidence of weathering, sedimentation, alteration, etc. | A | x |  |  |  | sedimentary rocks/ structures | n |  | 1m - 10+km | 10cm - 1+km |  |  |  |  |  |  |  | 1 | 6 |  | 1,2,3,4 | 4 | 1,2,3,6,8 | 3 |
|  | identify surface samples | A |  |  |  |  |  |  |  | 10-100 m | 1-10 m | 10cm-1m |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | identify distant sedimentary outcrops | A |  |  |  |  |  |  |  | 1 km | 10-100 m | 1-10 cm |  |  | 10 cm @ 1 km | |  |  |  |  |  |  |  |  |  |
| VIS/NIR Spectroscopy | surface mineralogy, texture | B | x | x |  | s | rocks, fines | n (abr) |  | cm - m |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| mini TES | mineralogy | B | x |  |  | s |  | n (abr) |  | m - km |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |
| long focal length imaging | identify distant sedimentary outcrops | A | x |  |  |  |  | n |  | 10m - km | cm-10m | cm |  |  |  |  |  |  |  |  |  |  |  |  |  |
| laser ranging | distance to target | A | x |  |  |  | boulders, vertical faces | n |  | 100m - km | cm spot |  |  |  | cm @ 100 m | |  |  |  |  |  |  |  |  |  |
| LIBS | elemental composition | B | x |  |  | s | boulders, slopes | n |  | 1 - 25m | mm - cm spot |  | low (l absorb.) | ppmw |  | ~ 10% | elements | laser ablation | | 2,4 |  |  | 2,5 | 3 |  |
| ground penetrating radar | ice, H2O, other | B, C | x |  |  | s | subsurface | n |  | m - 10s m |  |  |  |  |  |  |  |  | 3 |  |  |  |  |  |  |
| seismic sounding | ice, H2O, other | B, C | x | x |  | s | subsurface | n |  | 100's m - km |  |  |  |  |  |  |  |  | 3 |  |  |  |  |  |  |
| neutron spectroscopy | ice, hydrated minerals | B | x | x |  |  | drill cores, fines | n (acq) |  | 10's cm - m's |  |  | high | variable <%-% | |  |  |  |  |  |  |  |  |  |  |
| gamma ray spectroscopy | elemental composition | B | x | x |  |  | any | n |  | 10's cm - m's |  |  | med | variable <%-% | |  |  |  | 5 |  |  |  |  |  |  |
| x-ray spectroscopy | elemental composition | B, E2 |  | x |  |  | any | n (acq) |  | cm | cm+ |  | med | variable <%-% | |  |  |  |  |  |  |  |  |  |  |
| Raman spectroscopy | mineralogy, some geochemical/organic | B, E | x | x |  |  | rocks | n (abr) |  | cm - m | cm+ |  | med |  |  |  |  |  |  | 1,5 | 1,4 |  | 2 | 1 |  |
| micro-Raman spectroscopy | mineralogy, some geochemical/organic | B,D,E |  | x | x |  | rock chips | n (acq, abr) | | mm - cm | < mm |  | med |  |  |  |  |  |  | 1,5 | 1,4 |  | 2 | 1 |  |
| micro-LIBS | elemental composition | B,D |  | x | x | s | rocks, chips | acq,pos |  | mm - cm | < mm |  | low (l absorb.) | |  |  |  |  |  | 2,4 |  |  | 2,5 | 3 |  |
| hand-lens-scale imaging | phase texture/identity | D |  | x |  | s |  | n (abr) |  | cm - m's | 0.1-10 mm | grains |  |  |  |  |  |  |  |  |  |  |  |  |  |
| optical microscopy | fine morphology | D,E |  | x | x | s |  | n (abr) |  | mm - cm | 0.001-1 mm | subgrain |  |  |  |  |  |  |  |  | 2 |  |  | 1,2,3,6,8 |  |
| confocal microscopy |  | D,E |  |  | x | s |  |  |  | mm | 0.001-1 mm | subgrain |  |  |  |  |  |  |  |  | 2 |  |  | 1,2,3,6,8 |  |
| near-field microscopy | very high res imaging | D,E |  |  | x | s | flat chip | acq, pos |  |  |  | subgrain |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mossbauer | Fe-bearing mineralogy | B |  | x | x | s |  |  |  | mm - cm |  | avg |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Fe-NMR |  | B |  | x |  | s |  |  |  | mm - cm |  |  | high |  |  |  |  |  |  |  |  |  |  |  |  |
| XRD/XRF | mineralogy | B,D |  |  | x | s | drill cores, fines | acq, pow | mg's | 0 | whole sample | avg or grains | |  |  |  |  |  |  | 1 |  |  | 1,5 |  |  |
| FTIR | mineralogy, some geochemical/organic | B |  |  | x | s |  |  |  |  |  |  |  |  |  |  |  |  |  | 1,5 | 1,4,6 |  | 2,5 | 3 | 4 |
| VCD |  |  |  |  | x | s |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| deep UV fluorescence | organics: identity, oxidation state, … | B,D,E |  | x | x | s |  | n (abr) | surface? | mm - m |  | grain scale+ | |  |  |  |  |  |  |  | 1 |  |  | 3 |  |
| pyrolysis/GCMS | organic and some mineralogical/inorganic composition; isotopes | B,E |  |  | x | s,g,l |  | acq, pos, vac | mg-10's mg | 0 | whole sample | avg | low |  |  |  |  |  | 4 |  | 1,5 |  | 5 |  |  |
| chemical derivatization | less-tractable organics | E |  |  | x | s,l |  | liq |  | 0 | whole sample | avg |  |  |  |  |  |  | 4 |  | 1,4,7 |  | 5 |  |  |
| isotope ratio MS (IRMS) | C and other isotopes for bio-fractionation, age dating | E2 |  |  | x | s,l |  | acq, pos, vac | | 0 | whole sample | avg |  |  |  |  |  |  | 2 |  |  |  |  |  |  |
|  | compound-specific IRMS using sampling selectivity | E2 |  |  |  |  |  |  |  |  | whole sample | avg | cmpd isolated w/pyr, GC,or other proc. | | | |  |  |  |  |  |  |  |  |  |
| chiral GC | enantiomeric excess (ee) | E3 |  |  | x | s,g,l |  | acq, pos, gas | | 0 | whole sample | avg |  |  |  |  |  |  |  |  | 3 |  |  |  |  |
| circular dichroism | enantiomeric excess (ee) | E3 |  |  | x |  |  |  |  | 0 |  | avg |  |  |  |  |  |  |  |  | 3 |  |  |  |  |
| liquid chromatography (LC) | organics, ee | E |  |  | x | s,l |  | liq |  | 0 | whole sample | avg |  |  |  |  |  |  | 4 |  | 1,3,5 |  | 5 |  |  |
| 2D GCMS/TOF-MS | organic and some mineralogical/inorganic composition; isotopes | B,E |  |  | x | s,g,l | rocks/cores, fines | acq, pos, vac | 10's mg | 0 | whole sample | avg | low |  |  |  | ~1E3-1E5+ | | 4 |  | 1,5 |  | 5 |  |  |
| electrospray ionization MS (ESI/IMS/CIT-MS) | | E |  |  | x | s,g,l |  | acq, pos, vac | g's | 0 | whole sample | avg | low |  | Dm/m 1E2-1E3+ | |  | contact w/ fluidized sample | | |  |  |  |  |  |
| laser ablation TOF-MS | local elemental/isotopic composition | B,D |  |  | x | s | rock chips, fines | acq, pos, vac | surface | 0 | 10mm - 1 mm | grain scale+ | low (l absorb.) | ppbw-ppmw | Dm/m 1E2-1E3 | 5-25% | ~ 300 |  | 4 | 2,4 |  |  | 2,5 | 1,3 |  |
| LD/MALDI-TOF MS | high-MW organics; some inorganic molecules | D,E |  |  | x | s | rock chips, fines | acq, pos, vac (pow, liq) | surface/prep film | 0 | 100mm - 1 mm | grain scale+ | med (l absorb.) | fmol-pmol | Dm/m 1E3-1E4 | | ~1E3-1E5+ | |  |  | 1,2,4,7 |  |  | 3 | 4 |
| REMPI-MS/RIMS | organics, elements (trace) | E |  |  | x | s |  | acq, pos, vac | | 0 | 10mm - 1 mm |  | very high (l absorb.) | s. atom - pmol | Dm/m 1E2-1E4 | | ~ 1E3 |  |  |  |  |  |  |  |  |
| AP-MALDI-MS (TOFMS or ITMS) | organic, inorganic molec. | D,E |  | x | x | s | rocks, ices | vac | surface | mm | 10mm - 1 mm | grain scale+ | med (l absorb.) | fmol-nmol | Dm/m 1E3-1E4+ | | ~ 1E3-1E5 |  |  |  |  |  |  |  |  |
| electrospray TOF-MS | high-MW organics | E |  |  | x | s,l | rocks/cores, fines | acq, liq, pos, vac | | 0 | whole sample | avg | med |  | Dm/m 1E2-1E4 | | ~1E4-1E5+ | |  |  | 1,2,7 |  |  | 3 | 4 |
| TOF-SIMS | chemical imaging | B,E |  |  | x | s | rock chips | acq, pos, vac | surface | 0 | 50nm-50mm | sub-grain+ | low |  | Dm/m 1E3-1E4 | | ~1E3-1E4 |  |  |  |  |  |  |  |  |
| ICP-MS | trace elements | B |  |  | x | s,g,l | rock chips, fines | acq, pos, vac, gas | | 0 | whole sample | avg or grains | low | pptw-ppbw | Dm/m 1E3+ | 0.1-10% | ~ 300 |  | 2,4 |  | 1,3,5 |  | 5 |  |  |
| TIMS | isotope ratios (~IRMS) | B,E2 |  |  | x | s |  | acq, pos, vac | | 0 | whole sample | avg | low | pptw-ppbw | Dm/m 1E3+ | 0.1-1% | ~ 300 |  | 2 |  | 5 |  |  |  |  |
| AFM | nanoscale imaging | D, E3 |  |  | x | s | flat chip | acq, pos | chips |  | 1nm-1mm | sub-micron | |  |  |  |  |  |  |  |  |  |  |  |  |
| TEM/SEM | nanoscale imaging | D |  |  | x | s | flat chip | acq, pos, vac | chips |  | 1nm-1mm | sub-micron | |  |  |  |  |  |  |  |  |  |  |  |  |
|  | image microbes in ice cores | D,E3 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| XPS | chemical comp. and bond state | B,C |  |  | x | s,l |  | vac | 100's mg |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Auger spectroscopy | bond state of elements | B,C |  |  |  | s,l |  | vac | 100's mg |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| amino-acid sensors (eg MOD) | detection of amino acids | E |  |  | x | s,l |  | acq | 100's mg |  | whole sample |  | high |  |  |  |  |  |  |  | 1,3 |  |  |  |  |
| oxidant sensors | detection of oxidants | C |  |  | x | s,l |  | Acq, dry | 100's mg |  | whole sample |  | high |  | per sample weight | |  |  |  |  | 6 |  |  |  |  |
| bio-assay chip lab |  | E |  |  | x | s,l |  | liq | 100's mg | 0 | whole sample |  | high | pptw | per sample weight | | Kda |  |  |  | 7 |  |  | 1,5,6 | 1,5,6 |
| micro-array sensors |  | E |  |  | x | s,l |  | Liq | 100's mg |  | whole sample |  | high | pptw | per sample weight | | Kda |  |  |  | 7 |  |  | 1,5,6 | 1,5,6 |
| MORD |  |  |  |  | x | s |  |  | 100's mg | 0 | whole sample |  | high |  | per sample weight | |  |  |  |  |  |  |  |  |  |
| fluorescence staining | organics | E |  | x | x | s,l |  | Liq | 100's mg |  | whole sample | avg | high | single cell | per sample weight | |  |  |  |  | 7 |  |  |  |  |
|  | SYBR gold, SYTO, DAPI nucleic acid stains for counting microbes | E |  |  | x | s,l |  | Liq | 100's mg |  | whole sample |  | Medium | single cell | per sample weight | |  |  |  |  |  |  |  |  | 1.5.6 |
|  | CTC, tetrazolium salt redox stains for individual cells | E |  |  | x | s,l |  | Liq | 100's mg |  | whole sample |  | Medium | Single cell | per sample weight | |  |  |  |  |  |  |  |  | 1,5,6 |
| isotopic labelling | 14CO2 or 3H for total population activity | E |  |  | x | s,g,l |  | Lig | 100's mg |  | whole sample | avg | medium | single cell | per sample weight | |  |  |  |  |  |  |  |  | 1,5,6 |
| flow-cytometry |  | E |  |  | x | s,l |  | liq | 100's mg |  | whole sample | avg | medium | single cell | per sample weight | |  | If have required media | | |  |  |  |  | 1,5,6 |
| culturing/cell-growth assays |  | E |  |  | x | s,l |  | liq | 100's mg |  | whole sample | avg | high | single cell | per sample weight | |  | If have required media | | |  |  |  |  | 1,5,6 |
| ATP and LAL enzyme assays |  | E |  |  | x | s,l |  | liq | 100's mg |  | whole sample | avg | high | pptw | per sample weight | |  |  |  |  |  |  |  |  | 1,5,6 |
| DNA extraction/PCR |  | E |  |  | x | s,l |  | liq | 100's mg |  | whole sample | avg | high | 100 cells | per sample weight | |  | with correct primers | | |  |  |  |  | 5,6 |
| capillary electrophoresis (CE) |  | E |  |  | x | s,l |  |  | 100's mg |  | whole sample | avg | high | pptw | per sample weight | |  |  |  |  | 7 |  |  |  | 1,5,6 |
| microcalorimetry |  |  |  |  | x |  |  |  | 100's mg |  | whole sample | avg | medium | pptw | per sample weight | |  |  |  |  | 7 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **KEY:** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| n - can be operated with no sample acquisition/processing | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| abr - abrasion to remove surface layers | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| acq - sample acquisition from host matl (via whatever means) | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| pow - powdering of solid sample | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| pos - sample positioning (e.g., manipulation to oven, point of focus or extraction) | | | | | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| vac - vacuum processing |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| liq - liquid processing |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| gas - gas processing |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |