Probe Technology for the Direct Measurement and Sampling of Ellsworth Subglacial Lake

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The direct measurement and sampling of Ellsworth Subglacial Lake is a multidisciplinary investigation of life in extreme environments and West Antarctic ice sheet history. The project's aims are (1) to determine whether, and in what form, microbial life exists in Antarctic subglacial lakes and (2) to reveal the post-Pliocene history of the West Antarctic Ice Sheet. A U.K. consortium has planned an extensive logistics and equipment development program that will deliver the necessary resources. This will include hot water drill technology for lake access through approximately 3.2 km of ice, a probe to make measurements with sensors and to collect water and sediment samples, and a percussion corer to acquire an ~3–4 m sediment core. This chapter details the requirements and early stages of design and development of the probe system. This includes the instrumentation package, water samplers, and a mini gravity corer mounted on the front of the probe. Initial design concepts for supporting equipment required at the drill site to deploy and operate the probe are also described. A review of the literature describing relevant technology is presented. The project will implement environmental protection in line with principles set out by the Scientific Committee on Antarctic Research.

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This includes application of microbiological control and best practice in protection of pristine environments within a pragmatic and realizable framework. Appropriate environmental protection standards, methods, verification protocols, and technology are being developed by the Lake Ellsworth Consortium. A review of best practice, initial plans, and results is presented.

1. INTRODUCTION

The scientific aims of the direct access and measurement of Ellsworth Subglacial Lake (ESL) are (1) to determine whether, and in what form, microbial life exists in Antarctic subglacial lakes, and (2) to reveal the post-Pliocene history of the West Antarctic Ice Sheet. The project also aims to determine the organic geochemical, hydrochemical, physical, and biological characteristics of the lake. These aims necessitate the collection of water and sediment samples from the lake and favor the use of in situ sensor technology wherever feasible. The project will meet these requirements by deploying a probe consisting of a vehicle, imaging and sonar systems, in situ sensors, water samplers, and a sediment sampler into ESL. The probe (see Figure 1) is heavily negatively buoyant, is tethered to the surface and has only simple maneuverability (depth control via tether and limited rotation). It is ~3.5 m in length, 20 cm in diameter, and consists of two pressure cases separated by water samplers. The lower case houses the instrument package and is tipped with a short sediment sampler (increasing total length to ~4 m).

Microbial control measures will be used throughout to prevent confounding of microbial analysis and to preserve this unique environment. To minimize risk, the probe is being developed using a design approach that specifies and works toward a reliability target.

This chapter begins with a summary of the performance requirements and characteristics of the ESL probe technology as demanded by the aims of the project. We then review the relevant literature describing vehicles, imaging and sonar systems, in situ sensors, sampler systems, microbial control and targeted reliability. In each case, the implications for the ESL probe technology are discussed. The initial designs for the probe and supporting equipment are then detailed.

2. PERFORMANCE REQUIREMENTS FOR ESL PROBE TECHNOLOGIES

The requirements for the ESL probe technologies are based on the most up to date understanding of ESL from geophysical experiments and research on other subglacial habitats. The design is also constrained by the requirement to minimize the environmental impact of lake access, the use of hot water drilling (HWD) to access the lake, the timetable of the program, and nature of the logistics used.

2.1. Microbial Control

The primary requirement for the ESL probe technologies is to ensure environmental protection, particularly by minimizing transfer of exogenous microorganisms to the lake. In addition, it is imperative to avoid contamination of the samples taken from the lake, as this may confound their analysis. The design of the probe and support systems is required to simplify, as far as possible, the process of achieving microbial control and to be resilient to these processes. A review of published best practice and our proposed protection methods are described below.

2.2. Physical Characteristics

The ESL HWD will result in a 360 mm borehole that refreezes at approximately 6 mm h^{-1} [*Siegert et al.*, 2006] resulting in useable diameter of ~200 mm after only a day from first access. Before the hole reduces to this size, both the probe and a sediment corer [see *Bentley et al.*, this volume] must be deployed and retrieved. There will only be sufficient fuel to ream the hole once or twice (dependent on timetable), and once drilling has commenced, there can be no delay. The probe is therefore required to be reliable and to perform on time. To maximize the duration of possible borehole traverse, the probe is required to be <200 mm in diameter. To reduce the time required to complete its deployment, it must be able to descend rapidly (>1 m s^{-1}).

The ESL probe and support equipment will be shipped from the United Kingdom to Chile from August to November 2012 in containers dimensionally identical to standard 20' ISO shipping containers. From Chile, they will be flown to Antarctica by aircraft, which can transport a maximum weight of 17 tons but no more than 10 tons per shipping container. Once in Antarctica, a 2 day overland journey is required to reach the drill site. The probe and all support equipment is therefore required to fit into standard shipping containers (i.e., must be <5.8 m long) and to survive the environmental conditions of this logistics chain.

2.3. Resilience to Harsh Environments

In transport, and in operation, the probe and support systems must withstand harsh environments and some rapid environmental changes. It is possible for us to provide

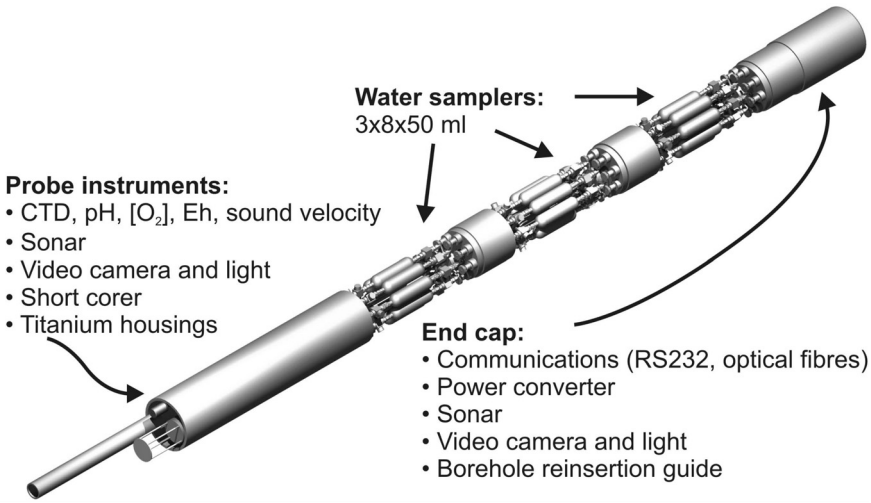

Figure 1. 3D CAD rendering of the Lake Ellsworth probe concept which consist of two gas-filled pressure cases separated by three carousels of water samplers (see Figure 2) all attached to a central core that is attached to the tether. The bottom pressure case houses the majority of the instrument package and is tipped with a short gravity core sediment sampler (increasing total length to ~4 m).

environmental control during transit (by sea, air, and overland in Antarctica), for example, temperature regulation and humidity management. However, it would be simpler and lower risk, if the systems could survive without such a system. For example, if the sample bottles are transported closed, containing sterile water, it would be advantageous if their design would enable them to survive freezing. This would mitigate the risk of failure of the temperature regulation system, while in transit to the drill site. The lowest ambient pressure (<0.7 bar (70 kPA)) will be experienced in flight to Antarctica, while the lowest temperature (<−25°C) will be experienced at the drill site and during overland transport to this location. The atmospheric pressure at the drill site is higher (~0.750–0.780 bar (75–78 kPa), measured at the ice surface over Lake Ellsworth in field season 2007/2008 (J. Woodward, personal communication, 20 August 2010)). In the top (air filled) section of the borehole, the temperature will be close to that of the surrounding ice at moderate depths (~−18°C). On entering the water at approximately 270 m depth in the borehole, the probe and tether will experience a rapid change in external temperature (~−18°C to 2°C in less than 1 s). During the descent of the borehole and lake traverse, the pressure will increase to approximately 30.2 MPa (assuming an ice thickness of 3170 m and a lake depth of 156 m) [see *Ross et al.*, this volume] and temperature will decrease from ~2°C to −5°C. On retrieval, the probe will pass through the water-air interface at the hydrostatic level in the borehole returning to atmospheric pressure and will experience a large thermal gradient (~2°C to −18°C in less than 1 s).

2.4. In Situ Measurements

To maximize the probability of successful characterization of ESL, the probe is required to make measurements of the environment with in situ sensors. The requirements for these sensors are shown in Table 1. These measurements enable the characterization of the lake environment in real-time, providing useful scientific data and informing the sampling strategies.

2.5. Water Samplers

While in situ measurements allow characterization of the environment during the deployment, retrieval of water and sediment samples is a primary aim because of the large number of detailed analyses this enables. The water sampler system is required to enable acquisition of more than 18 individual water samples of volume >50 mL enabling triplicate sampling at six locations. This gives a total of at least 150 mL per sample location, which will be divided between analysis techniques as shown in Table 2. To minimize the deployment duration, and hence maximize the probability of successful probe extraction through the borehole, the water samplers are required to trigger and fill rapidly. It should take less than 1 min to obtain a sample in each bottle. To ease sample processing and distribution to laboratories, the bottles are required to be removable (individually) from the probe and sampling system without compromising the physical seal or introducing contamination. Each bottle should be uniquely identified (e.g., carousel and

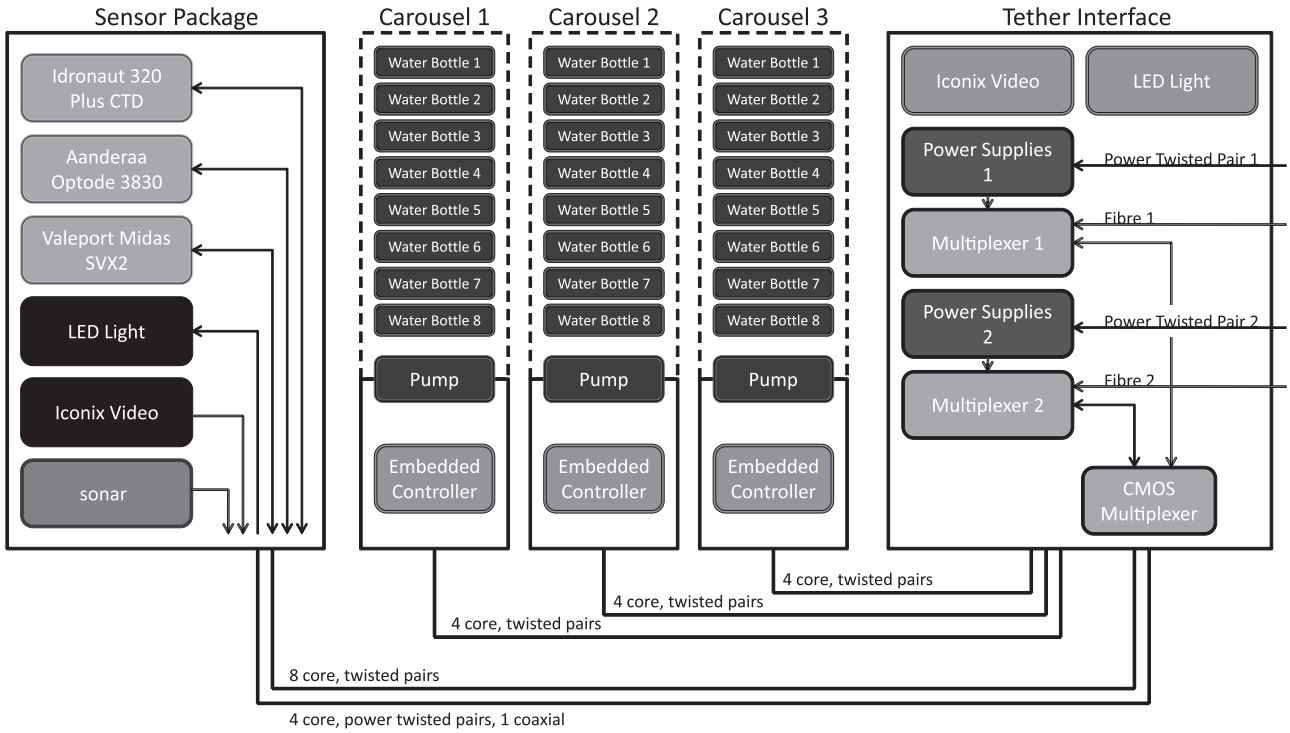

Figure 2. Schematic of the Lake Ellsworth probe highlighting electrical layout, cable connections, and electronic systems. The design depicted does not include backup batteries or an embedded microcontroller (included in design variants) to enable continued operation should the tether power or communications link fail. The current water sample protocol fires one bottle on each of the carousels simultaneously creating three >50 mL samples from each location. The provision of eight bottles allows sampling in six locations and two further locations as a reserve. Carousel 1 will be analyzed for microbiology, carousel 2 will be split between organic geochemistry and microbiology, and carousel 3 will be analyzed for hydrochemistry.

bottle number). One of the scientific aims is to characterize the dissolved gasses present in ESL, and therefore, the sample bottles are required to store water at the pressure it was collected (i.e., gas tight sampling). The design and materials selection of the bottles must minimize contamination with analytes of interest (see Table 2). Iron analysis is required, so stainless steel is not a suitable material (see sampler review below). Gas and organic geochemistry analyses may also be compromised by the use of polymer bottles or liners.

Table 1. Probe Instrumentation Requirements

Sensor	Range	Accuracy	Resolution	Time Constant
Pressure	0–1000 bar (0–100 MPa)	0.01% FS	0.001% FS	15 ms
Temperature	−5°C to −45°C	0.001°C	0.0001°C	50 ms
Conductivity	0–6400 μS cm^{-1}	5 μS cm^{-1}	0.1 μS cm^{-1}	50 ms
Oxygen (electrode)	0–1560 μM	3.1 μM	0.21 μM	3 s
pH	0–14 pH	0.01 pH	0.001 pH	3 s
Redox	−1000 to +1000 mV	1 mV	0.1 mV	3 s
Sound velocity (SV)	1400–1600 m s^{-1} (extended on request)	0.03 m s^{-1}	0.001 m s^{-1}	NC
Temperature (SV system)	−5°C to +35°C	0.01°C	0.005°C	NC
Pressure (SV system)	0–600 bar (0–60 MPa)	0.01% FS	0.001% FS	NC
Oxygen optode	0–500 μM	<8 μM or 5%, whichever is greater	<1 μM	25 s (63%)

Table 2. Analyses to be Applied to Samples Acquired Using the ESL Probe System

Analysis	Sample[a]	Details
Microscopy	Water[b] (25 mL), particles (filtrand[c]), sediment	Range of scales targeted: light microscopy ~0.2 μm, fluorescence ~30–50 nm (using DNA binding stains), SEM ~1–10 nm and TEM ~1 nm, epifluorescence to detect NADH (live organisms), FISH microautoradiography and CARD-FISH to identify specific cells and group and taxon
Molecular biology	Water (25 mL), filtrand[c], sediment	DNA will be used to construct a metagenomic library to screen for novel physiologies 16S rDNA-based community reconstruction PCR and RT-PCR to assess transcription
Microbiological physiology	Water (38 mL)[d], filtrand[c], sediment	Cultures. Incubations (including ^{14}C-labeled) to assess activity. Biomarkers including ATP, PLFAs, lipopolysaccharide, enantiomeric compounds, biogenically precipitated minerals, and stable isotopes
Organic geochemistry	Water (20 mL), filtrand[c]	Single derivatizing agent (BSTFA) and GC-MS[e] analysis to include phenols, alkylphenols, polyaromatic hydrocarbons (PAHs), fatty acids, alcohols, sterols, and amino acids HPLC[f] and (coupled) ICP-MS[g] targeting heteroatoms (e.g., organosulfur and organophosphorus compounds) and organometallic compounds (e.g., porphyrins)
Hydrochemistry	Water (requires minimum 30 mL) [*Siegert et al.*, 2006]	Geochemical reactivity, Cl^-, $\delta^{18}O$-H_2O and δD-H_2O, major cations and anions, DIC, DOC, pH, NO_3^-, NH_4^+, PO_4^{3-}, DON, DOP, O_2, $\delta^{13}C$-DIC, $\delta^{34}S$-SO_4^{2-}, $\delta^{18}O$-SO_4^{2-}, Eh, Mn(II), Fe(II) and other analytes depend on the concentration of the waters examined Gasses: N_2, O_2, CO_2, CH_4, $\delta^{13}C$-CO_2, and $\delta^{13}C$-CH_4
Sedimentology	Sediment	Environmental change: SEM, XRF, XRD, grain size, Nd-Sr, density, magnetic susceptibility, P wave, imaging, gamma radioactivity. Life detection (see microscopy, molecular biology and physiology above) SEM and microfossil analysis. Dating: magnetostratigraphy, cosmogenic isotopes (e.g., 10Be, 3He, etc.), other radioactive isotopes, carbonate isotopes, and U-Th dating

[a]Water volumes listed are the volumes analyzed per sample location of which there will be six.

[b]Water samples will be filtered for microscopic analysis. The filtrate will be retained for further hydrochemistry analysis.

[c]A total of at least 200 L of lake water will be filtered in situ with approximately 100 L filtered at a minimum of two locations. These specific analyses will examine filtrand from approximately 50 L of lake water.

[d]This water sample will be used in part to create cultures which will also be analyzed with microscopy and molecular techniques.

[e]Gas chromatography-mass spectrometry.

[f]High-performance liquid chromatography.

[g]Inductively coupled plasma-MS.

2.6. Particle Samplers

Particle samples are required to investigate the microbial and geochemical characteristics of the lake. The particulate sampling systems is required to acquire at least two samples of filtered particles at use-specified locations each from at least 100 L of lake water and must collect all particles ≥2 μm. A large volume is required to maximize the probability of capturing microorganisms or particles of interest. To enable quantitative studies, the volume filtered must be measured. It may be advisable to stop sampling prior to contact with the sediment when disturbance is likely to cause a small plume, hence triggering control from the surface is required. To ease analysis, the filter should be detachable from the probe and must be easy to isolate from the environment.

2.7. Sediment Sampler

Discussion of the coring operations planned for the ESL experiment is presented by *Bently et al.* [this volume]. The acquisition of a long sediment core (>3 m) will follow retrieval of the probe using a large corer controlled by the same tether as the probe. The main challenges in the design of this large corer are the use of a single tether, operation through a narrow borehole, and achieving sufficient penetration via onboard

percussion. For redundancy (e.g., to mitigate the risk that only the probe may be deployed), the probe is also required to capture at least one sample of sediment of >1 cm diameter and >20 cm length. This enables sampling of the sediment-water interface and the top layer of sediment, which are key targets for microbial analysis. A small diameter may be used, as preservation of sediment stratigraphy is not a priority for this sample. This will be investigated in samples from the larger percussion corer. However, disturbance or distortion of the sample should be minimized. It would be desirable to remove the sediment sampler from probe after probe recovery without losing, disturbing, or contaminating the sample.

2.8. Video

Video data is required to inform probe operation during the experiment including triggering and control of sampler systems. Images should be recorded looking downward from the bottom of the probe and upward to image the undersurface of the overlying ice. Color is not essential for imaging of ice and lake bed gross structure but may have uses in this unique environment. Resolution should be standard definition or better. Recording should be performed at the surface only, as in the event of tether failure, there will be insufficient power to run the lights.

2.9. Sonar

Sonar data is required primarily for measuring the distance to the lake floor and imaging the ice ceiling. This must be recorded simultaneously looking upward from the top of the probe and downward from the bottom of the probe. If an upward-looking camera is installed, the upward-looking sonar may be omitted. Sonar data should be recorded at the surface. If used, the upward-looking sonar must have a range of >200 m and a beam angle of >20°. The downward-facing sonar does not need to provide images but must have a range of >200 m. It would be advantageous if the downward-looking sonar enabled measurement of the sub-bottom sediment profile.

2.10. Reliability and Fault Tolerance

A high degree of system reliability is required, as the lake access borehole can only be maintained for approximately 36 h. Little or no repairs can be made on site and in addition, once HWD has commenced, there is little opportunity to alter the timetable of the experiment. The system must work on time. To achieve this reliability, a suitable risk model should be used to enable risk minimization and targeted reliability through design. In addition, testing of components and systems will provide data to improve reliability estimates. For example, early life failure of components may be reduced by stress testing, for pressure and temperature.

One possible mitigation method is to include fault tolerance, so that the system will continue to operate if one or more systems fail. For example, if power failure on the tether then occurs, the system should allow the probe to continue to communicate with the surface, take samples (water and sediment), and make in situ measurements and record data. If the communications link were to fail, the probe should take water and sediment samples in a predetermined fashion, continue to make all in situ measurements, and record the data. If both power and communications were lost, samples and data should still be acquired. A reduction in the quantity of sample and data returned may be permissible in each of these cases.

The ability to detect and isolate faults may also be necessary. For example, if one of the modules (e.g., a water sampler carousel or an individual pressure vessel) fails or is flooded, it should be possible to turn off the power and shut down this unit without compromising the performance of remaining functional systems.

In addition to the engineered robustness, automation, and fault tolerance described above, risk due to human factors should be mitigated by testing, procedural documentation, checklists, cross-checking, and rehearsal (including the use of simulation).

The final risk mitigation requirement is the construction of two probes, both of which should be delivered to site prior to the experiment.

3. REVIEW OF TECHNOLOGIES AND TECHNIQUES APPLICABLE TO SUBGLACIAL MEASUREMENT AND SAMPLING

3.1. Vehicles

Remotely operated vehicle (ROV) technology has been used successfully to explore the grounding zone at a glacier terminus [*Dawber and Powell*, 1995; *Powell et al.*, 1995] at moderate depths of approximately 100 m using commercial systems. These systems would need considerable adaptation to enable access through a borehole and to enable sterilization. Bespoke ROV systems are in development for sub-ice applications by *Vogel et al.* [2007, 2008]. This Sub-Ice ROV (SIR) is intended for exploration at the base of the Whillans Ice Stream [*Bindschadler et al.*, 2003; *Fricker and Scambos*, 2009; *Wiens et al.*, 2008] downstream of the grounding line. The vehicle transforms between a borehole decent configuration and a sub-ice exploration configuration. On decent, its

maximum diameter is 55 cm requiring a 70–75 cm borehole [*Vogel et al.*, 2008]. The tether for this vehicle is integrated with a HWD [*Bentley and Koci*, 2007; *Makinson*, 1993; *Craven et al.*, 2004] hose enabling reaming of the borehole on decent or extraction. In sub-ice configuration, SIR is a capable ROV carrying extensive oceanographic instrumentation, a water sampler, and a sediment corer. ROV operations in the vicinity of glacier-grounding zones have not required microbial control due to the open access to the marine environment in these regions and therefore an assessment of the amenability of the SIR to microbial control has not been published.

Autonomous Underwater Vehicle (AUV) technology has been successfully used under ice in West Lake Bonney, Taylor Valley, Antarctica [*Stone et al.*, 2010] and to survey cenote sinkhole environments [*Gary et al.*, 2008] using multidirectional sonar. The restricted access encountered in these environments is analogous to subglacial applications. The system has autonomy, mapping capability, and sophisticated navigation, including homing to an ice borehole. This vehicle is large (1.9 m diameter) [*Gary et al.*, 2008], has a cruise speed of 0.2 m s^{-1} and has not been designed to enable microbial control. Autosub, a large AUV developed by Natural Environment Research Council (NERC) in the United Kingdom, has successfully mapped ice topography, bathymetry, and hydrographic parameters under ice shelves in Antarctica [*McPhail et al.*, 2009; *Nicholls et al.*, 2006; *Jenkins et al.*, 2010] and under Arctic sea ice [*Wadhams et al.*, 2006]. This vehicle is large (0.9 m diameter) and is not designed for microbial control. *Bruhn et al.* [2005] propose the development of a tethered ROV/AUV for sub-ice applications; however, significant investment would be required to enable this concept to reliably deliver data for the ESL experiment. Under ice, deployments of REMUS AUV (19 cm diameter) [*Plueddemann et al.*, 2008] have been completed off Barrow, Alaska. A docking system has been developed [*Allen et al.*, 2006; *Stokey et al.*, 1997], opening the possibility of untethered lake exploration. Neither the docking system nor the AUV has been designed for microbial control. The GAVIA AUV (20 cm diameter) has been successfully deployed through ice [*Doble et al.*, 2009] though without an automated recovery system for free swimming operations. This vehicle has operated under ice while tethered [*Doble et al.*, 2009] to distances of a few hundred meters. While this has an impact on AUV performance, this is a promising low-risk solution for subglacial applications. The GAVIA AUV is depth limited to 1000 m and has not been designed for microbial control.

Melting probe technology has been used in Antarctic exploration [*Kasser*, 1960; *Ulamec et al.*, 2007; *Aamot*, 1970; *Hansen and Kersten*, 1984; *Kelty*, 1995; *Tüg*, 2003] achieving ice penetration to depths of 1000 m [*Aamot*, 1968]. This technology has also been proposed for astrobiological missions to explore the polar ice caps of Mars [*Zimmerman et al.*, 2002, 2001] and Europa [*Bruhn et al.*, 2005; *Zimmerman et al.*, 2001; *Biele et al.*, 2002; *French et al.*, 2001] where an extensive sub-ice ocean is expected [*Carr et al.*, 1998]. There is some similarity between vehicles designed for Europan and Antarctic exploration [*Ulamec et al.*, 2007; *Biele et al.*, 2002]. Heat generated within the vehicle or probe tip is used to penetrate the ice. Radioisotope thermo(electric) generators are proposed in astrobiological applications [*Zimmerman et al.*, 2001] but would not be permitted in Antarctica. Melt-induced penetration can be problematic in near-vacuum conditions [*Kaufmann et al.*, 2009] or if the ice contains dust or sediment, as this collects at the probe tip forming an impenetrable barrier [*Ulamec et al.*, 2007]. These systems communicate to the surface using a tether that spools out from the topmost part of the vehicle and is refrozen into the ice as the vehicle descends. In this manner, the ice cap seal overlying the water body is not broken at breakthrough into this environment. However, the volume available for the tether spool is limited resulting in either a short, or thin, tether. In deep sub-ice applications, a short tether necessitates a larger taxi vehicle [*Ulamec et al.*, 2007], whereas a thin tether necessitates a probe containing significant onboard power to enable penetration of the ice sheet. Rapid recovery of melting probes (e.g., for sample retrieval) is problematic.

3.2. Implications for the ESL Vehicle (Probe)

The measurement and sampling of ESL requires a vehicle with a unique portfolio of attributes. It must be designed to facilitate microbial control, whereas current ROVs and AUVs are not specifically designed to meet this requirement. Considerable investment would be required to develop protocols for microbial control and validation on these platforms. This development is not without risk. In addition, the combination of stringent requirements for reliability, environmental resilience, and small diameter cannot be met by existing AUV or ROV systems. Melting probes are attractive because of their simplicity, which eases microbial control and enhances reliability. However, problems with drilling caused by debris are well documented, and deep drilling (as required for ESL) is challenging. In addition, a melt probe cannot be rapidly retrieved from the lake, which is incompatible with rapid analysis of water and sediment samples from sub-ice environments, as required for the ESL experiment. It is these unique and unmet requirements that have motivated the development of a custom-made probe.

3.3. Imaging and Sensor Systems

3.3.1. Imagery. Imagery through ice access boreholes (typically formed by HWD) has been used in a number of studies [*Engelhardt et al.*, 1978; *Bruchhausen et al.*, 1979; *Lipps et al.*, 1979; *Craven et al.*, 2005; *Carsey et al.*, 2002; *Behar et al.*, 2001; *Harper et al.*, 2002; *Harrison and Kamb*, 1973]. As is used routinely in ROV technology, video may be streamed in real time via a fiber optic communications link [e.g., *Craven et al.*, 2005]. Borehole imaging systems typically obtain images over short distances and low turbidity is frequently observed in sub-ice environments. Therefore, scattering and optical absorption do not prevent the acquisition of high-quality images [*Craven et al.*, 2005; *Carsey et al.*, 2002; *Harper et al.*, 2002]. Imaging has been achieved with modest separation of the light source and the camera. However, turbidity has been observed as a consequence of thermal/mechanical [*Engelhardt et al.*, 1978] and HWD [*Craven et al.*, 2005] due to suspended glacial flour in drill fluids. Innovations in underwater imaging [*Kocak et al.*, 2008; *Caimi et al.*, 2008; *Kocak and Caimi*, 2005; *Mueller et al.*, 2006] include the extensive use of digital camera technology for still, video, three-dimensional (3-D), and holographic imaging. Digital technology is compact and low-cost with low-light, IR, high-resolution, and high-speed variants available. For subglacial applications, imaging system design must consider the tradeoff between resolution, low-light performance, and frame rate (e.g., CCD still cameras can offer improved resolution or low-light level performance at the expense of frame rate). High-definition video and even higher-resolution formats [*Kocak et al.*, 2008; *Caimi et al.*, 2008] are supported by commercially available camera technologies enabling a good compromise between resolution and frame rate while enabling color recording. Lighting technologies are critical for ensuring adequate range, color representation and minimizing scatter [*Kocak et al.*, 2008; *Caimi et al.*, 2008; *Kocak and Caimi*, 2005; *Jaffe*, 2010, 2005]. LEDs offer robust high-efficiency solutions, while angular separation from the camera and structured light sources dramatically reduce scatter and increase range [*Kocak et al.*, 2008; *Caimi et al.*, 2008; *Jaffe*, 2010, 2005]. Quantitative information, such as object size and location, can be obtained from images using laser spots or with a penalty of increased system size/complexity, with 3-D or holographic imaging systems [*Kocak et al.*, 2008; *Caimi et al.*, 2008; *Mueller et al.*, 2006].

3.3.2. In situ sensors. In situ sensors have been used in glacier and sub-glacial systems to measure temperature [*Engelhardt et al.*, 1990; *Hart et al.*, 2009], pressure [*Engelhardt et al.*, 1990; *Hart et al.*, 2009; *Stone and Clarke*, 1996; *Harrison et al.*, 2004], electrical conductivity [*Hart et al.*, 2009; *Stone and Clarke*, 1996; *Stone et al.*, 1993; *Gordon et al.*, 1998], and turbidity [*Stone and Clarke*, 1996; *Stone et al.*, 1993; *Gordon et al.*, 1998]. In situ oxygen measurements have been made under sea ice [*Kühl et al.*, 2001; *McMinn and Ashworth*, 1998; *Trenerry et al.*, 2002], in laboratory-based sea ice mesocosms [*Mock et al.*, 2002, 2003], and in glacial melt waters and cryoconite holes in Antarctica [*Bagshaw et al.*, 2011]. Larger sensor systems have also been deployed for simultaneous monitoring of carbon dioxide and oxygen concentrations in ice-covered lakes [*Baehr and DeGrandpre*, 2002].

The use of in situ sensor systems is more widespread in an oceanographic setting [*Daly and Byrne*, 2004], where there is often less restricted access, and there is large-scale logistical support (e.g., a research vessel). In this setting conductivity, temperature and pressure (depth) sensing is routine. Commercial sensors exist for many parameters including oxygen [*Tengberg et al.*, 2006; *Falkner et al.*, 2005], chlorophyll, and hydrocarbon fluorescence [*Falkowski and Kiefer*, 1985; *Suggett et al.*, 2009], carbon dioxide [*Degrandpre*, 1993], nutrients/inorganic anions [*Johnson and Coletti*, 2002; *Hanson*, 2005], pH [*Seidel et al.*, 2008], and methane [*Fukasawa et al.*, 2006]. However, sensor performance and suitability for sterilization must be carefully matched to the application to ensure high-quality studies. Larger analytical systems using membrane inlet mass spectroscopy are in the early stages of commercialization [*Camilli and Duryea*, 2007; *Short et al.*, 1999, 2006] and have overcome many of the difficulties in calibration and quantitative analysis [*Bell et al.*, 2007]. Biological and chemical sensor and analytical system development is an active area of research [*Daly and Byrne*, 2004; *Prien*, 2007].

3.4. Implications for Imaging and Sensor Technologies for ESL

The requirement for imaging and in situ sensing in ESL experiment are well matched by commercially available technologies that are proven in similar environments. Some care is required to ensure that these technologies meet the demanding performance specification, and further work is required to prove that microbial control is feasible. The requirement for proven performance and high reliability for any system used in the ESL experiment makes the use of research instrumentation unattractive in this application. While the effects on imaging of turbidity in boreholes formed by HWD are of concern, this phenomena is not anticipated in ESL. During the ESL experiment, HWD fluids will be brought to the surface at a rate equal to the injection rate at the cutting head (~3 L s^{-1}) and filtered (<0.2 μm)

before reinjection. This process will limit the accumulation of turbidity in the ESL borehole.

3.5. Sampler Systems

Sampler systems for water, dissolved gasses, and particles have been widely developed for subglacial and oceanographic applications. The requirement for multiple water samples and quantitative gas content analysis in the ESL experiment suggests either gas-tight water sampling or quantitative analysis of outgassing (and capture of samples of both the water and the escaping gas). The expected low-particulate concentration in ESL suggests filtration capture from large volumes of water.

3.5.1. Water sampling. Water sampling has been used effectively in glacier systems and boreholes enabling characterization of geochemistry and study of hydrological and glacial processes [*Tranter et al.*, 1997; *Blake and Clarke*, 1991; *Gaidos et al.*, 2007]. Typically formed from acrylic plexiglass, such systems are not gas tight or sufficiently pressure resistant for the ESL experiment. A compact (12 cm × 85 cm) and elegant gas-tight sampler developed by *Roman and Camilli* [2007] enables collection of 8 × 20 mL samples. Unfortunately, the depth rating is only 2000 m in seawater (~20.5 MPa pressure, ESL 30.2 MPa). It also uses a single multiport commutating valve (which represents a potential single point of failure) and has not been designed to be sterilized both internally and externally. Other gas-tight samplers [*Albro et al.*, 1990; *Tabor et al.*, 1981] offer additional useful design insights, but require significant re-engineering to make them sufficiently compact for the ESL experiment. A commercially available system (AquaLab Deep Ocean Water, EnviroTech LLC) using flexible titanium bags provides water sampling and retention of gas. This system does not maintain pressure, but gas can be retained within the limits of the flexible bag. Unfortunately, it also uses a multiport valve and would require compaction for ESL. Numerous systems exist for water sampling without maintaining pressure or retaining gas. The Niskin bottle [*Niskin et al.*, 1973] rosette is frequently used in an oceanographic setting. This system consists of an array of bottles (often tubes) with sealing end caps at either end, which when released are pulled together by an elastic or spring member. Release systems are commercially available (e.g., SBE 32, Seabird Electronics Inc.) but are not sufficiently compact for the ESL sampler. More compact systems based on a multiport valve exist (Aqua Monitor, EnviroTech LLC) but do not sample gas quantitatively. Alternative designs include variants of the end seal system as used in Niskin bottles [*de Resseguier*, 2000] and syringes (to pump and retain water samples) [*Di Meo et al.*, 1999]. Some include some form of microprocessor, e.g., to enable event-driven sampling [e.g., *Ruberg et al.*, 2000].

Care must be taken in the choice of materials, which come into contact with the sample. *Doherty et al.* [2003] recommend the use of titanium and estimate the contamination caused by commercially available (weakly alloyed) grades. They conclude that for seawater retained in 5 L bottles for 4 h, only titanium (approximately 1.3k%–18k% contamination) and tin (1.4%–118% contamination) quantification would be severely affected and that iron contamination would be less than 3.1% (~1.6 pM). Polymeric materials can also be problematic for trace gas analysis [*Doherty et al.*, 2003]. Other trace metal analysis systems using polymer bottles and acid washing [*Bell et al.*, 2002] release acid into the environment. Stainless steel is problematic for trace iron analysis. Even when coated and excluded from the interior of the sample, bottle contamination may result [*Hunter et al.*, 1996; *Martin et al.*, 1990].

Alternative strategies include sampling via a pipe or tube with a processing system above the hydrostatic level. Sampling can either be by direct suction pumping [*Stukas et al.*, 1999], which depressurizes the sample and results in degassing, or using an innovative 'U' tube design, which maintains the sample at pressure [*Freifeld et al.*, 2005]. In each case, the tube represents single point of failure, and for the U-tube, extensive processing equipment is required at the surface. Another approach collects only the analyte of interest by passing sample water through an adsorption column [*Johnson et al.*, 1987], but this limits the number of analyses that can be performed.

3.5.2. Particles. Particles may be analyzed by extraction (e.g., concentration using filtration) from water samples, but this may be problematic in the dilute samples expected in ESL. In situ systems for particle sampling typically use a pump and filter [e.g., *Johnson et al.*, 1987; *Behar et al.*, 2006]. Commercially available systems exist (e.g., WTS-LV, McLane Research Laboratories Inc.) but would require adaptation to enable miniaturization and sterilization required for ESL.

3.5.3. Corer technology. This technology is used widely in oceanographic, geological, and industrial (e.g., oil and gas) settings resulting in a significant market serviced by a number of commercial products. Innovations include pressurized systems for acquisition of gas hydrates [*Abegg et al.*, 2008], hydrostatically powered hammer corers [*Kristoffersen et al.*, 2006], and line-operated percussion corers [*Chambers and Cameron*, 2001; *Neale and Walker*, 1996], freezing sampling [*Neale and Walker*, 1996] and designs for fine-grained

[*Jahnke and Knight*, 1997] or soft [*Blomqvist*, 1991] sediments. Lacustrine sediment cores have been obtained (UWITEC gravity and percussion corers) from perennially ice-covered former subglacial Lake Hodgson in a region 93.4 m beneath the ice surface [*Hodgson et al.*, 2009]. Acquisition of short sediment cores has long provenance and enables microbial, faunal [*Tita et al.*, 2000], input rate [*Guevara et al.*, 2005], organic degradation [*Sun and Wakeham*, 1994], sediment, and paleosedimentology [*Meriläinen et al.*, 2000] studies. A number of systems for acquisition of a short core are available commercially.

3.6. Implications for ESL Sampler and Corer Technologies

The requirements for microbial control, multiple samples, small size, reliability, and compatibility with a wide range of organic geochemistry, hydrochemistry, and microbiological analysis are not met by existing water and particulate sampler technologies. In addition for water samples, the requirement for gas analysis necessitates a pressure-tolerant (gas-tight) design. While gas-tight sampling has been demonstrated widely, no system exists which meets the aforementioned constraints. Custom-made water sampler systems and particulate samplers, which draw on elements of existing systems, are therefore required for ESL.

Existing sediment sampler technologies are in contrast, far simpler, robust, and amenable to microbial control. This suggests the use of commercial solutions for both the large percussion corer and the short core mounted on the probe. The probe-mounted short core will require careful selection and testing to ensure effective sampling of the sediment-water interface and upper sediment.

3.7. Microbial Control

Microbial control during subglacial lake access experiments is recommended by the Scientific Committee on Antarctic Research (SCAR) [*Alekhina et al.*, 2009] and by the U.S. National Academies [*Committee on Principles of Environmental Stewardship for the Exploration and Study of Subglacial Environments, N.R.C.*, 2007]. Both Committees express a number of principles pertaining to stewardship of these unique environments, which may be summarized as follows:

1. Possible damage and contamination is minimized to safeguard the scientific value of subglacial lakes and to conserve these pristine environments.

2. The results of sampling and microbial analysis must not be confounded by contamination.

3. Any access to the base of Antarctic ice sheets should assume underlying liquid water forming part of a drainage network requiring particular attention to upstream sites.

4. That a living ecosystem is possible necessitating prevention of any permanent alteration of the biology (including introduction of alien species) or habitat.

5. Any contamination should not be expected to change the measurable properties of the environment and should be expected to have a less than minor and/or transitory impact.

6. That minor, transitory, and undetectable impacts are acceptable in pursuit of scientific understanding and that these should be mitigated as far as possible.

These principles have not as yet been translated into standards, methods, or verification protocols. These details are in development by the subglacial access community. However, microbial control is routinely employed in medical, pharmaceutical, food, and space exploration industries.

Microbial control can be achieved by cleaning, disinfection, and/or sterilization. "Sterilization" is defined [*Allen et al.*, 1997] as a process used to render an object free from viable microorganisms, including bacterial spores and viruses. "Disinfection" is defined as a process used to reduce the number of viable microorganisms but which may not inactivate some viruses or bacterial spores. "Cleaning," an essential prerequisite for disinfection and sterilization, is defined as a process which physically removes contamination and hence reduces the microbial load but does not necessarily render microorganisms nonviable [*Allen et al.*, 1997]. Cleaning can remove grease, soil, and about 80% of microorganisms, but chemical disinfection is essential to completely neutralize most viable microorganisms [*Ayliffe et al.*, 1992]. Surfaces that are clean and dry will not support the growth of most bacteria [*Wilson*, 2001].

Sterility assurance level (SAL) is a term used in microbiology to describe the probability of a single unit being nonsterile after it has been subjected to the sterilization process, according to ISO 11139: 2006 specifications of the International Organization for Standardization [*Rutala and Weber*, 1999; *McDonnell and Moselio*, 2009; *Mosley*, 2006]. Aseptically produced products are generally considered to have a SAL of 10^{-3} or more, while physical sterilization technologies used in component preparation resulted in a SAL of at least 10^{-6} [*Agalloco*, 2004].

3.7.1. Microbial control standards. The pharmaceutical industry has very strict regulations for the production of sterile drug products by aseptic processing [*Agalloco*, 2004]. The drug products can be either produced under aseptic processing or sterilized at a terminal stage. The U.K. National Health Service (NHS) has recently published a policy on cleaning, disinfection, and sterilization [*Holmes*, 2008]. Also, National Specifications for Cleanliness in the NHS are available [*National Patient Safety Agency*, 2007]. Bacterial contamination is closely monitored in the food

industry [*Haysom and Sharp*, 2005]. Both the U.K. *Food Standards Agency* [2004] and the European Union have published guidelines on food hygiene practices. The Food and Drug Administration utilizes the ISO 14644 standards for manufacturing facilities in the pharmaceutical industry (see Table 3).

These standards control the environment of manufacture, but require that components and materials enter the process precleaned. They are most suitable for equipment assembly and further processing. While control to ISO 14644 class 5 would achieve a high level of cleanliness, it would require extensive infrastructure, which is not easily or economically available to the Ellsworth consortium. ISO 14644 class 6 and below also require significant infrastructure but are unlikely on their own to enable sufficient control particularly for our most stringent requirement, i.e., maintaining contamination below that which will compromise our measurement of samples with potentially very low numbers of endogenous microorganisms.

The most applicable standards to our project are those used by the space exploration industry. The space science community has a long history of microbial control both for forward contamination (i.e., protection of the explored environment) and backward contamination (resulting from the return of samples). The acceptable terminal sterilization bioburden level for Viking vehicles is 30 bacterial spores per vehicle. A series of guidelines has been published by NASA for the purpose of planetary protection [*Barengoltz*, 2005; *DeVincenzi et al.*, 1996]. Ice coring in Mars employed alcohol, bleach, and flame sterilization, and very low counts using the ATP luciferin-luciferase assay were enough to validate sterility [*Steele et al.*, 2006]. Contamination control during the MARTE drilling project (intended for Mars missions) was achieved by using a suite of procedures depending on material compatibility, laminar flow assembly for hardware, and bagging during transport and testing [*Miller et al.*, 2008]. Microbial load was validated by a negative ATP luciferin-luciferase assay, which corresponds to less than four cell number equivalents per square centimeters [*Eigenbrode et al.*, 2009]. It must be noted that extrapolating cell numbers from the luciferin assay is difficult; hence, the method is semiquantitative.

3.7.2. Disinfection and sterilization. For microbial control, disinfection and sterilization can be either physical or chemical. The current approaches have been thoroughly reviewed [*Rutala and Weber*, 1999; *McDonnell and Moselio*, 2009], but a short review follows.

3.7.3. Physical methods. Physical methods include wet and dry heat, radiation, and filtration. Wet heat, or autoclaving, typically involves sample exposure to steam for 15 min at 121°C or 3 min at 134°C. Autoclaving still remains the most popular method for sterilization of health care surgical equipment [*National Patient Safety Agency*, 2007] and glass and elastomeric components used in the pharmaceutical industry [*Agalloco*, 2004]. This method is problematic for electronics, water sensitive, or temperature-intolerant materials, which are used on the ESL probe. However, autoclaving can be attractive for robust subsystems (e.g., the water sampler bottle). Dry heat is used extensively for sterilization of glassware and reduces bacterial endotoxins and spores resulting in a SAL of 10^{-6} [*Agalloco*, 2004]. Until 2005, dry heat was the approved sterilization technique of NASA for planetary protection for instruments and probes [*DeVincenzi et al.*, 1996]. It remains the most practical technique for large hardware, but NASA has replaced it for electronics parts with hydrogen peroxide vapor (HPV; see combined physical and chemical treatments which are reviewed below) [*Chung et al.*, 2008]. Dry heat is not applicable to most polymers, sensors, and electronic systems used in the ESL probe. While suitable for components

Table 3. Pharmaceutical Industry Permissible Limits for Cleanliness as Per ISO 14644 Standard[a]

Clean Area Contamination ($\geq$ 0.5 μm particles/ft^3)	ISO 14644 Designation	$\geq$ 0.5 μm particles/m^3	Microbiological Active Air Action Levels[b] (CFU m^{-3})	Microbiological Settling Plates Action Levels[c]
100	5	3,520	1[d]	1[d]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

[a]Reproduced from *Department of Health and Human Services* [2004].

[b]The number of colony-forming units (CFU) per cubic meter of air.

[c]Number of CFU collected on a 90 mm culturing media (settling) plate with 4 h exposure.

[d]Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

(e.g., metal items prior to assembly), larger units require extensive facilities. Alcohol and flame sterilization is another form of dry heat treatment that is particularly attractive because of its simplicity [*Richardson*, 1987]. However, flame sterilization is restricted to resistant materials, and there is also a lack of data on its efficacy, making its use for ESL unattractive.

Radiation methods involve the irradiation of the samples with energy as particles or electromagnetic waves. Ionizing radiation treatment with beta or gamma rays is very effective but costly, requires an isolated site, and may affect the bulk properties of polymers by disruption of chemical bonds [*Henn et al.*, 1996]. The nonionizing alternative of UV radiation treatment is suitable for heat-sensitive materials and thus could be used for electronic components. Also, UV irradiation will irreversibly degrade DNA. The most effective wavelength for sterilizing bacterial spores from *Bacillus atrophaeus* (a robust spore forming model organism) is from 235 to 300 nm [*Halfmann et al.*, 2007]. UV is attractive for the ESL experiment in a number of applications (e.g., borehole and tether sterilization), as systems can be compact, noncontact, and fast-acting. However, it is only effective on exposed surfaces and is nonpenetrating, necessitating alternative strategies for recessed components and closed systems.

Filtration is the last alternative of physical disinfection and sterilization. Sterile filtration can be performed in a normal flow or a dead-end configuration, and microorganisms are excluded from filtered liquids or gases [*van Reis and Zydney*, 2001]. First, gases are usually filtered using depth filters, which are made of mineral, glass, or cotton wool. Second, membrane filters retain particles based on their size depending on the diameter of membrane pores. Finally, nucleation track filters are similar to membrane filters, but consist of irradiated thin polycarbonate films.

Sterilizing filters were originally manufactured with a 0.45 μm pore-size specification, but current regulatory standards have decreased the pore size to 0.22 μm to enable retention of bacterium *Brevundimonas diminuta*, formerly known as *Pseodomonas diminuta* [*Jornitz et al.*, 2003]. However, some organisms cannot be retained by 0.22 μm filters [*Wallhausser*, 1979]; thus, a 0.1 μm pore size filter has to be used if viruses, mycoplasmas, and small bacteria need to be retained. Sterilizing filtration for liquids has been critically reviewed by the Parenteral Drug Association, a leading authority of pharmaceutical science in the United States [*Antonsen et al.*, 2008]. A biological filtration system, which uses a modular cartridge to remove all bacteria and many viruses, as small as 45 nm, has been incorporated in the concept design of the hot water drill used for the exploration of Lake Ellsworth [*Siegert et al.*, 2006].

3.7.4. Chemical treatment. Chemical treatment includes use of hypochlorite (household bleach), acids, bases, alcohols, halogens, epoxides, phenols, metals, oxidizing agents, quaternary ammonium compounds, and aldehydes. The method is attractive, particularly in preparation of materials prior to encapsulation for shipping, because of the limited infrastructure required to achieve high efficacy. However, handling of these materials requires care, and they are less attractive on site in Antarctica, as risk reduction and disposal will require further on site infrastructure. Treatment with 2-glutaraldehyde for 2–3 h has been traditionally used for the inactivation of most viruses, vegetative bacteria, and mycobacteria on surgical instruments but is now less common due to its high chemical toxicity [*Manzoor et al.*, 1999]. However, toxic effects can be significantly diminished by subsequent washes of the sample with a saline solution [*Tosun et al.*, 2003]. Ethylene oxide gas (EtO) is traditionally used for sterilization of plastics and other nonheat stable components used for packing of drug products [*Agalloco*, 2004]. Despite environmental and occupational safety hazards, it is still used in the pharmaceutical industry [*Mendes et al.*, 2007]. It is suitable for heat-sensitive materials and thus could be used for electronic components. Commercial chemical disinfectants include Virkon© [*Hernandez et al.*, 2000], which contains suspected neurotoxicant sodium dodecylbenzenesulfonate [*Gasparini et al.*, 1995]. Enzymes and peptides, although not strictly "chemicals," can also be used for disinfection and sterilization because of their biocidal activity. Different types of enzyme include esterases, nucleases, and lysozyme. Lysozyme, for example, hydrolyzes cell walls and membrane components and can be an effective biocide against bacteria, fungi, protozoan, and viruses [*Benkerroum*, 2008]. Biocidal peptides include nisin and magainin. Nisin is a lactic acid bacteria metabolite, which is commonly used as a food preservative because of its antimicrobial properties [*Maqueda and Rodriguez*, 2008]. Similarly, magainin, a peptide first extracted from the African clawed frog *Xenopus laevis* in the 1980s [*Zasloff*, 1987], has since revolutionized the use of antimicrobial peptides for food industry and agriculture [*Meng and Wang*, 2010].

3.7.5. Combined physical and chemical treatments. The most recently developed techniques for disinfection and sterilization combine physical and chemical treatments and are termed as physiochemical. The most common example is the aforementioned HPV. HPV is suitable for heat-sensitive materials and, thus, could be used for electronic components. HPV acts as a biocide using the free radicals produced when hydrogen peroxide is heated beyond its liquid-vapor conversion point and forced to evaporate.

After decontamination, the HPV can be converted (either catalytically or using ventilation) into water vapor and oxygen, leaving no toxicity. HPV is currently used for planetary protection by NASA [*Chung et al.*, 2008] and has been found to completely deactivate bacterial spores of *Clostridium botulinum*, *Clostridium* spp. and *Geobacillus stearothermophilus* dried onto stainless steel surfaces [*Johnston et al.*, 2005]. Large-scale applications of this method are currently developed for the decontamination of whole buildings from *Bacillus anthracis* [*Wood and Blair Martin*, 2009]. This method is attractive for ESL in both the preparation of engineering structures and on site. The duration of ventilation required to enable complete conversion to water and oxygen is volume and temperature dependent (e.g., 2 h at 20°C for large rooms). The suitability of the method must therefore be carefully assessed for moving objects (e.g., the tether or drill hose) or during time-critical processes (e.g., loading the probe into the borehole). Other alternative physiochemical treatments employ plasmas, which are highly energized gases. Low-temperature plasma treatment (LTPT) is suitable for heat-sensitive materials, such as electronic components. LTPT exposes any microorganisms present in the sample to an electrical discharge with biocidal effects [*Moisan et al.*, 2001]. Low-pressure plasma treatment is used for surgical instruments and usually includes a UV irradiation step for genetic material destruction [*Kylian and Rossi*, 2009]. Chlorine dioxide vapor is suitable for heat-sensitive materials and thus could be used for electronic components. Large-scale applications of this method are currently developed for the decontamination of whole buildings from *B. anthracis* [*Wood and Blair Martin*, 2009]. While effective, these methods are less suited to the ESL experiment either because of disposal, the infrastructure required, or flexibility.

3.7.6. Other techniques. Techniques which could be defined as neither physical nor chemical include anodic protection [*Nakayama et al.*, 1998] and freeze-thaw cycling [*Walker et al.*, 2006]. Also, special sample manipulation is used for sediments [*Lanoil et al.*, 2009], permafrost [*Vishnivetskaya et al.*, 2000], or ice cores [*Bulat et al.*, 2009; *Christner et al.*, 2005]. Material is removed from the innermost portion of the solid sample, while the outer layers of the core protect the sample used in the measurement. While these processes may be applicable to the treatment of samples, they are not suitable for the engineered structures used in the ESL probe systems, as they would either be ineffective, would create engineering challenges, or a better result could be obtained using alternative methods.

3.8. Microbial Assessment

Microbial assessment is undertaken once disinfection and sterilization has been affected to probe components. This is required to verify the processes which we use to control microbiology. This provides assurance that we are working in accordance with the recommendations (SCAR [*Alekhina et al.*, 2009], U.S. National Academies [*Committee on Principles of Environmental Stewardship for the Exploration and Study of Subglacial Environments, N.R.C.*, 2007]). However, it is likely that our requirement to prevent confounding of samples analyzed for microbiology is harder to achieve. Assessment methods also help us to develop techniques that enable us to reach this target.

A number of techniques are available to assess and validate the efficacy of disinfection and sterilization. While microscopy (see below) may be performed on engineered surfaces, other techniques require creation of an aqueous sample. This can be achieved using swabs or washing both with and without subsequent concentration steps (e.g., centrifugation). Such additional sample preparation steps can induce errors and sample contamination, which must be accounted for in protocol design.

The simplest assessment methods provide data on cell numbers. Quantitative information can be gained utilizing fluorescence microscopy [*Kepner and Pratt*, 1994] or flow cytometry [*Hoefel et al.*, 2003; *Lebaron et al.*, 1998; *Lemarchand et al.*, 2001]. Fluorescent cell staining dyes are used to increase contrast and include 4′,6-diamidino-2-phenylindole (DAPI) [*Kepner and Pratt*, 1994] and can be used to discriminate between live and dead cells [*Boulos et al.*, 1999]. Low cell density requires careful contamination control, differential measurement versus blanks, and may be aided by a preconcentration step to raise the measured cell number above any background. However, such additional processing can also lead to contamination and loss of whole cells.

A total viable count enumerates cell density or population typically using serial dilution, culturing on appropriate growth media, and counting of colony-forming units (CFU) [*Miles et al.*, 1938]. As growth rates are culture- and species-dependent, the result is semiquantitative. Different culture media can be used to selectively grow different target microorganism, but only culturable species can be investigated. The process is also slow, particularly for psychrophilic organisms, which take weeks or months to culture. Therefore, there is limited applicability to field monitoring under Antarctic conditions.

Finally, adenosine triphosphate (ATP) and other similar biomarkers can be used as a marker of cell presence on probe components. ATP concentration can be measured using the bioluminescence luciferin-luciferase assay [*Lin and Cohen*,

1968]. The concentration of ATP found in solution in environmental samples is subject to processing and matrix effects as ATP binds efficiently to surfaces [*Webster et al.*, 1984].

More complex analyses for microbial load assessment include qualitative and quantitative nucleic acid detection. Tested surfaces can be swabbed and followed by total DNA or RNA extraction and purification. The pure nucleic acids can then be quantified by UV spectrophotometry and gel electrophoresis using intercalating chemical dyes like ethidium bromide, SYBR®, RiboGreen®, or Hoechst stains. The sensitivity of intercalating dyes is within the range of 10 ng mL^{-1} to 50 μg mL^{-1} of nucleic acids. To improve sensitivity and detection limits, quantitative molecular biology techniques can also be employed for detection and speciation of contaminant organisms. The state-of-the-art methods include quantitative polymerase chain reaction (qPCR), fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal-restriction fragment length polymorphisms (T-RFLP), and automated ribosomal intergenic spacer analysis (ARISA).

qPCR is a quantitative DNA amplification protocol with real-time detection of the amount of amplified DNA using a fluorescent reporter molecule. Use of generic markers and fluorescent probes for terrestrial bacteria and archaea and comparison of the results of samples of unknown concentration with a series of standards can determine accurately the amount of template DNA in samples and help to assess how DNA-free surfaces really are. The technique can be applied to difficult sample matrices such as soil [*Picard et al.*, 1992]. FISH, in optimum conditions, is a quantitative nucleic acid detection technique that employs fluorescent probes with sequences complementary to known sequences. In optimum conditions, it is a nondestructive (i.e., cells remain intact) and can therefore be used together with flow cytometry or microscopy. However, to ensure the fluorescent probes gain access to cellular contents, the cell membrane must be made permeable. This process is rarely perfect, either resulting in impermeable membranes and unstained cells, or cell lysis. Both problems result in an underestimation of populations in uncompensated analyses. As in qPCR, generic markers for terrestrial bacteria and archaea could be used to validate whether our sterilization methods are effective. FISH also targets 16S ribosomal RNA (rRNA); thus, only viable cells or recently moribund cells are detected. Most recently, FISH has been used to monitor bacterial community fouling on polyvinylidene fluoride and polyethylene filter membranes used for freshwater treatment [*Fontanos et al.*, 2010].

DGGE is another DNA-based technique, which is most often used to look at the community structure of samples rather than to quantitatively measure cell numbers. It can separate (often amplified) DNA amplicons of PCR of the same length but with different sequences and involves the construction of clone libraries [*Muyzer and Smalla*, 1998]. DGGE has been used to study bacterial populations in a variety of samples, from antique paintings [*Piñar et al.*, 2001] to processed water from waste water plants [*Boon et al.*, 2002]. The disadvantage of DGGE is that it may lack the specificity to enable separation of unknown species from known ones, and if used in the Antarctic environment where new species might inhabit, novel organisms may be overlooked.

T-RFLP is based on the analysis of bacterial 16S rRNA, which is a small subunit of nucleic acid, which is highly conserved in bacteria [*Liu et al.*, 1997]. Archaeal diversity in deep-sea sediments has been estimated using T-RFLP [*Luna et al.*, 2009]. Length Heterogeneity-PCR is very similar to T-RFLP [*Suzuki et al.*, 1998] and similarly takes advantage of naturally occurring sequence length variation. A further variant is amplified rDNA restriction analysis [*Liu et al.*, 1997]. These variations can be small, and therefore, the technique requires single base pair resolution of sequence length. The 16S rRNA genes are often targeted.

ARISA was originally developed for studying populations in soil [*Borneman and Triplett*, 1997] and then also applied to freshwater samples [*Fisher and Triplett*, 1999]. ARISA involves total community DNA isolation, PCR amplification using a fluorescent forward primer and ARISA-PCR fragments analyzed using automated gel electrophoresis. Nucleic acid analysis in Antarctic samples is a challenge, as gene sequences for novel organisms are not known a priori, which is a requirement for methods such as qPCR and FISH.

As an alternative to microbial enumeration and speciation, chemical tracers can be used to estimate transfer of contaminant materials after disinfection and sterilization. This technique is particularly useful, if the limit of detection of the chemical tracer is lower than that of the contaminant. Multiple tracers could also be used to verify the source of contamination [*Smith et al.*, 2004]. For example, the drill fluid could be loaded with a tracer, and the surfaces of engineering structures loaded with another. Tracers can also be used in solid samples, such as ice or sediment cores, in order to evaluate penetration of contamination [*Christner et al.*, 2005]. Perfluorocarbon and fluorescent-microspheres-based tracers have been used widely in the International Ocean Drilling Programme [*D'Hondt et al.*, 2004; *Lever et al.*, 2006]. The use of tracers for subglacial exploration would have to be carefully controlled to prevent the tracer from becoming a chemical contaminant in the environment or in the samples.

3.9. Implications for Microbial Control for the ESL Experiment

The protection of subglacial lake environments and assurance of microbiological sample validity through microbial control remains the most challenging aspect of the development of probe and support system technologies for the ESL experiment. While progress has been made in the development of universally accepted standards for subglacial environmental protection, further work is required to translate these to agreed targets, methods, and verification protocols. In contrast, the science of microbial control and assessment is advanced, particularly in analogous disciplines such as space science and in health care. While microbial control and assessment techniques will need to be modified and assessed when applied to ESL technologies, widely used techniques are promising in this application. The development of appropriate methods of microbial control and assessment should be undertaken in parallel with the design and construction of technologies for the ESL experiment.

3.10. Targeted Reliability

The short duration during which the borehole into ESL will remain open necessitates a high degree of reliability of the engineered systems used in the experiment. There are a number of techniques that may be applied to engineering systems to enable estimation of risk enabling a reliability assessment [*O'Connor*, 2002]. Inclusion of this assessment within the design cycle and feedback into this process enables design to a reliability or availability target. Availability is the probability that a sequence of successful phases of the deployment will take place, at the time required. A similar analysis may be used with existing technology to assess availability during operational phases of a deployment [*Brito and Griffiths*, 2011]. Targeted reliability has been applied to the design and operation of AUVs [e.g., *Brito and Griffiths*, 2011] including under-ice missions. Methods of assessment include the use of expert judgment [*Brito and Griffiths*, 2009], Markov chain models [*Brito and Griffiths*, 2011] (which can be combined with Bayesian theory, Monte Carlo methods, and event trees [*Furukawa et al.*, 2009; *Chu and Sun*, 2008]), fault tree analysis (FTA) and mean time between failure (MTBF) analysis [*O'Connor*, 2002].

3.11. Implications for Reliability of ESL Technologies

The use of targeted reliability in engineering design is gaining acceptance in the development of platforms and systems for environmental science. The existing tools and techniques can be adapted for use in the development of Antarctic subglacial lake probe technologies, and this should be undertaken for the ESL experiment.

4. THE DEVELOPMENT OF ESL PROBE TECHNOLOGIES

In addition to risk management through documentation, peer review, training, and testing, we are currently using availability analysis employing a Markov chain model of the deployment process to determine reliability targets for systems used in different phases of the deployment. The reliability of systems and subsystems is estimated using FTA. The system FTA model combines MTBF (determined from datasheets, testing, or expert judgment) of components to calculate the overall system reliability.

At time of writing, we have developed an initial design concept and are undertaking detailed design of components, subsystems, and systems. Within this concept design, we have attempted to minimize risk and cost by keeping the number and complexity of elements to a minimum and by using proven commercial off-the-shelf (COTS) technology wherever possible. This limits the risk and cost of bespoke system development.

Scientific return is maximized by the combined use of instrumentation returning real-time data and acquisition of water and sediment samples for postretrieval analyses. This provides redundancy and enables informed deployment of the sampler systems. An overview of the concept design is given below.

A key engineering challenge is to enable microbial control while maintaining minimal cost and reliability in an extreme yet delicate environment. The standards that we propose to attain and the methods selected to enable microbial control and verification are discussed below prior to discussion of the engineering structures. This illustrates that microbial control is both central and dominant in our design process.

4.1. Microbial Control

In anticipation of the development of detailed standards and protocols approved by the subglacial community, we have proposed standards and identified a number of microbial control and assessment techniques that could be applied to the experiment. These pragmatic approaches have been developed in response to the principles recommended by SCAR [*Alekhina et al.*, 2009] and U.S. National Academies [*Committee on Principles of Environmental Stewardship for the Exploration and Study of Subglacial Environments, N.R.C.*, 2007]. In addition, they also prevent confounding of microbial analysis of samples.

4.1.1. Standards. We propose the following standards:

1. That exogenous microflora populations are reduced to prevent confounding of sample analysis. This will be achieved using population reduction techniques (described below) to reduce the exogenous background below the detection limit of the analysis techniques used (see Table 2) wherever possible. Where this is not possible (and we do not foresee this), the exogenous microorganism population must be sufficiently low and repeatable to allow the use of correction using an appropriate control and differential measurement, for example, parallel analysis of the water that was stored in one of the sample bottles while in transit to the experiment site.

2. That the final assessment of all engineered structures should verify an exogenous population at or below the detection limit of the analysis used.

3. That following final assessment, a method that is proven to reduce population further is applied. The efficiency of this final population reduction step will be quantified using a positive control contaminant on representative models.

4.1.2. Verification and assessment. To enable assessment of the efficacy of each method, we will use a positive control bacterial species to contaminate engineered surfaces and components. The level of contamination will be assessed (see below), a microbial reduction protocol applied, and a repeated measurement of population used to calculate the reduction achieved. We propose to use both adherent (*Pseudomonas fluorescens*) and nonadherent (*Escherichia coli*) species. This method allows accurate efficacy assessment while minimizing error by raising the number of cells well above the limit of detection.

4.1.3. Analytical methods

4.1.3.1. Qualitative. We propose visualization of cells with fluorescence microscopy poststaining with DAPI, 5-cyano-2,3-di(*p*-tolyl)tetrazolium chloride (CTC), Light Green SF Yellowish (Acid Green) or LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen). Fluorescence microscopy is advantageous, as it has a low limit of detection (one cell per field of view) and allows discrimination of live/dead cells. Wherever possible, this will be performed directly on engineered surfaces. This is preferred to analysis of samples collected by eluting or swabbing, as these additional processing steps can introduce errors and further contamination.

4.1.3.2. Quantitative. We propose bacterial enumeration of liquid samples, eluents, and eluted swab samples using flow cytometry post staining with SYBR Green II. Cytometry is advantageous, as it is quantitative and enables rapid enumeration of large numbers of cells. However, it is difficult to achieve contamination-free analysis and to discriminate target cells from the background if very low cell numbers are present. We propose to use this technique, where microscopy and qPCR are not possible, and we will use positive controls (see above), as then, the cell numbers will be well above the limit of detection. To reduce the limit of detection and to investigate use for low cell numbers, we are developing appropriate controls and the use of preconcentration (filtration and centrifugation).

4.1.3.3. Semiquantitative. We propose qPCR enumeration using domain-specific primers (Archaea, Bacteria, and Eukaryota). This will be performed on swab/eluted samples prior to cleaning to estimate the total population in each domain. This will be repeated postcleaning to assess efficacy. The bacteria primers will also be used in the evaluation of population reduction techniques using positive control species (see above). qPCR is a robust (if experimental contamination is controlled) and quick analytical technique with very low detection limits [*Burns and Valdivia*, 2008]. The technique could also be employed in the field with modest infrastructure. Also, ATP luciferin/luciferase assays will be undertaken.

Each of these techniques will be used in the preparation of engineered systems. Microscopy facilities will also be available on site at a minimum. In all cases, the effect of sample matrix variation on the result will be quantified and resuspension/elution with standard buffers used if matrix effects become dominant.

4.1.4. Population reduction methods. We propose the following methods:

1. Hydrogen peroxide vapor will be used in both construction and at the field site to reduce exogenous microorganism populations. Despite requiring a dedicated machine, heat, and ventilation for a significant duration (~2 h), this technique is attractive because it enables treatment of engineered structures with complex topography and small recesses, it can be used on a wide range of polymers and all electronic components, it has high and proven efficacy, and does not result in a toxic end product requiring disposal.

2. Autoclaving will be used in the construction and preparation of the probe (and the water sampler in particular). This method offers a proven and convenient method of treating resistant structures and is effective for closed volumes (e.g., water retained within a sample bottle). On-site facilities will be available as a backup, as little infrastructure is required.

3. UV radiation (254 nm, 30 W) will be used at the field site for treatment of the probe, the drill hose, and wellhead structures (including the air-filled section of the borehole). A minimum dose of 30 mJ cm^{-2} will be applied to moving surfaces and higher doses used in static applications. UV has high efficacy, is portable, requires modest infrastructure, and is fast acting on surfaces with limited topography.

4. Chemical wash (70% ethanol and also hypochlorite) will be used in preparation of equipment where persistent microorganisms are encountered. We will only use 70% ethanol on-site to reduce the complexity of environmental protection and site clean up.

4.1.5. Control through design. To improve the efficacy and extent of application of these methods, there are a number of steps that can be taken during engineering design. These are primarily the following:

1. Materials selection. In general, hard materials (e.g., titanium) are easier to clean than those with thick oxides (aluminum) or soft materials (elastomers and rubbers which may be porous). Titanium will be used extensively on the probe. This eases microbial control but also enables trace Fe analysis (see Table 2) and reduction of the thickness of load-bearing structures giving more room for ancillary equipment. Samples of all candidate materials will be exposed to cleaning and the population control measures to identify any material degradation and the efficacy of these treatments.

2. Minimization of recesses. Recesses and intricate surface topography has been shown to promote microbial growth [*Ploux et al.*, 2009]. Autoclaving is the only procedure that can reliably kill organisms in blind recesses but cannot be used for all materials, components, and subsystems. It is therefore desirable to limit the number and extent of recesses, which we will do through design. For example, seals are traditionally placed in recessed grooves; we will explore alternative geometries and alternative sealing designs. Where a recess cannot be avoided, we will use sterile liquids (for pressure communication and compensation) and elastomeric capping (potting) to provide a recess-free and cleanable surface. All fluidic systems (e.g., the valve and pump system for the water sampler) are designed to enable flushing to enable cleaning with HPV and/or chemical wash.

3. Limited handling. The design should facilitate operation while requiring minimal handling. This requires simplicity, durability, and reliability. For example the probe is designed to operate without being touched after final assembly, cleaning, and bagging. Targeted reliability design has and will be used to ensure the sterile bagging is not opened to affect repairs or adjustments.

4. Containment. Once the engineered systems are assembled and cleaned, they must be protected against recontamination. All the systems are designed to be placed within protective environments such as sterile bags, which protect them against unavoidable handling.

4.1.6. Methodological control. In each phase of the ESL experiment, we will ensure efficient and effective microbial control. In the "construction phase," we will use a combination of postmanufacture cleaning and population reduction to ensure components are clean. The population reduction methods selected (from the shortlist above) will depend on the material and design of the component. Assessment and verification will be undertaken at a process level in all cases and at a component level where required. The components will be assembled where necessary (e.g., where an inaccessible void is created such as in a gas-filled pressure case) in a clean room environment to ISO 14644 (cleanliness for equipment used in clean rooms) working to Class 100,000 (ISO 8) of this standard (Pharmaceuticals industry permissible limits for cleanliness of equipment in clean rooms as per ISO 14644). Terminal cleaning (i.e., at the end of the assembly) will be used in all cases and may be sufficient for simple structures and subsystems. Subsequent to final terminal cleaning, all equipment will be placed in a protective environment (e.g., heat-sealed bagging or hard case). For transport, the bagged probe will be placed inside a lightweight 20' ISO container together with the winch, tether, and an HPV generator. This sealed container will be shipped to site without breach of access. All other ancillary equipment will be shipped in sterile bags and protected from mechanical damage. Microbial control procedures used immediately prior and during deployment are described in the section below describing the probe support systems.

4.2. The Probe

The probe is heavily negatively buoyant, is tethered to the surface, and has only simple maneuverability (depth control via tether and limited rotation). A melting probe design has not been used because of the difficulties using this technology (e.g., sediment accumulation at the drill tip) in deep ice and because it would be difficult to rapidly retrieve such a probe. A borehole will be created prior to deployment using a hot water drill giving a 36 cm hole that will refreeze at approximately 6 mm h^{-1}.

To facilitate microbial control, we have developed a design with minimal components, reduced dead volumes, few exposed threads, minimized recesses, and using materials suited to microbial control protocols. The probe will be

cleaned during manufacture and assembly and delivered to the drill site in a protective bag. Prior to insertion into the borehole, the exterior of this bag will be cleaned inside a sealed wellhead structure. The tether will be cleaned prior to entering the borehole.

The probe (see Figure 1) is ~3.5 m in length, 20 cm in diameter and consists of two gas-filled pressure cases separated by three carousels of water samplers (see Figure 3), all attached to a central core that is attached to the tether. The bottom pressure case houses the majority of the instrument package and is tipped with a short gravity core sediment sampler (increasing total length to ~4 m). A schematic of the probe systems and their interconnections is depicted in Figure 2.

The upper pressure case contains the power and communications link to the tether. We are completing a trade study of the use of an onboard microprocessor and data logger to enable continued operation (with reduced functionality, e.g., sampling at predetermined intervals) and archiving of instrument data in case of communications failure. The trade study evaluates the impact of this design decision on the reliability of the system (i.e., the likelihood of data and sample return) using formal methods (see above) to assess any advantage over a simpler (and hence less prone to fault) design that controls the probe and logs all data at the ice surface via the tether. Probe-to-surface communications (two-way) will be via an optical link and backup wire modem. The data link is provided by two (one is redundant for robustness) pairs (one of each pair is at the surface end of the tether) of optical multiplexers (Focal 907-HDM). Each pair will provide 1× High Definition Video channel, 1× RS422 and 2× RS232. An additional daughterboard with each pair enables an additional 16× RS232 channels. Power will be supplied through the tether as high voltage DC and down converted within the upper pressure case (e.g., using DC-DC converters (Lambda)). Duplicate power supplies will be used for the 5 and 12 V systems for redundancy. A bespoke multiplexor and power control board will monitor the status of the multiplexors and power supplies and will select between systems in the event of an error. We are also evaluating the use of on board batteries that are sufficient to complete the mission but with limited video footage. The upper pressure case also includes an upward pointing camera and lights to enable imaging of the underside of the overlying ice.

4.2.1. Probe instrumentation. Including instrumentation within the probe enables acquisition of data on the properties of the lake. The approximate chemical and physical properties of the lake have been estimated from geophysical survey, consideration of glaciological history of the lake, and estimates of ice melt and accretion rates [*Siegert et al.*, 2006]. This enables estimation of the required performance and measurement range for each of the instrument systems and testing in simulated lake conditions in the lab. The communications link with the surface allows this data to be recorded and available in real time at the surface. This enables the operations team to plan and execute deviations from the deployment and sampling plan in response to environmental conditions as detailed in the requirements specification. COTS technology will be used to obtain all in situ data. This enables purchase at an early stage in the project and facilitates extended testing prior to deployment. This testing, coupled with quality control measures implemented by the suppliers reduces the risk of instrumentation failure during the deployment. The instruments selected are all available with titanium casings (or will be converted reducing chemical and biological sample confounding) and have designs amenable to microbial control. The extent of microbial control possible is being determined experimentally in our laboratories. The instrumentation package is based around the 320 plus CTD instrument (Idronaut, Italy). This has been selected, as it has a proven pedigree, is available in a variant with conductivity range suited to subglacial deployment, and has the capability to include proven electrochemical sensors for Eh, pH, and O_2. In addition, the manufacturer has facilities to test and develop these sensors in simulated lake conditions and at the low temperatures expected in transport. A Midas SVX2 (Valeport, United Kingdom) is also included; this provides duplicate CTD data which enhances robustness through redundancy. In addition, it provides measurement of sound velocity with an accuracy of ± 0.03 m s^{-1}. A dissolved oxygen optode (e.g., model 3830 AADI, Norway) will be included to provide duplicate oxygen data and to mitigate the risk of electrochemical (Idronaut) sensor damage due to electrolyte freezing during transport. A camera (Iconix High Definition Colour Video, CA, USA) and light system are included to provide images of the ice borehole, the lake, and sediment. Our preferred light design includes LEDs for flood lighting and a tungsten filament lamp with retroreflector to create a long-range (>15 m) spot with power over a wide spectrum, maximizing color intensity. This gives a high-performance imaging system, while minimizing the surface area occupied on the tip of the probe. Sonar ranging systems will be employed for measuring the distance from the probe to both the lake floor and ice ceiling. These measurements will be augmented with laser spot altimetry imaged using the onboard cameras. The measurement of altitude from the lake floor is particularly important prior to and during acquisition of the short core.

Instrumentation systems for nutrient, dissolved gas, and other biogeochemical parameters are commercially available but have not been included in the Lake Ellsworth instrumentation suite. This decision is motivated by the

difficulty in attaining microbial control, by poorly matched performance to the conditions expected in ESL, by physical space on the probe, and by a desire to increase robustness through simplicity.

4.2.2. Sampler systems

4.2.2.1. The water sampler. The water sampler (see design concept depicted in Figure 3) consists of three carousels each containing eight pressure-tolerant bottles (tubes) capped at each end with pressure-tolerant valves. This design enables preservation of samples at in situ conditions and quantitative measurement of gas content. The inlet valves are each connected to the lake via short inlet tubes, while the outlet valves are connected to a common pump, which pulls sample through the tubes when the appropriate valves are open. This design limits the interaction of the sample with the engineered system and limits the flushing volume required to obtain a discrete sample. Valves at each end of the bottle are actuated simultaneously using a gear and rod driven via a magnetic coupling attached to an electric motor inside a pressure case. The simple exposed design facilitates cleaning and robustness. The pressure case also contains electronics that interface to the multiplexer unit (via RS 232) and control the valve and pump motors. We are comparing the reliability of this design with one that includes a battery and a more advanced water sampler control unit to enable continued operation (i.e., sampling at predetermined depths) should the tether power or communications link fail. Commercial valves and sample bottles (Swagelock) are available with a pressure rating of

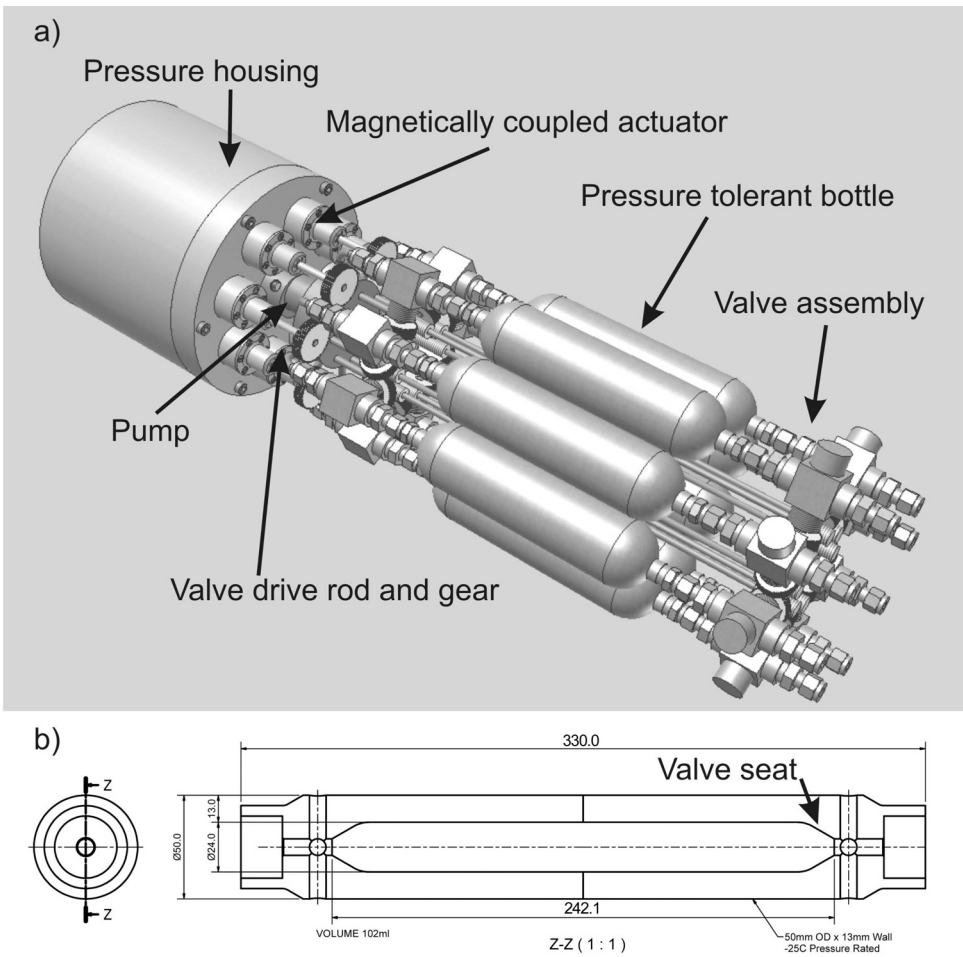

Figure 3. Water sampler design concept: (a) 3D CAD rendering of water sampler carousel concept using commercial valves and (b) engineering drawing of titanium bottle design to withstand sample freezing to −25 °C. The valve seat (cup) for bespoke cone and cup valves (not shown) is annotated.

34 MPa (bottles) and 46 MPa (valves). While this is sufficient to return liquid samples from the lake bottom to the surface at or above approximately −2°C, this is not sufficient to resist the additional pressure should the sample be cooled further. In addition, these components are not available in Titanium as standard, but are manufactured in stainless steel, which is problematic for iron metrology. To address these problems, we have developed a bottle and valve design that can be manufactured in titanium and resists greater pressures (see Figure 3b). These are formed by welding two separately machined halves together with a bespoke internal cone and cup valve included before welding. This design facilitates flushing and cleaning of the bottle and valve and is designed to withstand an internal pressure of 226 MPa.

4.2.2.2. The particle sampler. The particle sampler is integrated with the water sampler, and there are duplicate systems in each carousel. The sampler is based on filter membranes and a gear pump (GJ-N23, IDEX Health & Science Group) that pulls a measured volume through the filter. The pump is controlled by the electronics unit within the water sampler carousel pressure case. This unit enables measurement of the number of pump rotations (and hence volume passed through the filters). The power consumption and rotation rate can also be used to estimate back pressure and can therefore be used to estimate filter clogging. The particle samplers will not be run simultaneously to further mitigate the risk of rapid clogging before a sufficient depth range of the lake is sampled. We are currently evaluating variants of the design using MicropreSure, 0.45 μm mixed ester of cellulose membrane, filters (Millipore, UK). In the first design, three filters are connected in parallel to the pump to reduce the back pressure and to enable the flow rate required (>3.3 L min^{-1}). The filters are contained within the sterile plastic housing in which they are supplied. This has the advantage of low-cost, while facilitating microbial control. In the second design, the same filter material is used but is repackaged into a cone or blind-ended tube, which is contained within a retainer within a pressure-tolerant water sample bottle. This design also enables microbial control (using the same methods applied to the water sampler) and maintains the pressure of the sample until analysis. This method is extremely robust and allows the filter samples to be treated in the same way as the water samples on site.

4.2.2.3. The short corer. The short corer (UWITEC, Austria) will be tested with a range of sediment types including with indistinct sediment water interfaces. We propose to use a double "orange peel" core catcher (UWITEC) to enable retention of loose sediment but are developing bespoke core catcher solutions (both flap and multiple offset elastomeric iris designs) to provide risk mitigation. Prior to acquisition of the short core, the distance between the corer tip and the sediment water interface will be measured using sonar.

4.3. The Tether

The tether provides a flexible mechanical link between the probe, the top sheave (of the gantry), and the winch system provided by the probe support system (see below). The tether is also used to control the large corer. A tether that meets the preliminary specification of both the probe and corer has been identified (for details see Table 4). Detailed design of the probe, corer, and support systems, together with assessment of microbial control procedures, is underway to confirm the suitability of this choice. Optical and electrical links are provided by conductors within the cable, while strength members (aramid/aromatic polyester fiber) take the mechanical load. An encapsulating sheath (polyurethane) provides mechanical protection and prevents microbial egress from the tether interior. Preliminary tests evaluating microbial control on the outer surface of the encapsulation have been conducted. These incubated sections were cleaned with Teknon Biocleanse™ biocidal cleaner (Fisher, United Kingdom) and uncleaned sections with synthetic (simulated) lake water for 1 week. Samples of the unexposed synthetic lake water and of both water incubated with the cleaned and uncleaned sections were

Table 4. Specification of Tether Used to Provide Mechanical, Power, and Communication Links to the Probe

Tether Property	Specification
Optical conductors	Six single-mode communications fibers inside a protective stainless steel tube placed in the center of the tether layup. Water blocked
Electrical power conductors	4 × 2.5 mm^2 (12 AWG) copper conductors (operating voltage >340 DC). Water blocked
Electrical communication conductors	2 × 20 AWG twisted pairs. Water blocked
Binder	Mylar
Strength	Kevlar (Aramid) or Vectran (aromatic polyester) to provide breaking strain >6000 kg
Weight in air	332 kg km^{-1}
Weight in sea water	132 kg km^{-1}
Weight in pure water (0°C)	137 kg km^{-1}
Estimated volume on reel (4 km tether)	3 m^3

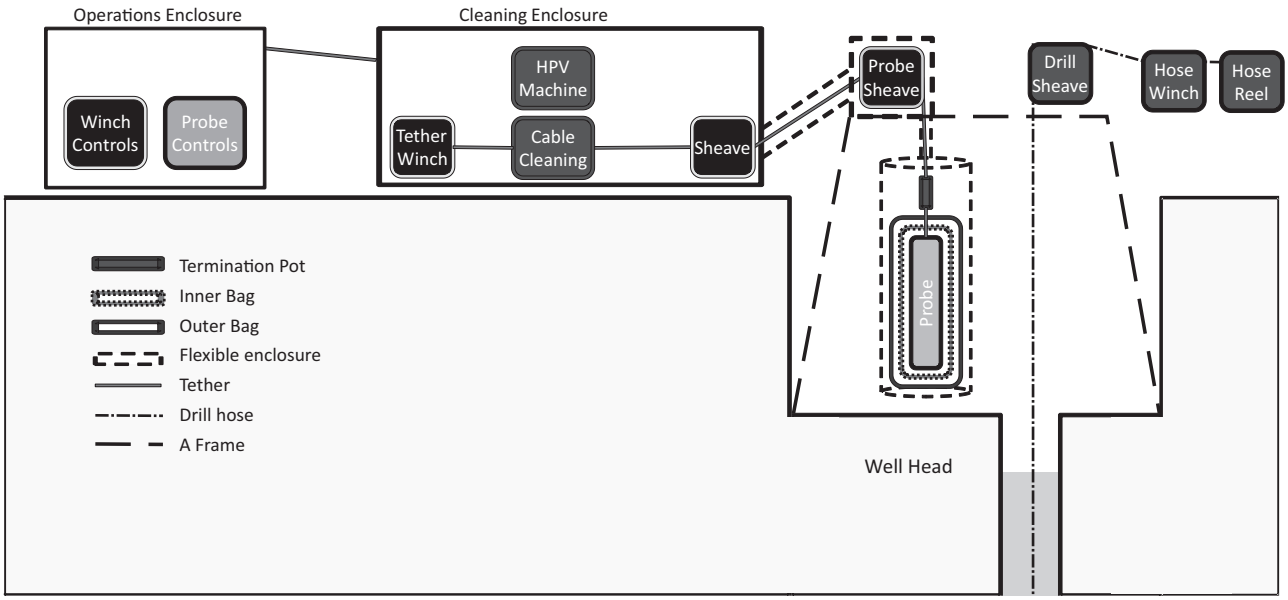

Figure 4. Schematic of probe support systems illustrating key components and zones for microbial control. The three main enclosures are (1) the operations enclosure which houses the probe (or corer) operator, winch operator, and scientist; (2) the cleaning enclosure, which contains the tether winch, equipment for microbial control (depicted here using HPV (that permeates the enclosure) and a separate cable cleaning system); and (3) the wellhead that provides an enclosed space for operations above the borehole. The winches and reels for the hot water drill (HWD) is depicted outside the wellhead and cleaning enclosure; however, we are investigating the use of a cleaning unit immediately above the wellhead, which would act on the hose and tether.

analyzed with flow cytometry SYBR Green II dye. This enumerated bacterial populations and confirmed that the polyurethane sheath could be cleaned and did not harbor significant microflora uncleaned. Further quantitative tests are underway to confirm this initial result.

4.4. Probe Support Systems

Probe support systems present at the ice surface are illustrated in Figure 4 together with representations of the probe and hot water drill. The three main enclosures are (1) the operations enclosure, which houses the probe (or corer) operator, winch operator, and scientist; (2) the cleaning enclosure, which contains the tether winch, equipment for microbial control; and a separate cable cleaning system, and (3) the wellhead that provides an enclosed space for operations above the borehole.

The operations enclosure includes the probe command and control unit, video screens for visualization of images and data from the probe, and facilities of data logging and recording. The system is based on two (for redundancy) ruggedized PCs with data acquisition cards to interface to other systems. The data link from the command and control unit passes through one (of the two) multiplexor units where an optical link is generated. Power systems for the probe (also used for the large corer) are also housed in this unit. The optical link and power supply connects the operations enclosure to the cleaning enclosure.

The winch is housed inside the cleaning enclosure and consists of a lightweight reel, electric rotation control with indexing (cable out measurement), a render (a torque limiting clutch) rotating optical connections, and slip rings for electrical power connection. The HPV machine is used, together with heat and ventilation, to further clean the tether winch and the outside of the probe protective bagging prior to deployment.

As illustrated in Figure 4, the probe top sheave is connected to the cleaning enclosure via a flexible link. This enables the probe and tether to pass over the sheave in a protective environment and to be positioned in the wellhead ready for deployment.

The presence of gas hydrates and high gas concentrations in the lake are being investigated by the Lake Ellsworth Consortium. We are applying formal risk estimation techniques to evaluate the blowout risk. If required, blowout protection will be developed to mitigate the risk that the water in the borehole is ejected by expanding gas. In our current design, the borehole itself is capped with a gland

that is securely fixed to the wellhead structure, which includes a large plate weighted with snow. This gland includes a shut-off valve and lining through the porous fern ice to enable blowout protection. Our current calculations suggest that pressurization of the air-filled headspace (nominally 270 m at hydrostatic equilibrium) to only 5 bar would sufficiently compress any outgassing enough to prevent blowout. The details of the blowout protection system are in development, but it is likely that an additional high-pressure water input (using sterile and stored hot water drill water) will connect to the gland beneath the shut-off valve to enable large quantities of water to be added to the borehole under pressure. This would enable any clathrate or supersaturated water to be pushed back into the lake and would provide hydrostatic head. This would rapidly reestablish equilibrium, and any remaining gas pressure at the gland could be vented while replacing this volume with water. This will be done if outgassing in the lake or borehole forces water up the borehole depressurizing the lake. The addition of large quantities of high-pressure water compresses any gas and re-pressurizes the borehole and the lake preventing a runaway situation.

The gland, lining, and any exposed fern ice at the top of the borehole will be irradiated with UV prior to deployment of the probe. Together with the heat and filter sterilization used in the HWD operation, this ensures that a microbial-controlled environment extends from the ice surface to the lake. The flexible enclosure link is then connected to the gland (and further irradiated with UV). The probe and tether is then lowered through the gland to be deployed.

The probe then descends to the lake to conduct the experiment as per the requirements specified above. On retrieval, the probe is guided from the lake into the ice borehole with the aid of conic guides on the top pressure vessel and images from the upward-looking instrumentation. On assent, the probe exterior is flushed with borehole water and passes back through the gland into the controlled environment of the sealed wellhead. This enables two-way contamination control at the drill site. All probe surfaces, samples, and data will be preserved for appropriate analysis to maximize the impact of this pioneering project.

5. OUTLOOK

The development of probe technologies and methodologies for clean access for the direct measurement and sampling of ESL will provide significant equipment and knowledge for future exploration of pristine and extreme environments. This will include the novel probe and probe management systems specifically developed to enable measurement of pristine environments. All equipment developed for the project is designed for multiple use, and key items will be NERC capital assets made available for future subglacial studies.

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