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Synchronous in-field application of life-detection techniques in Icelandic Mars analogue sites

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Abstract: Field expeditions that simulate the operations of robotic planetary exploration missions at analogue sites on Earth can help establish best practices and are therefore a positive contribution to the planetary exploration community. There are many sites in Iceland that possess heritage as planetary exploration analogue locations and whose environmental extremes make them suitable for simulating scientific sampling and robotic operations.

We conducted a planetary exploration analogue mission at two recent lava fields in Iceland, Fimmvörðuháls (2010) and Eldfell (1973), using a specially developed field laboratory. We tested the utility of in-field site sampling down selection and tiered analysis operational capabilities with three life detection and characterization techniques: fluorescence microscopy (FM), adenine-triphosphate (ATP) bioluminescence assay, and quantitative polymerase chain reaction (qPCR) assay. The study made use of multiple cycles of sample collection at multiple distance scales and field laboratory analysis using the synchronous life-detection techniques to heuristically develop the continuing sampling and analysis strategy during the expedition.

Here we report the operational lessons learned and provide brief summaries of scientific data. The full scientific data report will follow separately. We found that rapid in-field analysis to determine subsequent sampling decisions is operationally feasible, and that the chosen life detection and characterization techniques are suitable for a terrestrial life-detection field mission.

In-field analysis enables the rapid obtainment of scientific data and thus facilitates the collection of the most scientifically relevant samples within a single field expedition, without the need for sample relocation to external laboratories. The operational lessons learned in this study could be applied to

future terrestrial field expeditions employing other analytical techniques and to future robotic planetary exploration missions.

1 **Synchronous in-field application of life-detection techniques** 2 **in Icelandic Mars analogue sites**

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7 Keywords:

8 Astrobiology

9 Life detection

10 Mars

11 Analog

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13 **1 Abstract**

14 Field expeditions that simulate the operations of robotic planetary exploration missions at
15 analogue sites on Earth can help establish best practices and are therefore a positive contribution

16 to the planetary exploration community. There are many sites in Iceland that possess heritage as
17 planetary exploration analogue locations and whose environmental extremes make them suitable
18 for simulating scientific sampling and robotic operations.

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25 distance scales and field laboratory analysis using the synchronous life-detection techniques to
26 heuristically develop the continuing sampling and analysis strategy during the expedition.

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28 data. The full scientific data report will follow separately. We found that rapid in-field analysis
29 to determine subsequent sampling decisions is operationally feasible, and that the chosen life
30 detection and characterization techniques are suitable for a terrestrial life-detection field mission.

31 In-field analysis enables the rapid obtainment of scientific data and thus facilitates the
32 collection of the most scientifically relevant samples within a single field expedition, without the
33 need for sample relocation to external laboratories. The operational lessons learned in this study
34 could be applied to future terrestrial field expeditions employing other analytical techniques and
35 to future robotic planetary exploration missions.

36 **2 Introduction**

37 Extreme environments on Earth are used as analogs to inform both the science and
38 operations of future planetary exploration missions (Amils *et al.*, 2007, Amato *et al.*, 2010, Billi
39 *et al.*, 2013). In particular, Icelandic lava fields have an especially good heritage as Mars analog
40 sites (Farr, 2004, Warner and Farmer, 2010, Cockell *et al.*, 2011, Cousins and Crawford, 2011,
41 Mangold *et al.*, 2011, Ehlmann *et al.*, 2012, Cousins *et al.*, 2013). Lava fields are relevant for
42 astrobiological science due to the presence of extreme conditions, including desiccation, low
43 nutrient availability, temperature extremes (e.g. due to high elevation or close proximity to
44 fumaroles), relatively young ages, and their isolation from anthropogenic contamination (Allen
45 *et al.*, 1981, Bagshaw *et al.*, 2011). From an operational perspective, many Icelandic lava fields
46 are remote enough to require that field expeditions address several sampling operational
47 constraints that are also experienced in robotic planetary exploration (Arena *et al.*, 2004, Preston
48 and Dartnell, 2014).

49 Terrestrial field campaigns designed to conduct scientific studies of planetary analogs can
50 also serve as operational analogs for robotic planetary missions. Field campaigns typically
51 involve *in situ* sampling, followed by preservation of any collected samples and subsequent
52 return to an institutional laboratory where the samples can then be analyzed, analogous to
53 planetary sample return missions. However, some field expeditions may carry limited
54 instrumentation for *in situ* analysis (Ehlmann *et al.*, 2012), and like robotic planetary missions,
55 these instruments must be chosen ahead of time. Limited on-site consumables further constrain

56 the amount that can be accomplished in the field by both terrestrial field expeditions and
57 planetary exploration robots. Furthermore, sending samples to an institutional laboratory with a
58 delay of potentially several months before full scientific analysis is possible. This may prevent
59 results of prior sampling being available to influence sampling strategy throughout the
60 expedition, and this applies to whether on Earth or elsewhere in the solar system. Although the
61 results obtained might be available to assist in the planning stages of future field campaigns or
62 missions, such follow-up expeditions might be weeks, months, years or decades in the future.
63 The ability to maximize science return from limited in-field planetary exploration analyses is far
64 more critical given that a sample return mission from Mars, or other astrobiologically relevant
65 planetary bodies, is still decades away (McLennan, 2012), and raises significant planetary
66 protection issues (Bridges and Guest, 2011).

67 The capacity for rapid sample analysis and interpretation can alleviate the problems posed
68 by terrestrial or planetary expeditions. Firstly, it allows for the down-selection of sampling sites
69 in the field. Rather than being dependent solely on previous mission data or remote sensing
70 provided by partner programs, sampling choices can be made in the field based on near-real-time
71 results. Secondly, it allows for 'tiered analysis', in which a single sample may be subject to a
72 faster or lower-cost analysis (either non-destructively or by partitioning) to determine whether it
73 is sufficiently interesting to warrant a second, more resource-intensive or more limited-capacity
74 analysis. These features can be combined to maximize science return if a balance is struck

75 between the cost of carrying additional resource-light 'pre-sampling' instruments and the
76 increased science return from more resource-intensive instruments.

77 Choosing the exact locations and samples that a field team, rover, or lander will analyze is
78 critically important given the operational constraints. The planetary mission team must select a
79 location to sample using the vehicle's remote sensing instruments (*e.g.* the ChemCam instrument
80 on the Mars Science Laboratory (Meslin *et al.*, 2013)) and assume that this site is representative
81 of the area of interest. If a difference in sampling location of a few meters, centimeters, or even
82 hundreds of microns could make a significant difference in the results, it may mean that science
83 objectives are not met. This will be especially critical when life-detection is the primary goal,
84 given the inherent variability in the distribution of living things as we know them on Earth.
85 Successfully characterizing multiple parameters across the multiple scales of a field site will help
86 to reduce the number of initial sampling rounds needed.

87 We conducted a planetary exploration analog expedition to two recent Icelandic lava fields,
88 Fimmvörðuháls (2010) and Eldfell (1973), with a specially developed field laboratory. Our main
89 goal was to prove the feasibility of real-time sampling and site down-selection in a life detection
90 robotic exploration context through quick-turnaround 'pre-sample' analysis and extrapolation of
91 the likely presence of biomarkers. To inform the development of current and future *in situ*
92 planetary missions, this was broken down into three interrelated operational sub-goals:

- 93 1. Demonstrate the feasibility of performing multiple rapid cycles of sample selection,
94 sample analysis and interpretation in-field under simulated robotic exploration
95 constraints.
- 96 2. Demonstrate the synchronous application of multiple life detection techniques
97 within these multiple cycles.
- 98 3. Demonstrate the potential of fluorescence microscopy (FM), adenosine
99 triphosphate (ATP) bioluminescence, and quantitative polymerase chain reaction
100 (qPCR) assays as quick-turnaround terrestrial life detection techniques.

101 Here, we report upon the operational and logistic lessons learned during the expedition,
102 which could influence the design of future field studies. Scientific results of the expedition will
103 be reported separately (manuscript in preparation). Follow-up expeditions are planned and will
104 be reported upon in relation to this work.

105 **3** **Methodology**

106 The expedition consisted of cycles of sampling, rapid preliminary analysis, and follow-up
107 based on the results from the previous sampling and analysis cycle. The expedition personnel
108 were split into two teams, allowing two of these repeated sampling and analysis cycles to be run
109 in staggered parallel, thus increasing the expedition's throughput and ensuring that the field lab
110 was neither idle nor acting as a bottleneck. After sampling cycles were completed all samples

111 were more extensively analyzed over three additional days in the field lab to address the more
112 detailed question of sample site homogeneity.

113 **3.1 Field Sites**

114 Two lava fields were chosen for our expedition: Fimmvörðuháls (63° 38' 12.30" N, 19° 26'
115 49.20" W) and Eldfell (63° 25' 08.30" N, 20° 14' 38.70" W) (Figure 1). The Fimmvörðuháls
116 lava field formed between 20 March and 12 April 2010, from a basaltic effusive eruption
117 associated with the 2010 Eyjafjallajökull eruption located approximately 7.5 km away. The field
118 site is located in a saddle between the larger Eyjafjallajökull and Myrdalsjökull volcanic
119 structures (Figure 2) (Edwards *et al.*, 2012). The Eldfell volcano, associated with the
120 Vestmannaeyjar volcanic system, began erupting on 23 January 1973 on the island of Heimaey.
121 The Eldfell eruption had both effusive and explosive alkali basalt eruptions and lasted for five
122 months, producing $\sim 0.23 \text{ km}^3$ of volcanic material (Thorarinsson *et al.*, 1973, Higgins and
123 Roberge, 2007). Both field sites have very similar basaltic tephra sediment types with limited
124 vegetation cover. The two sites are approximately 45.0 km apart.

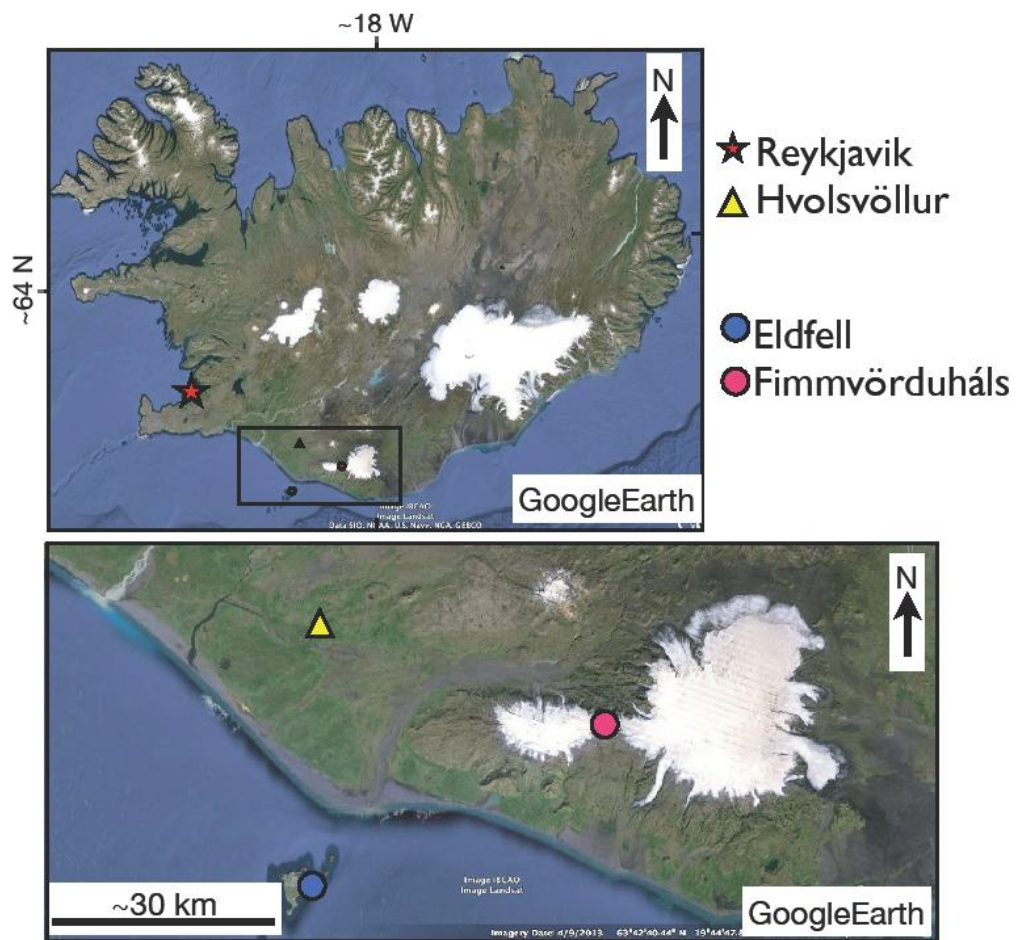
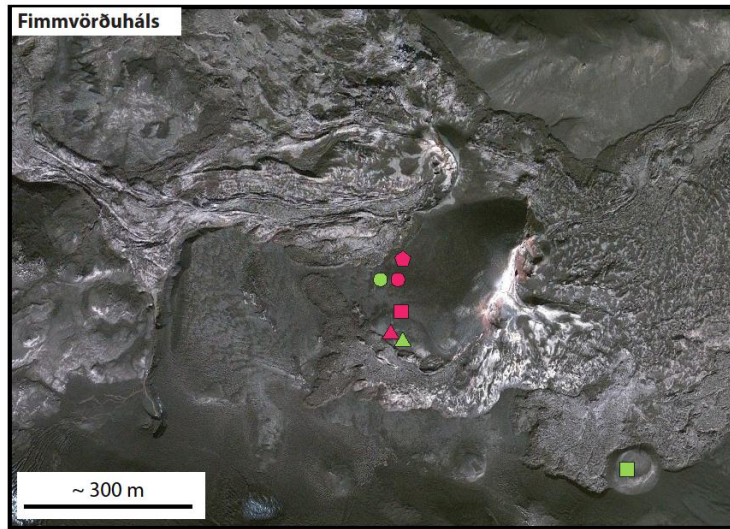
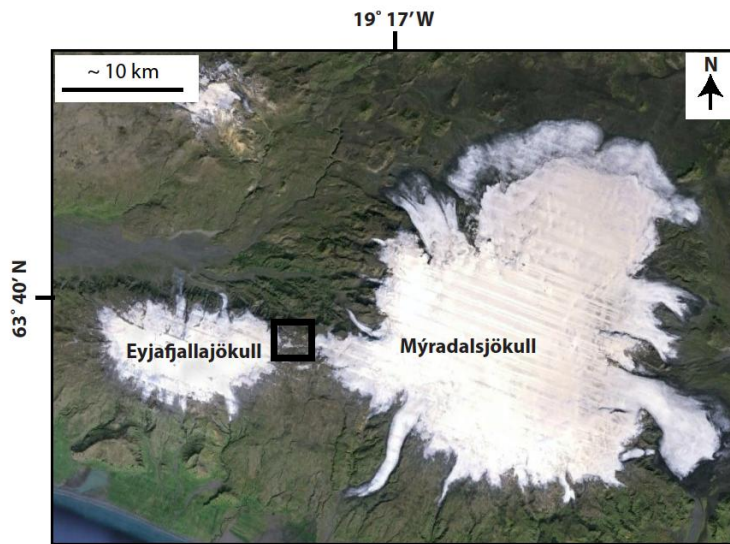


Figure 1 - A map of Iceland with the expedition's field sites marked.

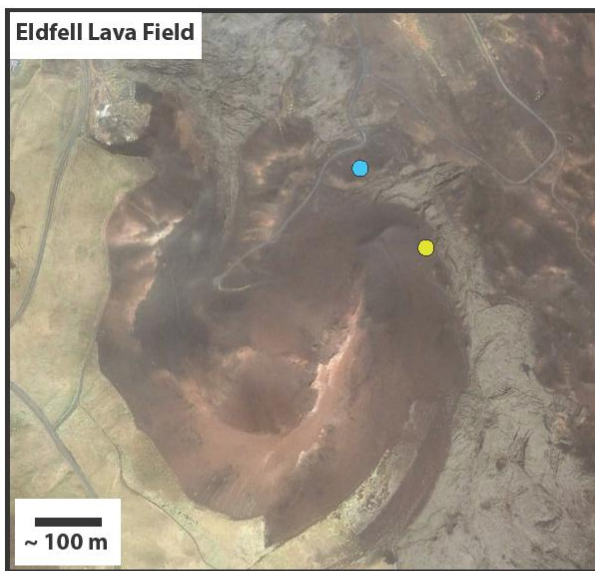
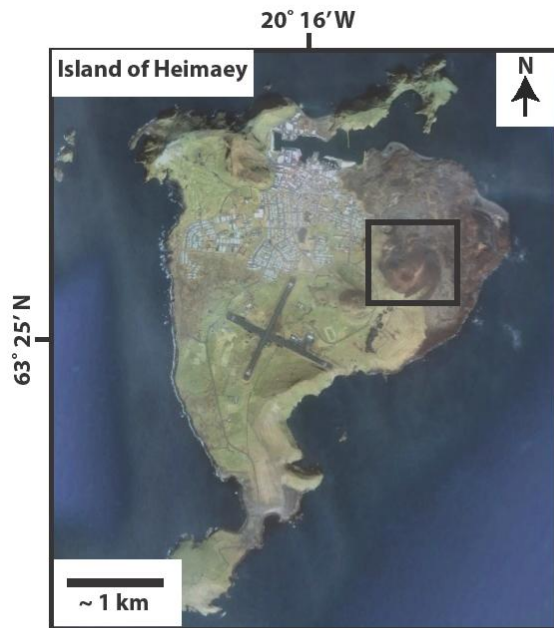
125
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July 27, 2013	July 29, 2013
▲ Fimm. 1 - (1-2) - (1-3)	▲ Fimm. 1 - (1-2) - (1-3)
● Fimm. 2 - (1-2) - (1-3)	● Fimm. 2 - (1-2) - (1-3)
■ Fimm. 3 - (1-2) - (1-3)	■ Fimm. D - 1 - (1-3)
	◆ Fimm. R - 1 - (1-3)

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Figure 2 - Sampling locations within the Fimmvörðuháls field site.



July 28, 2013

- HEI. 1 - (1-2) - (1-3)
- HEI. 2 - (1-2) - (1-3)

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Figure 3 - Sampling locations within the Eldfell field site.

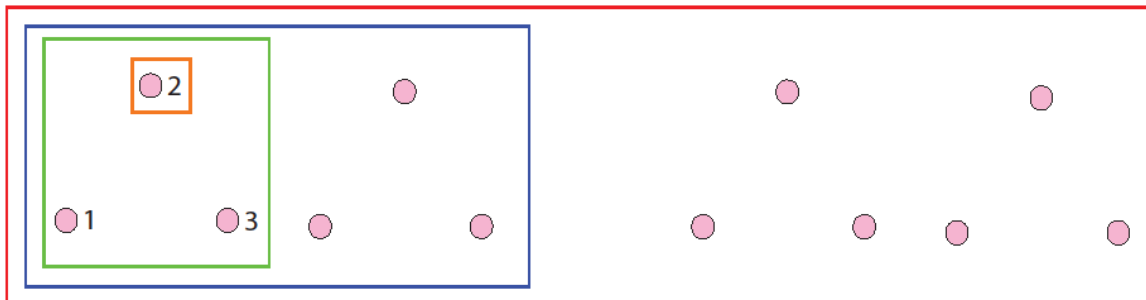
132 **3.2 Sampling**

133 A grid of sample locations spaced at 1 m, 10 m, and 100 m intervals (see Figure 4 and
134 Figure 5) was established at each of the two main sites in an area where the basaltic tephra
135 appeared to be visually homogeneous by color, morphology, and grain size. A triplicate sample
136 set was taken at each grid point.

137

Field Sampling and Naming Protocol

● Sampling Site Naming Procedure: Field Site - 10² m scale - 10¹ m scale - 10⁰ m scale
Example: HEI. - 1 - 1 - 2



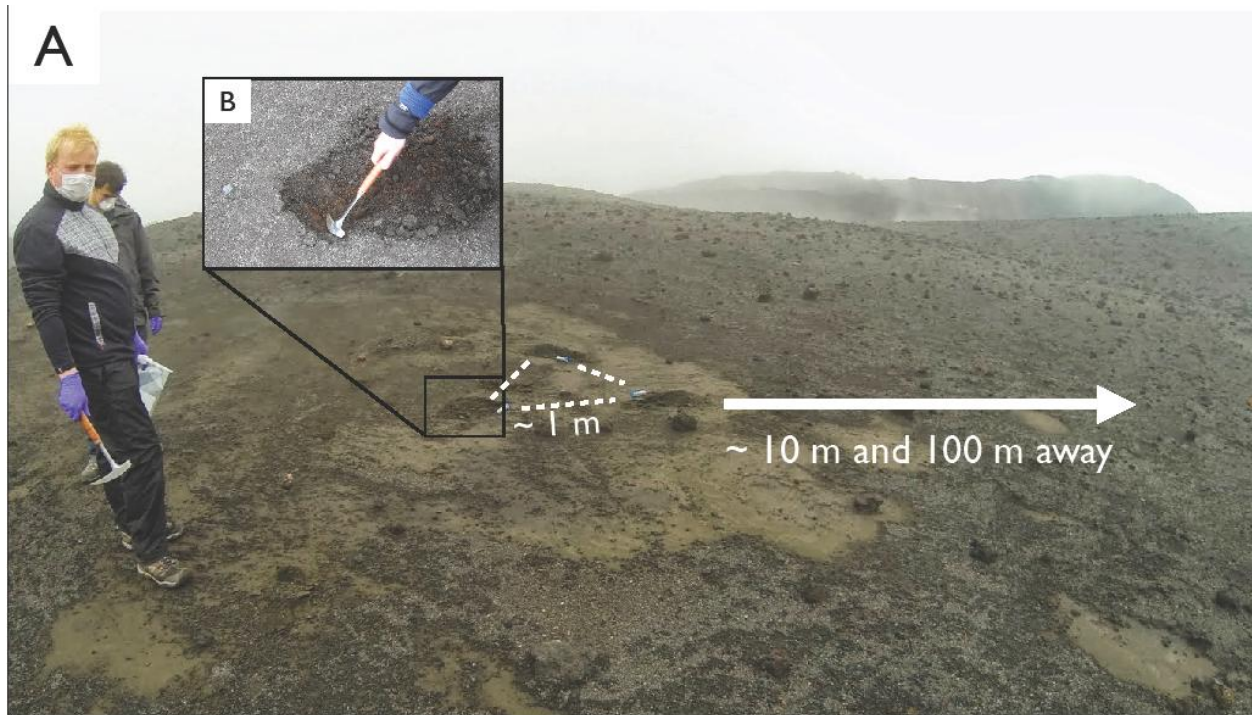
Note: For Fimvordulhas, where multiple samples were taken with this process, the 10² m scale region is subdivided into A, B, and C.

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Figure 4 - Field sampling and naming protocol.

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Figure 5 - (A) Samples being collected at Fimmvörðuháls by A. Stevens (front) and E. Schwieterman (back) using the sampling protocol shown in Figure . (B) A closer view of how the samples were collected.

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Each sample was taken from approximately 5 cm below the surface by digging with a rock hammer that was wiped with isopropanol before each sample collection (Figure 5) and scooping the uncovered tephra with a sterile 50 mL falcon tube, which was sealed and returned to the field lab. During sample collection, team members wore facemasks and gloves and approached the pristine sampling site from a downwind direction to minimize anthropomorphic contamination. Gloves were rinsed with isopropanol between samples. Caution was taken to avoid stepping on or otherwise disturbing any potential sampling site.



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153

Figure 6 – Example of a sample collected from the Eldfell lava field.

154 Fimmvörðuháls samples were collected along the base and in the surrounding area of the
155 Magni cinder cone (Figure 2), which appeared to have had little foot traffic compared to the rest
156 of the lava field. The basaltic tephra collected ranged from coarse ash to lapilli and reached up to
157 50 cm in thickness. On 24 July, four samples were collected from a small area of the cinder cone
158 using the general method described above. Along with these, a ‘positive control’ sample was also
159 collected from the ash around the roots of a small tuft of grass, on the assumption that this would
160 be a likely habitat for microorganisms. A separate positive control was also collected from the
161 grassy area around the field laboratory. First-round analysis of these initial samples was used to
162 inform follow-up sample collection at Fimmvörðuháls (27 and 29 July), which were taken from
163 the same general region (Figure 2).

164 The samples collected from the Eldfell lava field (28 July) were lapilli-sized or smaller and
165 came from a large scoria cone associated with the main explosive eruption (Figure 6). As with
166 the Fimmvörðuháls site, care was taken to collect samples from regions with little to no apparent
167 anthropomorphic disturbance, but this was much more difficult given the status of Heimaey and
168 Eldfell as a site for tourism. Further samples were taken from a site off the main eruption cone in
169 an attempt to reduce the anthropomorphic contamination.

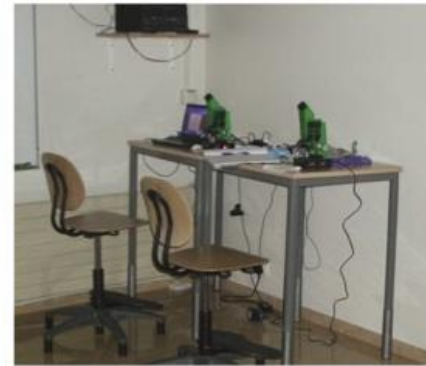
170 **3.3 Field Laboratory**

171 All analytical work was performed in a field laboratory located within a day's travel of the
172 field sites at the Hvolsskóli school in Hvolsvöllur (Figure 1). While the classroom used had very
173 little equipment itself, it provided the basis for a field laboratory with running water and access
174 to power.

175 The majority of the scientific equipment used in the expedition was shipped directly to
176 Hvolsvöllur in a single container. Some equipment (such as isopropyl alcohol) was purchased
177 locally. A number of items required for the field lab were adapted from household items – for
178 example, the autoclave used to sterilize equipment was a domestic pressure cooker. The
179 equipment that was shipped to the field laboratory included a benchtop vortex mixer and
180 centrifuge, sterilized pipettes and tips, a commercial water filtration system and ultrasonic
181 cleaning system, Bio-Rad MiniOpticon™ real-time PCR system, the Partec CyScope®
182 fluorescence microscope and the Merck HY-LiTE® 2 portable luminometer used to quantify the
183 ATP immunoassay. Consumables including sterile sample containers, gloves and masks, PCR

184 and microcentrifuge tubes, filters, commercial qPCR assay kits, and the reagents for use with the
185 ATP assay and FM were also shipped.

186 On arrival, the classroom was rearranged to form three analytical stations, one for each
187 analytical technique, and each cleaned thoroughly with isopropanol. A second classroom was set
188 up as a separate sample preparation room to reduce cross-contamination. A third classroom was
189 assigned to be a darkroom for use with the FM.



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Figure 7 - The three workstations as set up in our field laboratory. (l) DNA extraction, purification, and qPCR area, with general supply table in foreground. (c) ATP bioluminescence area, with FM staining area in the background. (r) 'Darkroom' established for fluorescence microscopy.

195 **3.4 Analytical Methods**

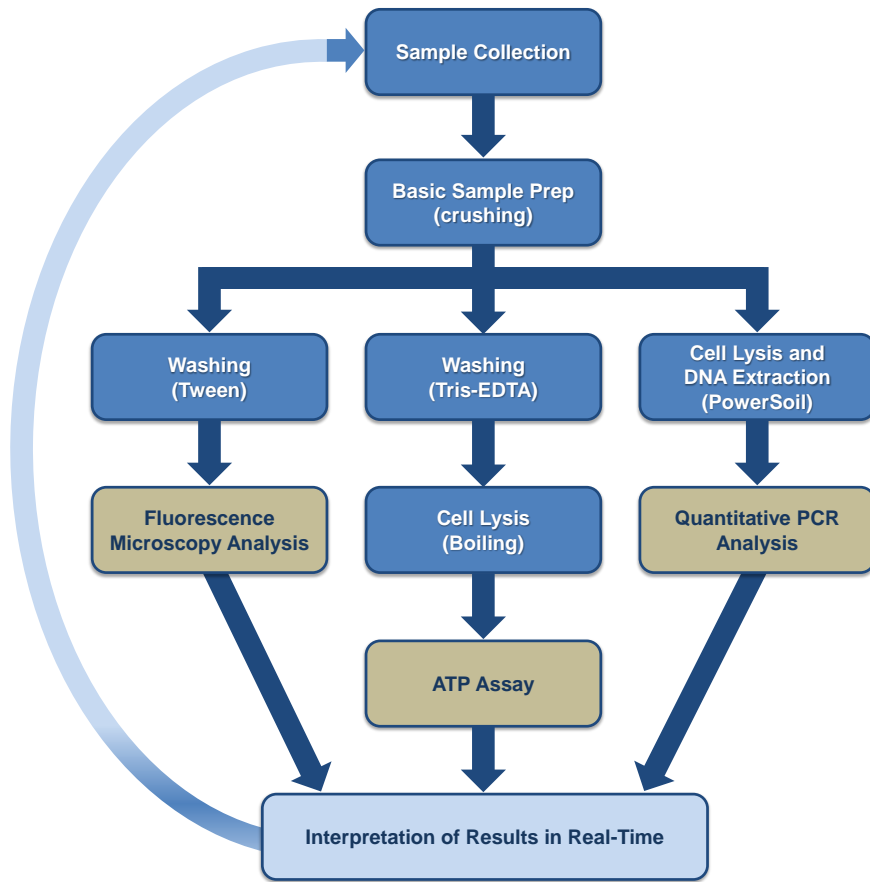
196 The analytical techniques used in the expedition were selected for their minimal laboratory
197 overhead, low cost and consumables requirements, and established history in terrestrial life
198 detection. Adenosine triphosphate (ATP) has long been considered a high-priority biomarker due
199 to its indication of bioavailable energy and ease of detection (Parnell *et al.*, 2007), and can be
200 implemented with standard, commercially available solutions and a benchtop luminometer.

201 Fluorescence microscopy (FM) offers the ability to directly quantify microorganisms under a
202 microscope using a dye that binds to double-stranded DNA and fluoresces when illuminated
203 under UV light. Counting cells via FM has been widely used in environmental samples (Kepner
204 and Pratt, 1994), and can be implemented with standard, commercially available stains and
205 buffer solutions and a microscope with appropriate illumination and filters. The quantitative
206 polymerase chain reaction (qPCR) assay allows for simultaneous amplification and
207 quantification of DNA that matches the set of primers used. By choosing an array of primers
208 that correspond to different taxonomic groups, it is possible to assess both the quantity of DNA
209 present in a given sample and the relative diversity (defined for our purposes as the relative ratios
210 of the DNA recovery of different types of organism *e.g.* bacteria:fungi:archaea) of the organisms
211 from which that DNA was extracted. The qPCR technique requires pre-synthesized primers,
212 standard buffer solutions, and a thermocycler equipped with a well-plate fluorometer. We
213 recognize that our chosen analytical suite is terrestrial extant-life-centric, and that other
214 techniques could be more appropriate for extraterrestrial planetary expeditions.

215 The analytical workflow was designed to maximize overlap between techniques and
216 stagger use of limited equipment. The same basic sample preparation techniques, including
217 crushing to homogenize particulate size, were used for all three life-detection techniques.
218 Samples were then split into sub-sample groups for washing (used for the fluorescence staining)
219 and cell extraction and lysis (used for both the ATP and qPCR analyses). The lysate was then

220 subject to a final DNA extraction step for qPCR only. The cell wash for the fluorescence
221 staining required an additional filtration step.

222 Expedition personnel on laboratory duty for a given day were divided into sample
223 preparation, qPCR, ATP and FM teams, as shown in Figure 8. The FM and qPCR teams took
224 sample input directly from the sample preparation team; the ATP team was split into preparation
225 and analysis groups to account for their additional secondary sample preparation step. Analytical
226 protocols are briefly described below, and will be reported in full detail in a future manuscript.



227
 228 **Figure 8 - Sample preparation and analysis throughput chart. Data are**
 229 **analyzed in real-time, informing the next round of sample collection and**
 230 **analysis.**

231

232 3.4.1 ATP Bioluminescence

233 The ATP bioluminescence assay was implemented using the Roche ATP Bioluminescence
 234 Assay Kit HS II following the manufacturer's recommendations for a tube assay, with some
 235 modifications as discussed in Barnett *et al.* (2012).

236 **3.4.2 Fluorescence Microscopy**

237 The FM protocol follows that of (Kepner and Pratt, 1994) with some minor modifications
238 and serves to remove any microorganisms from the sample matrix and allow them to be stained.
239 Briefly, a 1 mL portion of crushed sample was combined with 0.75 mL of PBS/Tween buffer,
240 vortexed, sonicated, then centrifuged for 5 minutes at $600 \times g$. The supernatant was removed into
241 a separate sterile tube and the extraction process was repeated. The combined supernatant
242 (extract) was loaded into a 2 mL syringe, pushed through a Whatman Nuclepore Track-Etch
243 Membrane 0.2 μm filter, and flushed through twice with sterile water and twice with air. The
244 sample filter was removed, stained with 100 μL of SYBR Gold Nucleic Acid Gel Stain
245 (Invitrogen), covered with a cover slip, and incubated in the dark for at least 15 minutes before
246 imaging with a Partec Cyscope (fluorescence and transmitted light microscope) equipped with a
247 455 nm (RB) “royal blue” emission light source and 500 nm (DM) “yellow/green” dichroic
248 mirror long pass filter. The 100 \times (oil) objective was used in conjunction with the imager to
249 record digital micrographs of sample fields. The cell counts were documented with manual
250 counting from five randomly chosen locations in the field of view, and images were recorded for
251 later verification and analysis.

252 **3.4.3 Quantitative PCR**

253 DNA extraction and purification was performed on a 1 mL portion of crushed sample
254 using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) following the
255 manufacturer’s protocol. Real-time qPCR was performed in duplicate for each sample on a 1:10

256 dilution of purified DNA extract using a BioRad MiniOpticon™ real-time PCR system. Primers
257 were selected for breadth of taxonomic range, and included primers to identify bacterial, fungal
258 and Archaeal DNA. The primers chosen also had similar extensions to increase the amount of
259 samples that could be analysed at the same time. Soil gathered outside of the field laboratory in
260 Hvolsvöllur was used as a positive control; the negative control was sterile deionized water.

261 **4 Results and Discussion**

262 **4.1 Rapid Site-Selection Lessons**

263 One of the main objectives of the expedition was to demonstrate the feasibility of
264 performing multiple rapid cycles of sample selection, sample analysis and interpretation in a
265 field setting. To accomplish this, we performed several cycles of sampling and analysis, allowing
266 methods and protocols to be developed throughout the expedition.

267 **4.1.1 Preliminary Sampling and Protocol Testing**

268 After arriving at Hvolsvöllur and establishing the field lab, the first day of sampling was
269 spent on a preliminary expedition to both potential sampling locations. The following day was
270 spent analyzing these preliminary samples in the field laboratory. There were two goals: 1) to
271 survey potential areas for sampling in terms of accessibility and anthropogenic disturbance and
272 2) to take preliminary samples with which to test our field protocols and ensure all participants
273 were suitably trained on both field sampling protocols and the three analytical techniques.

274 After determining a common sample preparation protocol that could be used for all three
275 techniques, preparation of all 22 samples took approximately two hours. However, samples were
276 continually transferred into analysis as they were prepared, avoiding an initial two hour delay.
277 All three analytical methods – FM, qPCR and ATP – were performed in parallel.

278 Fluorescence staining followed by microscopy proved to be the most rapid technique when
279 used in a qualitative mode (i.e., gauging relative levels of microorganism abundance compared to
280 the positive or negative controls). At this level of analysis, a day's sample set could be stained
281 and measured in approximately one hour. The ATP bioluminescence assay took approximately
282 two hours to produce a quantitative report regarding the activity of the microorganism abundance
283 of the samples. The qPCR analysis required an entire day (~10 hours) to analyze the same
284 number of samples as the other two techniques. Approximately five hours were required to
285 perform the DNA extraction and purification; the remaining five hours were spent running the
286 temperature cycling on a single qPCR instrument. On subsequent days, sample preparation for
287 this analysis was given priority, and two team members were assigned to parallelize the
288 extraction procedure in the morning.

289 **4.1.2 First-Round Sampling and Analysis**

290 Our rapid site selection protocol was put into place starting on Day 1, with one team
291 departing to gather samples at the Fimmvörðuháls site while the other implemented and tested
292 protocol changes resulting from our preliminary analyses the previous day. Day 1 samples were
293 then analysed on Day 2 while the second team took samples from the Eldfell site. The results of

294 samples collected on Day 1 were reviewed in the evening of Day 2 to inform decisions about the
295 follow-up sampling trip to Fimmvörðuháls on Day 3.

296 The samples from Fimmvörðuháls taken on Day 1 and analyzed on Day 2 showed
297 generally low cell counts and low levels of ATP. The qPCR assays were used with both 10x and
298 100x dilutions, and it was determined that a 10x dilution factor was the most effective; however,
299 due to the complexity of qPCR data return, these results were not immediately available to guide
300 first round analysis. The Eldfell samples, as expected given the older age and increased regional
301 vegetation, showed a higher level of ATP than the results from Fimmvörðuháls, but still a lower
302 level than the positive controls. Cell counts were also generally very low, but difficult to
303 distinguish in magnitude from the Fimmvörðuháls samples. The results from the qPCR assays
304 were mixed, indicating that the diversity of different cell types in our sampling area was not
305 consistent.

306 A final change was made to our FM procedure after Day 1. Quantitative cell counts for all
307 samples were proving to be infeasible in a single day's time frame under the constraints of our
308 field laboratory and number of personnel. Fluorescent micrographs of the stained filters were
309 taken for later detailed counts, and qualitative-level comparisons were used to guide future
310 sampling decisions.

311 A final change was made to our qPCR procedure after Day 2. By reducing the number of
312 replicate samples for qPCR analysis each day, the analysis could be completed in a shorter

313 timeframe (about eight hours). The resulting data therefore could be made available in time for
314 high-level judgments, and additional replicates could be run later from the same DNA extract.

315 These initial results from both sites showed that assessment of heterogeneity in the field
316 sites was crucial. The purpose of our follow-up sampling was at this point defined as measuring
317 internal site variance at each area for each of the three life detection techniques.

318 **4.1.3 Subsequent Sampling and Analysis**

319 Samples were prepared as in the first round of analysis, with minor changes in protocols to
320 maximize throughput and minimize material use. Qualitative FM and ATP assays were
321 performed in the morning while a reduced set of samples was selected for qPCR analysis. After
322 the final round of on-site sampling, an additional day was included in the schedule to allow for
323 follow-up analysis of particularly interesting samples and any additional work that early results
324 indicated should be performed. The qPCR analysis was completed on this day, as was initial top-
325 level analysis of site homogeneity.

326 **4.2 Field Laboratory Lessons Learned**

327 Our experience of establishing a field laboratory for real-time on-site life detection provides
328 several valuable lessons, which could be applied more generally to similar expeditions to
329 planetary analogue sites, or the future in-field testing of life detection instrumentation.

330 The minimum requirements for locating a field laboratory are power, water, protection from
331 the elements, and control over entry and exit (to minimize contamination). This can be cost-

332 effectively provided by any reasonable local structure and has been achieved in far more modest
333 locations than our expedition (see Barnett *et al.* (2012)). Appliances for low-temperature
334 preservation (refrigerator and freezer) and heat (stove top) are highly recommended. At least
335 two separate areas (in our expedition, two classrooms) are strongly recommended to allow
336 sample preparation to be physically separated from analytical measurements.

337 All necessary equipment and reagents can be shipped in a single trunk (in our case, a KA64
338 Defender aluminium box, 1190 x 790 x 520 mm). Reagents can be shipped within the main
339 container in a secondary insulating box with dry ice and will remain preserved for ~48 hours.

340 All consumables should be packed and shipped pre-sterilized whenever possible. The small
341 autoclave (adapted from a consumer pressure cooker) was often a significant bottleneck.

342 Provisions should be made to take detailed data for later analysis. This was most notable with
343 the fluorescence microscopy. The microscopes were excellent, but the low-cost camera
344 attachments provided low image quality. A single higher-resolution camera and adapter would
345 have been a worthwhile investment.

346 We have demonstrated the feasibility of performing complex life-detection analysis in a
347 field-based laboratory in a single day, which enabled heuristic development of techniques,
348 methods and protocols and offered significant flexibility over the expeditionary standard of
349 bringing samples back to an institutional laboratory. While some highly sensitive techniques and
350 instruments could not be used in this context and would therefore require sample transport,

351 advances in miniaturization technology means that more analytical techniques are becoming
352 viable to use in a field context.

353 **4.2.1 Lessons Learned: Techniques**

354 Our initial FM protocol for cell quantification was impractical. The filters used for cell
355 isolation and staining were challenging to use and to transfer between the filtering apparatus and
356 the microscope slides. A potential alternative for future work is to stain and view the cells in
357 solution. Manual cell counting by eye also proved too slow for meaningful sample throughput.
358 The use of this technique in a similar field lab could be substantially improved with the use of a
359 higher-quality microscope camera and automated image-processing software. Alternately, given
360 that qualitative comparisons were of adequate value for rapid-turnaround analysis, a low-cost
361 fluorometer could be used to rank sample fluorescence levels post-staining without the need for a
362 microscope.

363 The ATP bioluminescence assay proved generally robust and reliable under the conditions
364 of our field laboratory. The protocols were easy to follow and required simple laboratory
365 equipment (*i.e.* vortex, hot plate) beyond the ATP luminometer. The assay provided useful data
366 in a relatively short amount of time, and therefore enabled the processing of a significant number
367 of samples each day. ATP bioluminescence is definitely suitable for future use in rapid
368 examination of biomass distribution during field expeditions to extreme environments.

369 The qPCR assay was largely successful as a field technique. DNA was successfully
370 extracted from all samples. Amplification protocols for all three primer sets (bacteria, fungi and
371 archaea) were successful as confirmed by C_q values of 17.5-27.5 in the positive controls (soil
372 gathered outside of the field laboratory in Hvolsvöllur). The clearest need for improvement is
373 the throughput, due to the serial-batch nature of the technique. Each batch of samples required at
374 least 4 hrs to run on the qPCR instrument, enabling a maximum of 48 individual samples
375 (including replicates and different primers) to be run in a single day. Given the results of our site
376 homogeneity analysis, it may be possible to reduce the number of samples taken in a given area
377 to achieve the same level of confidence, though using more primers would require more sample
378 wells. Alternately, the throughput could be increased by the addition of a second qPCR
379 instrument or the substitution of one with a larger capacity.

380 **5 Conclusions**

381 Our experience shows that rapid in-field analysis to determine subsequent sampling
382 decisions is operationally feasible for planetary analogue expeditions. A single-day turnaround
383 for biomarker analysis was achievable on a relatively low budget with the use of basic local
384 facilities. Similarly, no additional difficulties were encountered in running analyses
385 synchronously (*i.e.*, in parallel) and by using sample subdivision, results were acquired from
386 three separate analytical techniques each day. With carefully chosen equipment and protocols,
387 ATP bioluminescence and nucleic acid staining assays can be used to provide same-day input
388 into sample down-selection for quantitative PCR, conserving the most expensive equipment and

389 reagents without increasing the one-day analysis turnaround. We recognize that the techniques
390 were chosen for this study to meet the desired field science and are therefore centric upon
391 terrestrial life – missions to extraterrestrial locations cannot make the same assumptions we were
392 at liberty to make and therefore might include a different analytical suite. There is no *a priori*
393 reason other analytical techniques could not be used in the same manner, so this decision making
394 methodology could be used in other contexts.

395 These methods have a number of advantages over the standard sampling methodology of
396 taking samples back to institutional laboratories, especially in the context of life detection, where
397 changes over the course of even the days or weeks before analysis could severely affect the
398 results. Additionally, assuming that all samples are partitioned appropriately while performing in-
399 field analysis, samples can still be retained for return to an institutional laboratory if they show
400 particularly interesting results and would benefit from more extensive analysis.

401 Our expedition also has implications for robotic planetary missions. We have shown that
402 with careful planning of experimental sequences and shared resources, multiple analytical
403 techniques can be used synchronously and rapidly to provide information that improves further
404 sampling. In the context of future biomarker detection missions, such as the ExoMars or Mars
405 2020 rovers, instruments may face severe limits on their operation and the ability to increase
406 their science return within those limits will be of extreme importance. Decision making as
407 described here can help to increase science return. Future human planetary missions will also

408 need robust decision making tools, though their sampling limitations will likely be reduced as
409 compared to robotic missions.

410 The developments presented here are appropriate to the context of Icelandic lava fields, but
411 may need to be modified for other analogue sites. For example, the analogue sites of Iceland are
412 less remote than those such as the Haughton impact crater or remote regions of the Atacama
413 desert, and it may be much more challenging to assemble a fully equipped field lab in some of
414 these other analogue sites. Testing these methods in a variety of analogue sites will increase their
415 robustness under varying levels of infrastructure and demonstrate their applicability to planetary
416 missions.

417 The feasibility of implementing fluorescence microscopy with nucleic acid staining, ATP
418 bioluminescence assay and qPCR as a set of quick-turnaround life detection techniques in the
419 context of a field campaign at a Mars analogue location has been demonstrated. With the use of a
420 field laboratory such as that described here, these methods can be used and extended to increase
421 the science return of terrestrial planetary analogue expeditions.

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429 **7 Abbreviations**

430 ATP, adenosine triphosphate; Cq, quantification cycle; DNA, deoxyribonucleic acid;
431 EDTA, ethylenediaminetetraacetic acid; FIM, Fimmvörðuháls; FM, fluorescence microscopy;
432 HEI, Heimaey; qPCR, quantitative polymerase chain reaction; RLU, relative light units; Tris,
433 tris(hydroxymethyl)aminomethane; UV, ultraviolet.

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