Water and microbial monitoring technologies towards the near future space exploration

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Authors

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Abstract

Space exploration is demanding longer lasting human missions and water resupply from Earth will become increasingly unrealistic. In a near future, the spacecraft water monitoring systems will require technological advances to promptly identify and counteract contingent events of waterborne microbial contamination, posing health risks to astronauts with lowered immune responsiveness. The search for bio-analytical approaches, alternative to those applied on Earth by cultivation-dependent methods, is pushed by the compelling need to limit waste disposal and avoid microbial regrowth from analytical carryovers. Prospective technologies will be selected only if first validated in a flight-like environment, by following basic principles, advantages, and limitations beyond their current applications on Earth. Starting from the water monitoring activities applied on the International Space Station, we provide a critical overview of the nucleic acid amplification-based approaches (i.e., loop-mediated isothermal amplification, quantitative PCR, and high-throughput sequencing) and early-warning methods for total microbial load assessments (i.e., ATP-metry, flow cytometry), already used at a high readiness level aboard crewed space vehicles. Our findings suggest that the forthcoming space applications of mature technologies will be necessarily bounded by a compromise between analytical performances (e.g., speed to results, identification depth, reproducibility, multiparametricity) and detrimental technical requirements (e.g., reagent usage, waste production, operator skills, crew time). As space exploration progresses toward extended missions to Moon and Mars, miniaturized systems that also minimize crew involvement in their end-to-end operation are likely applicable on the long-term and suitable for the in-flight water and microbiological research.

Keywords:

International Space Station; space missions; biomonitoring; water biological contamination

Contents

1 Introduction

Liquid water is essential for all known Earth-derived life forms living in space conditions, including microbes (McKay, 2014). The on-going space exploration has not yet demonstrated whether the presence of extra-terrestrial water could indicate itself the occurrence of microbial life, but the prevailing paradigm is that living microorganisms necessitate temporal and spatial proximity with aqueous solutions for their metabolism (Martín-Torres et al., 2015; Merino et al., 2019; Stevenson et al., 2015). In turn, the microbial contamination cannot be thoroughly eliminated from Earth's waters but only controlled and attenuated on the long-term time scale (Lopez et al., 2019; Rettberg et al., 2019). Since space exploration has been demanding longer lasting missions, the analysis of waterborne microorganisms turned out of utmost importance for future human spaceflights, planetary outposts, and life-support systems (Horneck et al., 2010).

78 The prevention of crew infectious waterborne diseases is retained among the highest
79 priorities particularly for long duration missions (Ott et al., 2014), since emergency resupply
80 is unrealistic and recycled water could represent the only suitable source for the on-board
81 activities. Most of the aquatic microorganisms found aboard the International Space Station
82 (ISS) do not generally constitute a severe hazard for human health (Blaustein et al., 2019;
83 Checinska Sielaff et al., 2019; Sobisch et al., 2019). However, they may threat astronauts
84 with reduced immune response, mostly following the microgravity stress conditions (Garrett-
85 Bakelman et al., 2019; Ott et al., 2016). Other concerning issues arise from microbial
86 influences on spacecraft integrity and function, owing to the potential corrosion and
87 degradation of stainless steel and other materials associated with the electronic equipment
88 and life support systems (Horneck et al., 2010; Yang et al., 2018; Zea et al., 2018). Therefore,
89 there is an increasing interest to improve the spacecraft water monitoring systems to identify
90 and possibly counteract contingent events of microbial contamination (Van Houdt et al.,
91 2012; Yamaguchi et al., 2014).
92 The definition, identification, and test of the microbial monitoring approaches suitable for the
93 on-board water quality control are challenged by several technical constraints (e.g., material
94 safety compatibility, resistance to launch vibration) and a minimal availability for managing
95 excess power, storage, volume, mass, and crew time (Allen et al., 2018). Moreover, selected
96 devices and their supporting reagents must remain viable for years, while operating safely
97 and reliably in extreme conditions (e.g., in the absence of gravity). Technology flexibility is
98 also critical, since monitoring systems should be able to detect different microbial targets
99 (e.g., fungi, protists, prokaryotes, viruses) and to accept samples of various origin, spanning
100 from biomedical (e.g., blood, urine, saliva samples, routine chemistry, cell cultures) to water
101 and environmental samples (Nelson, 2011).

In the next years, the Chinese modular space station, built on the experience gained from its precursors Tiangong-1 and Tiangong-2, will be placed and operating in the Low Earth orbit (Gibney, 2019). The sustainable human exploration of the Moon is programmed in the meanwhile (El-Jaby et al., 2019; Pittman et al., 2016), whereas the human missions to the surface of Mars are envisioned before 2040 (ISECG, 2018). Therefore, it is hypothesized that only prospective methodological applications at a high technology readiness level (i.e., at least validated in a flight-like environment; Straub, 2015) will be selected by following their basic principles and current uses in Earth and space-analogue settings.

A number of review papers has recently emphasized the need for high-throughput technologies to timely monitor and achieve the stringent microbial quality requirements of future crewed space habitats (De Middeleer et al., 2019; Karouia et al., 2017; Liu, 2017; Moissl-Eichinger et al., 2016; Mora et al., 2016; Yamaguchi et al., 2014).

In this article, we narrow the focus on promising bio-analytic technologies for quality assessments of waters in space, with the aim to explore advantages and limitations beyond their current applications on Earth. Starting from the rigorous housekeeping program and the consolidated results of water monitoring activity on the ISS (Duncan et al., 2008; Limero and Wallace, 2017), we provide a critical overview of the microbial monitoring approaches, based on flexible technologies for the identification of microbial components and a total contamination assessment (total microbial burden) in waters circulating on crewed space vehicles.

2 Water recycle and microbial monitoring aboard the International Space Station

2.1 The ISS water cycle

The water recycling system innovations required to support ISS activities have been listed among the major benefits for humanity (Detsis and Detsis, 2013; NASA et al., 2019). The

ISS is provided with potable water from different suppliers, coordinated by the space agencies of United States (National Aeronautics and Space Administration - NASA), Russia (Russian Federal Space Agency - Roscosmos), Europe (European Space Agency - ESA), and Japan (Japanese Aerospace Exploration Agency - JAXA) (Bruce et al., 2005; Van Houdt et al., 2012). All the possible necessary precautions to prevent external contamination are applied throughout water transferring and loading steps over the entire treatment period before the liftoff of supply modules. For instance, the American and Russian waters are produced in conditioned and limited-access areas and preparation facilities, with no risk of accidental water quality modifications during the production process. At the research center of the Italian Società Metropolitana Acque Torino (SMAT), purified waters for space travels are also processed upon selecting well and spring waters that most closely meet the physical, chemical, and bacteriological quality standards for astronauts (Lobascio et al., 2004). Currently on ISS, waters for direct human consumption are regularly delivered and recovered in order to guarantee approximately 4 L per person per day (Figure 1). A reserve of potable water (up to approx. 2000 L) is stored in contingency containers to maintain ISS operations in response to emergency scenarios (Carter et al., 2018). Although routinely monitored and kept constant, the overall water mass balance represents a recurrent major challenge owing to the various ISS water needs (Pickett et al., 2020).

Beside the on-demand crew consumption, on-board waters are distributed for different purposes, comprising hygiene and cleaning practices, urinal flushing, oxygen generation via electrolysis, life-support systems, and flexible water-based experimental activities (e.g., vegetable and food production systems, animal physiology and behavioral adaptation tests) (Baiocco et al., 2018; Chatani et al., 2015; Massa et al., 2016; Niederwieser et al., 2018; Ronca et al., 2019; Wolff et al., 2018). Wastewaters are continuously collected and recycled at high efficiency level (Pickett et al., 2020). In the US segment, the Water Recovery and

Management System was reported to recuperate up to 85% from crew urine and flush water, along with the water content from liquid wastes and humidity condensate from the cabin. Various containers, reservoirs, tanks and bellows are also necessary to maintain water pressure and circulation through the distribution network (Carter et al., 2018).

Since microbial growth is unavoidable in persistent stagnation zones and at varying residence times along the water distribution network (Lautenschlager et al., 2010; Ling et al., 2018), the pre- and in-flight addition of biocides is used for residual microbial control. Molecular iodine is applied in the U.S. segment, while the ionic silver level is amended in Russian waters, both at low concentrations (i.e., not detrimental for human health) (Artemyeva, 2016; Lobascio et al., 2004). Moreover, high temperature in the catalytic reactor, multifiltration beds within the Water Processor Assembly, UV-C LEDs within the CO_2 Concentration Assembly of the Advanced Closed Loop System, and novel antimicrobial coatings on various ISS surfaces were proven effective against potential microbial biomass growth (Bockstahler et al., 2017; Carter et al., 2018; Perrin et al., 2018; Petala et al., 2020; Roman et al., 2006; Sobisch et al., 2019). Finally, the ISS is maintained at pressure and oxygen levels very close to those at sea level on Earth, with a cabin temperature of about 22°C and a relative humidity of about 60%, in order to minimize detrimental growth of microbial biofilms on cabin surfaces (Pierson et al., 2013).

2.2 On-board water monitoring and microbial contamination

The achievement and maintenance of water quality standards are evaluated by systematic monitoring procedures (Limero and Wallace, 2017). Major water physical-chemical parameters including conductivity, pH, total organic, total inorganic and total carbon, nitrate, potassium, chloride and ammonium are monitored in-flight, together with iodine and silver levels (Artemyeva, 2016). Moreover, a robust monitoring program was implemented to verify

177 that risks of microbial contamination were within acceptable limits in samples collected from
178 different sites of the Russian and US segments, respectively once every three months and
179 each month (Pierson et al., 2013; Van Houdt and Leys, 2012). Crewmembers use handheld
180 equipment to monitor ISS waters and humidity condensate from surfaces. Chemical and
181 biological samples are taken concurrently and at a frequency that may change due to real-
182 time flight necessities (Pierson et al., 2013).
183 Water samples can be processed on-board by cultivation-based methods using the US-
184 supplied Water Microbiology Kit for the quantification of total heterotrophic bacteria and
185 coliforms (Bruce et al., 2005). The maximum total number of aerobic heterotrophic viable
186 bacterial cells, counted as colony forming units on a rich agar medium, was internationally
187 defined according to the concentration levels that are achievable with the current prevention
188 and monitoring technologies available and applicable for space (i.e., HPC $\leq$ 50 CFU/ml).
189 Microbial quality standards are also set for ISS internal surfaces, from which humidity
190 condensate is collected (maximum bacterial load = 10000 CFU/100 cm^2; maximum fungal
191 load = 100 CFU/100 cm^2) (Van Houdt and Leys, 2012). Along with the on-board monitoring
192 activities, archival water samples are regularly collected in teflon bags (Figure 1), preserved,
193 and returned to Earth approximately every three months for post-flight analyses (Limero and
194 Wallace, 2017).
195 The microbial contamination level was above the former acceptability limits several times
196 during previous in-flight monitoring surveys. Events of microbial biofilm growth within
197 space vehicles were mainly associated with the water layer covering internal surfaces and life
198 support systems (La Duc et al., 2004; Novikova et al., 2006; Roman et al., 2006). Thus,
199 waters collected from humidity condensate were retained among the major sources of
200 microbiological hazard for potable water quality deterioration (Horneck et al., 2010).
201

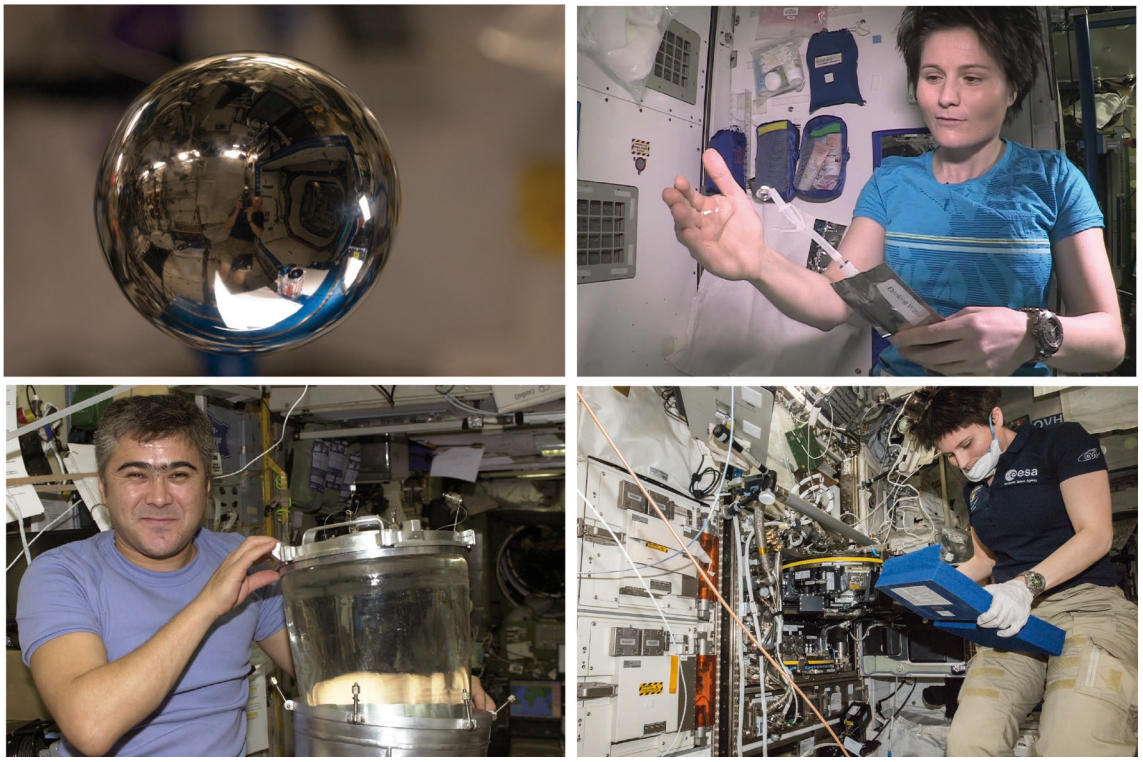


Figure 1. A water drop floating on the ISS (upper left). The Italian astronaut Samantha Cristoforetti and the Russian cosmonaut Salizhan Sharipov showing the Teflon bag (upper right), the water tank (lower left) and removal system (lower right), used for drinking purposes and water storage on the ISS (Credits: ESA and NASA).

Several cultivable microbial isolates obtained from spaceflights were mainly affiliated to Bacteria and Fungi (Bruce et al., 2005; Coil et al., 2016; Novikova et al., 2006). For the purposes of this review, it is worth noting that the analysis of the microbial cultivable fraction were likely to provide only a limited snapshot of the highly diverse community found on the ISS by cultivation-independent methods (Checinska Sielaff et al., 2019; Coil et al., 2016; De Middeleer et al., 2019; Ichijo et al., 2016; Lang et al., 2017; Mora et al., 2019; Morris et al., 2012). Despite major advantages arise from target-specific isolation and characterization of different types of waterborne microorganisms and pathogens in pure culture, a number of technical and logistic disadvantages characterizes the space application of cultivation-based methods. The microgravity conditions provide conflicting results on microbial growth and

virulence of opportunistic human pathogens (Morrison et al., 2017; Zhang et al., 2019), while the analysis of archival samples generates a detrimental time gap for a flight-supportive result interpretation (Huang et al., 2018; Novikova et al., 2006). HPC was proven to be also affected by carbon sources in cultivation media, the incubation time, and the initial microbial load level (Amalfitano et al., 2018b). Finally, the prompt development and requirement update of alternate bio-analytical technologies for microbial monitoring during space exploration is pushed fundamentally by the need to avoid microbial regrowth from analytical wastes (Wong et al., 2017).

3 Nucleic acid amplification-based methods for microbial identification in space waters

Methods based on nucleic acid amplification offer the advantage of specific and fast detection, easy automatization and standardization. Many of the limitations of cultivation-based techniques adopted for bacterial detection and water quality monitoring are overwhelmed by largely reducing the time required for the microbial identification including also viable but not cultivable bacteria and un-cultivable pathogens.

3.1 Sample pre-treatments and nucleic acid extraction in space

Sample pre-treatment and nucleic acid extraction are key starting points for most of the molecular approaches and product developments dedicated to microbial water quality monitoring. Nucleic acids extracted from water samples are amplified to specific markers. The extraction and purification of nucleic acids rely on cell lysis and the selective binding of cellular DNA and RNA to solid surfaces through filtration and column-based protocols. A fast biomolecular analysis can be conducted in space settings with the in-line implementation of optimized protocols for fast DNA and RNA extraction and sample

preparation for sequencing. Published studies reported new promising field-deployable amplification devices and approaches for on board applications (Boguraev et al., 2017; Montague et al., 2018) (Figure 2). Simplified, sample processing and DNA purification, strategies have currently been tested aboard the International Space Station (ISS). The Wetlab-2 project has already developed and tested on the ISS a Sample Preparation Module (SPM) to lyse cells, and to provide high quality extraction of nucleic acids extract, by circumventing operational issues related to microgravity, surface tension alteration, reduced operational space and handling expertise (Parra et al., 2017).

Numerous commercialized kits for solid-phase extraction allow handy and rapid nucleic acid purification procedures, in which potential contaminants are removed through sequential washing steps based on centrifugation or DNA separation by paramagnetic beads (Tan and Yiap, 2009). Methods for the direct PCR amplification without DNA extraction were also developed (Williams et al., 2017). Recent papers reported simplified methods for nucleic acid purification using filtration membranes and DNA amplification form the nucleic acid directly on filters (Kaliyappan et al., 2012; Rodriguez et al., 2016). Notably, a cellulose-paper-based dipstick was used to efficiently bind, wash, and elute purified nucleic acids from different matrices without any pipetting or electrical equipment (Zou et al., 2017).

Nevertheless, a major bottleneck for the in-flight application of amplification based approaches still lies on the need for time-consuming and waste-producing sample concentration and DNA extraction (Girones et al., 2010). In particular, the method sensibility is lowered when amplifying targets present at low levels over the total extracted DNA (Brandt and Albertsen, 2018). Suboptimal DNA extraction and purification are known to affect results of biomolecular analysis, mainly owing to uncomplete lysis of more resistant bacterial populations and poor removal of DNA polymerase inhibitors (Albertsen et al., 2015; Girones et al., 2010). DNA purification requires effective cell disruption, inactivation of

nucleases, and purification of DNA from contaminants that might interfere with the amplification efficiency. Residual extracellular DNA could not be easily discriminated from that of viable dangerous microorganisms, thus leading to an overestimation of risk for human health (Girones et al., 2010). Moreover, waterborne substances can concentrate together with DNA during sample processing and inhibit polymerase enzymes, thus influencing the sensitivity and reliability of the PCR-based microbial detection. Overall, the relative accuracy and precision (i.e., repeatability and reproducibility) of nucleic acid amplification based methods are affected by sample type and pre-treatment methods (concentration and DNA extraction), thus requiring to be evaluated across the whole sample processing (Hospodsky et al., 2010; Kralik and Ricchi, 2017).

3.2 Target-based techniques for detection and quantification of nucleic acids

The polymerase chain reaction (PCR) is widely used for detecting genes and microorganisms of health concern in water (Ramírez-Castillo et al., 2015). Among these target-based detection approaches, the loop-mediated isothermal amplification (LAMP) of nucleic acids was retained as a rapid and sensible option (Zhao et al., 2015), with minimal requirements for the in-flight water quality monitoring and pathogen detection (Ott et al., 2014). The amplification takes place at isothermal temperature (60-65°C) and positive reactions can be visualised by naked eye (i.e., without post-amplification steps) following the increase of sample turbidity or colour owing to the addition of fluorescent dyes (Notomi et al., 2015). Being less sensible than PCR to inhibition and not significantly influenced by non-target DNA, direct LAMP assays are currently used with good analytical performances (Etchebarne et al., 2017; Samhan et al., 2017). Low amount of DNA can be amplified up to generating 10^9 copies within 1 h and producing as final amplification product a complex stem-loop DNA, with several inverted repeats of the target and cauliflower-like structures. Commercial kits for

293 the rapid on-site detection of water pathogens are already available for terrestrial
294 applications, also comprising quantitative real-time LAMP, reverse transcription RT-LAMP,
295 *in situ* LAMP, and viable LAMP (Notomi et al., 2015). For the scopes of this review, it is
296 worth noting that a rapid method for detecting approximately 1 CFU/100 ml of *Legionella*
297 *pneumophila* in tap water was developed and efficiently applied on field in less than 2
298 working hours through a direct on-filter LAMP amplification with live/dead propidium
299 monoazide (PMA) differentiation (Samhan et al., 2017).
300 Among the target-based quantification approaches, the quantitative PCR (qPCR) represents a
301 popular technique for the in-flight water quality monitoring (Oubre et al., 2013). The
302 quantification of target sequences is based on the development of a fluorescent signal
303 proportional to the amount of amplified product obtained during the PCR thermal cycles.
304 Multiplex qPCR can be used for the simultaneous detection and quantification of multiple
305 pathogens, consistently reducing analytical time and costs (Ibekwe et al., 2002; LaGier et al.,
306 2004). With low detection limits (<400 cells per sample) and volume requirements (<100 µl
307 of sample), qPCR assays are routinely applied in monitoring plans for the detection and
308 quantification of waterborne pathogens (Girones et al., 2010; Ramírez-Castillo et al., 2015).
309 Good repeatability and reproducibility of qPCR outcomes were reported for gene targets of
310 fecal origin and a standardized workflow achieved consistent results, with low intra- and
311 inter-laboratory coefficients of variation (median CV = 0.1-3.3% and 1.9.7.1%, respectively)
312 (Ebentier et al., 2013; Orin et al., 2012). A large body of the scientific literature has
313 documented the development and use of qPCR for pathogenic viruses, bacteria, protozoa, and
314 fungi (Kralik and Ricchi, 2017; Ramírez-Castillo et al., 2015). Through a pre-treatment with
315 cell membrane impermeant DNA intercalating dyes, the so-called Viable qPCR was applied
316 for discriminating between viable (with intact membrane) and dead (with damaged
317 membrane) bacteria, allowing the quantification of water- and food-borne pathogens such as

Campylobacter, *E. coli* O157:H7, *Legionella pneumophila, Salmonella*, *Cryptosporidium* (Banihashemi et al., 2012; Brescia et al., 2009; Delgado-Viscogliosi et al., 2009).

Given the versatile applications of the numerous available assays, both LAMP and qPCR have been retained as suitable water monitoring methods for long-term exploration missions. Owing to the high sensitivity, specificity, and simple post-amplification steps to detect the amplified targets, LAMP was proposed by the Japanese Aerospace Exploration Agency as alternative microbial contamination monitoring system for the ISS (Ott et al., 2014). For the analysis of crew health related genetic modifications, qPCR technologies have been successfully tested on-board the ISS within the projects Gene in Space and Wet-lab2, devoted to definition of a robust, user-friendly nucleic acid extraction and sequencing approach aboard ISS (Boguraev et al 2017; https://www.genesinspace.org/; https://www.nasa.gov/mission_pages/station/research/experiments/1913.html; https://www.nasa.gov/ames/research/space-biosciences/wetlab-2). Moreover, the RAZOR EX PCR, launched on Space-X 9 (July 2016) within Water Monitoring Suite project will allow performing direct PCR amplification from water samples.

In all current space applications, however, the selected target-based detection and quantification approaches require the use of disposable materials and labour intense protocols, which will inevitably reduce their long-term applicability in space conditions. The general precautions used on Earth to limit contamination risks, including the most stringent procedures applied in clean rooms (Rettberg et al., 2019), might also represent a limiting practical issue in the small close spacecraft environment, since the high sensibility of PCR-based detection increases the chance of amplifying carry over contamination, with the consequent production of false positive results. This is crucial for LAMP owing to the limited accessibility to degradation of DNA products, while a major limitation of qPCR is also the occurrence of inhibitors that can be co-concentrated or extracted along with nucleic acids

343 from the target microorganisms (Gibson et al., 2012). The presence of qPCR inhibitors
344 introduces a number of problems, ranging from low amplification efficiency and reduced
345 assay sensitivity to complete reaction failure and false negative results (Radstrom et al.,
346 2008).
347
348 **3.3 Sequencing-based "-omics" approaches for microbial community characterization**
349 The High-Throughput Sequencing (HTS) encloses a popular suite of technologies,
350 methodological approaches, and data elaboration workflows used to characterize the
351 phylogenetic composition of the total microbial community in different aquatic matrices. The
352 taxonomic classification is based on the huge amount of sequences generated by either a
353 portion of the cellular nucleic acid content or the whole genome, through the so-called
354 amplicon and shotgun sequencing approaches, respectively (Peabody et al., 2015).
355 The amplicon analysis represents the extension of sequencing based methods of organisms'
356 classification, defined by specific protocols under the general name of "genetic" or
357 "molecular barcoding" (Hebert and Gregory, 2005), which is based on the information
358 carried by a single conventional marker, such as the 16S rRNA for microorganisms.
359 The shotgun sequencing consists in generating millions of DNA fragments of different
360 lengths from a starting pool of genomes. These fragments cover a quote of the original
361 genomes inversely proportional to the genome lengths, and taxonomic assignment may be
362 quantitative according to the proportion of fragments classified for each taxon. The amplified
363 products are sequenced, controlled for quality, and assembled in longer contigs. The obtained
364 sequences are classified at different taxonomic levels, according to the best match with a
365 reference sequence database and following different similarity criteria (Segata et al., 2012).
366 Currently, the most used sequencing platforms are characterized by different technical
367 principles and include Roche 454 GS-FLX (pyrosequencing), Illumina MiSeq and HiSeq

368 (reversible terminator sequencing by synthesis), Ion PGM (semiconductor based sequencing
369 by synthesis), and the nanopore GridION and MinION™ (Check Hayden, 2015; Clooney et
370 al., 2016; Ghanbari et al., 2015; Glenn, 2011).
371 Performance evaluation and standardization of HTS workflows are still limited for water
372 microbial community characterization. The DNA extraction procedures and primers sets were
373 reported to influence the assessment of the bacterial community composition in drinking
374 waters, with a generally effective representation of the core abundant taxa at total cell
375 abundance levels of 10^3-10^5 cells/ml (Brandt and Albertsen, 2018). Moreover, the method
376 sensitivity was detrimentally affected owing to the occurrence of contaminating bacteria in
377 extraction kits and laboratory reagents (Salter et al., 2014).
378 HTS-based technologies have been already tested under microgravity conditions and directly
379 on the ISS (Carr et al., 2020; Castro-Wallace et al., 2017; McIntyre et al., 2016). The most
380 suitable candidate technology to be transported and mounted on the ISS was the MinION
381 pocket-size device (Figure 2), which can provide rapid identification up to 300 kb with single
382 strand and 60 kb with double strands reads (Jain et al., 2016). The integrity and activity of
383 nanopores, in which DNA/RNA molecules pass through during base reading, were not
384 adversely affected by storage conditions, launch, cosmic radiations or handling in
385 microgravity, and reusability of flow cells was warranted. Specific indications to avoid air
386 bubbles interference at the nanopores were implemented (Castro-Wallace et al., 2017;
387 Rizzardi et al., 2016). Moreover, MinION performances in sequencing a mixture of genomic
388 DNA from a virus, a bacterium, and of mammal mtDNA, were comparable to those of
389 MinION, Illumina MiSeq and PacBio RSII run on ground. Accuracy respect to MiSeq and
390 RSII was slightly inferior (89% identity to the reference genome, respect to > 99%), but
391 sufficient for sequence analysis. Sequencing reads were successfully assigned to the correct
392 reference sequences in 90% of cases. Likely, with the novel improved versions of flow cells,

393 the molecular detection of aquatic microorganisms will improve notably (Kilianski et al.,
394 2015). Tests also demonstrated that real-time metagenomics analysis was also possible by
395 using a laptop device that can be integrated in the ISS hardware. Considering genomic data
396 analysis, one of the least demanding platforms has been tested successfully simulating flight
397 conditions and hardware availability onboard of the ISS, demonstrating its realistic
398 applicability even on a laptop base (Castro-Wallace et al., 2017). Provided the adaptation of
399 hardware and instruments to the spatial and logistic limitations onboard, both the shotgun and
400 amplicon approaches, can be considered as suitable tools for microbial community
401 characterization and pathogen detection on the ISS.
402 The amplicon approach requires straightforward PCR amplification protocols, downstream
403 bioinformatics, and database searches. Nevertheless, this technique is prone to fail to detect
404 unknown or highly divergent 16S rRNA gene sequences, which may be associated to novel
405 or previously undetected pathogens. Moreover, taxonomic assignment is highly sensitive to
406 the gene region selected as a marker and to the assignment method (Claesson et al., 2010; Liu
407 et al., 2008; Tremblay et al., 2015), while accuracy of quantification can be affected by
408 the variability in the number of gene copies shown by microbial taxon and even between
409 individual cells of the same species (Větrovský and Baldrian, 2013). Finally, a common
410 scientific view of suitable markers for viruses is still missing (Chakraborty et al., 2014).
411 In comparison to amplicon analysis, the shotgun sequencing offers a wide genome coverage
412 of organisms, allowing the detection of unknown contaminants and the possibility to screen
413 for both bacteria and viruses. In the field of water quality monitoring, the shotgun sequencing
414 was used, for instance, as the benchmark method to assess the occurrence of potential
415 multiple waterborne pathogens, novel indicators for human sewage contamination, and
416 microbial safety of drinking water in relation to the efficiency of reclamation treatments
417 (Chao et al., 2013; Lu et al., 2015; McLellan and Eren, 2014; Newton et al., 2015). Although

the possibility to obtain up to a few thousands of base pairs for each read improved the microbial identification specificity, non-trivial disadvantages include the initial skimming of the huge number of produced reads, their quality check and assemblage in contigs useful for classification, which require high computational effort and time (Tan et al., 2015).

Figure 2. The NASA astronaut Peggy Whitson performing the Genes in Space investigation on the ISS using the miniPCR and MinION. Credits: NASA

4 Real-time technologies for early warning microbial monitoring

The total contamination assessment (total microbial burden) through the accurate quantification of microbial cell abundance and viability in waters circulating is a recognized necessity on space crewed vehicles (Morris et al., 2012). Different early-warning real-time methods that target parameters at the single-cell level (e.g., cellular biomolecules, membrane integrity, enzyme activity, substrate uptake) have been developed for water monitoring, but only few can efficiently operate in flight-like and space settings.

435 **4.1 ATP-metry**

436 An option for real-time monitoring of biological contamination in water samples is the
437 analysis of adenosine triphosphate (ATP) cell content. The analysis is carried out through the
438 chemical and/or enzymatic extraction of this molecule from microbial cells, followed by the
439 measurement of light emission derived when the dissolved ATP, in presence of magnesium,
440 reacts with the luciferine (substrate) - luciferase (enzyme) complex. The emitted light
441 intensity is linearly related to the ATP concentration, easily measurable using a luminometer,
442 and gives virtually instant information (within minutes) of the metabolically active microbial
443 population. This peculiarity makes it suitable as an early-warning approach for measuring
444 bacterial contamination and monitoring water treatment efficacy in near real-time (Hammes
445 et al., 2010). The method is robust, easy to perform, and suitable to detect both cultivable and
446 uncultivable cells, with better estimates of total active microorganisms compared to
447 heterotrophic plate counts. The concomitant use of traditional cultivation-based approaches
448 showed levels of cultivable cells order of magnitude lower that those estimated by ATP
449 (Siebel et al., 2008; Zhang et al., 2019).
450 The ATP analysis was performed on pre-flight and post-flight ISS water samples and
451 revealed a biological contamination ranging between 0 (drinking water) and $4.9x10^4$ cells/ml
452 (humidity condensate) (Bacci et al., 2019; La Duc et al., 2004). Recently, the viable microbial
453 contamination on-board ISS was reliably monitored on surface samples by intracellular ATP
454 measurement (Perrin et al., 2018; Venkateswaran et al., 2003). ATP assays were also proven
455 effective in monitoring microbial contamination on surfaces from an inflated lunar/Mars
456 analogous habitat during long-term human occupation (Mayer et al., 2016). The total (ATP
457 content from both dead and live microbes) and viable microorganisms (intracellular ATP
458 content) were in the range of 10^5-10^6 relative luminescence unit (RLU)/m^2. These results
459 were in line with ATP data measured directly on-board within the experiment T2 carried out

460 during Euromir-95 mission (Guarnieri et al., 1997). Recently, the ATP-metry was selected for
461 the real-time monitoring of the biological contamination on board ISS within the H2020
462 European project BIOWYSE (Biocontamination integrated control of wet systems for space
463 exploration, http://biowyse.eu), aimed at developing a compact, automatic, and microgravity-
464 compatible on-board systems for the prevention, monitoring and control of microbial load in
465 waters and on wet surfaces (Figure 3). A humid area sampler was recently developed and
466 patented (ref. 102018000009137, dated 03/10/2018) (Detsis et al., 2018; Guarnieri et al.,
467 2019).
468 Today, commercial kits for quantitative ATP-metry are available to monitor the microbial
469 biomass level in water and to validate cleaning and disinfection procedures, with a wide
470 number of bulk and intracellular ATP measurements on microbial communities reported from
471 natural and engineered aquatic environments (Abushaban et al., 2019; Fillinger et al., 2019;
472 van der Wielen and van der Kooij, 2010; Vang et al., 2014). Although the apparent
473 advantages, major technical limitations are related to the low sensitivity and result
474 reproducibility at low cell concentration and sample volume (< 100 µl), along with the
475 susceptibility to environmental conditions (e.g., pH, temperature, occurrence of enzyme
476 inhibitors). Some drawbacks of the method are partly circumvented by using external and
477 internal standards and by operating under controlled reproducible settings. Therefore, the
478 correlation between intracellular ATP content and microbial cell counts will rely on a robust
479 cross-calibration with results from other reference methods (Amalfitano et al., 2018b;
480 Hammes et al., 2010). It has been calculated that the average ATP-per-cell content is
481 approximately 1.75 x 10^{-10} nmol/cell or 6.87 x 10^{-17} g ATP/cell (Zhang et al., 2019).
482 However, the amount of per-cell ATP can be significantly influenced by the phylogenetic
483 affiliation and the cellular physiological status, with diverse ATP-content reported between

484 either eukaryotic or prokaryotic cells from the same cultures (Bajerski et al., 2018; Yaginuma
485 et al., 2015).
486
487 **4.2 Flow cytometry**
488 Ubiquitously applied from the bio-medical research to environmental sciences, flow
489 cytometry (FCM) is considered an unparalleled high-throughput technology for single cell
490 analysis (Robinson and Roederer, 2015). This generic technology allows the measure (-
491 metry) of the optical properties of cells (cyto-) transported by a liquid sheath (flow).
492 Following sample intake, a pressurized laminar flow is generated and suspended particles are
493 individually forced to cross a light source excitation point for scanning and evaluation. Light
494 scatter and fluorescence signals (either with or without a secondary staining with fluorescent
495 dyes) are detected at the single-cell level and instantly converted into digital information to
496 be shown on multidimensional plots, along with real-time data analysis and statistics
497 (Shapiro, 2005).
498 Flow cytometry is included in the roadmaps of national space agencies worldwide and
499 deemed as a necessary technology for defining and monitoring spaceflight-associated
500 requirements since early 80's. Owing to the high versatility for diagnostic medicine (e.g.,
501 hematology, immunology, and physiology), this technology was so far retained as a prime
502 asset for health monitoring and clinical laboratory diagnostics for astronauts, in view of the
503 upcoming deep-space exploration missions (Crucian and Sams, 2012). A flow cytometry
504 platform was already successfully tested on-board the ISS to monitor and understand the
505 physiological adaptations of astronauts to microgravity (Dubeau-Laramée et al., 2014; Phipps
506 et al., 2014) (Figure 3). Advanced developments of the prototype were based on
507 commercialized flow cytometer with significant additional engineering modifications, mostly
508 aimed to generate laminar particle flow (distinct from the standard sheath fluid based

509 method), and to reduce the significant amount of liquid biohazardous waste and energy
510 operating requirements. The laminar flow within the flow chamber was found to be
511 dramatically altered by microgravity. In turn, the unavoidable elimination of the fluid
512 mechanical setting for particle hydrodynamic focusing was proven to significantly reduce
513 liquid waste and the total system operational load, in terms either of instrument size and
514 weight or energy consumption (Cohen et al., 2011; Crucian and Sams, 2005).
515 Notwithstanding the recent system design improvements, peripheral blood cells has been
516 retained as the only target for first studies in space environments (McMonigal and Crucian,
517 2015). Since a flow cytometer can provide support to a wide range of scientific applications
518 (e.g., biology, microbiology, and environmental science), it is needless to point out that a
519 spaceflight compatible machine could satisfy the unmet flight requirements for water
520 monitoring and treatment to complete future long-duration missions in closed healthy
521 environments.
522 The high sensitivity for scanning very small objects (i.e., from virus-like-particles to
523 prokaryotes, to pico- and micro-eukaryotes) and the wide detectable cell concentration range
524 (generally between 10^2 and 10^7 cells ml^{-1} without concentrating or diluting the samples)
525 represent unmatched features of this technology for water monitoring. A broad suite of assays
526 is available for microbial quality assessments in natural and engineered aquatic ecosystems
527 (Amalfitano et al., 2014; Boi et al., 2016; Gasol and Morán, 2015; Van Nevel et al., 2017).
528 Successful applications are reported to provide early warning indications of unexpected water
529 contamination events through the rapid detection of a variety of microbiological threats,
530 including pathogenic and potentially toxic microorganisms (Vital et al., 2010; Weisse and
531 Bergkemper, 2018; Yang et al., 2010). Moreover, flow cytometric measurements are suitable
532 to evaluate the efficiency of various industrial microbial bioprocesses (e.g., food and
533 pharmaceutical preparations) (Díaz et al., 2010), and the performances of engineered systems

for water treatment (Besmer and Hammes, 2016; Safford and Bischel, 2019). Remarkably, FCM was also useful to determine the presence of nonliving organic and inorganic substances, including nano- and micro-sized particles, suspended solids, flocs and aggregates of various origins (Aulenta et al., 2013; Casentini et al., 2016; Liu et al., 2016). On-going instrumental developments for on-site applications are directed to install flow cytometers on either mobile units (e.g., ships and vehicles) or fixed locations (e.g., treatment plants, marine buoys, off-shore platforms), with the possibility for automatic programmable staining of aquatic microorganisms and remote data transfer (Buysschaert et al., 2018; Pomati et al., 2011; Thyssen et al., 2007).

Leaving aside the high costs of all sophisticated systems, the need for specially trained staff is likely to represent a major drawback in the daily scheduled monitoring practices. Moreover, the reproducibility of cytometric data was reported to be adversely affected by changing cell staining protocols (e.g., fixatives, type of fluorescent dye), incubation conditions (e.g., temperature, time to analysis), instrumental settings (e.g., fluidic and signal amplification systems), and the source water (e.g., from natural or engineered systems) (Nescerecka et al., 2016; Prest et al., 2013). Further work is also required to establish user-independent strategies for gating and data handling (Amalfitano et al., 2018a; Koch et al., 2014). One more specific challenge is the lack of phylogenetic resolution. Despite the enumeration of targeted taxa may rely on specific fluorescence staining procedures (Couradeau et al., 2019; Manti et al., 2011; Neuenschwander et al., 2015), the cytometric information characterizing different subpopulations of microbial cells is generally ataxonomic. A direct link between cytometric fingerprinting and microbial diversity, assessed by 16S rRNA gene amplicon sequencing, was demonstrated in recent studies (Props et al., 2017, 2016). However, the computational workflows are convoluted and still under development (Rubbens et al., 2019).

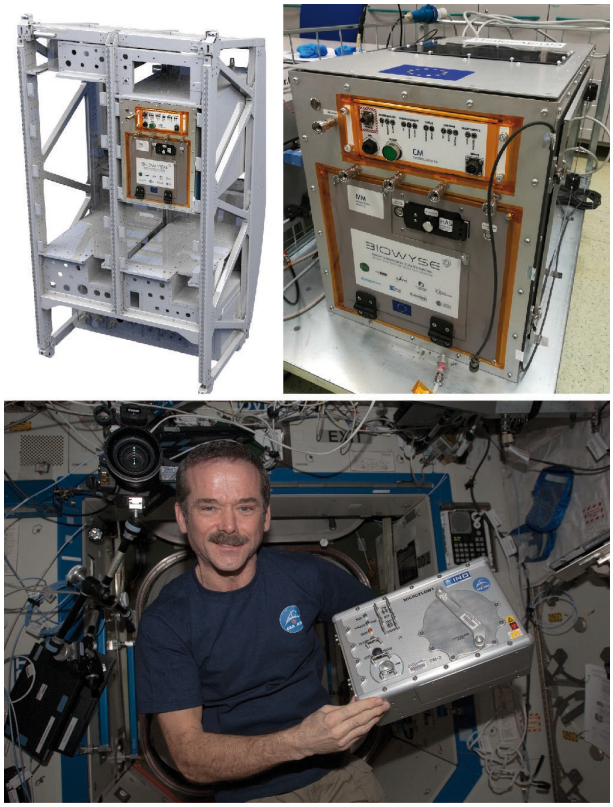
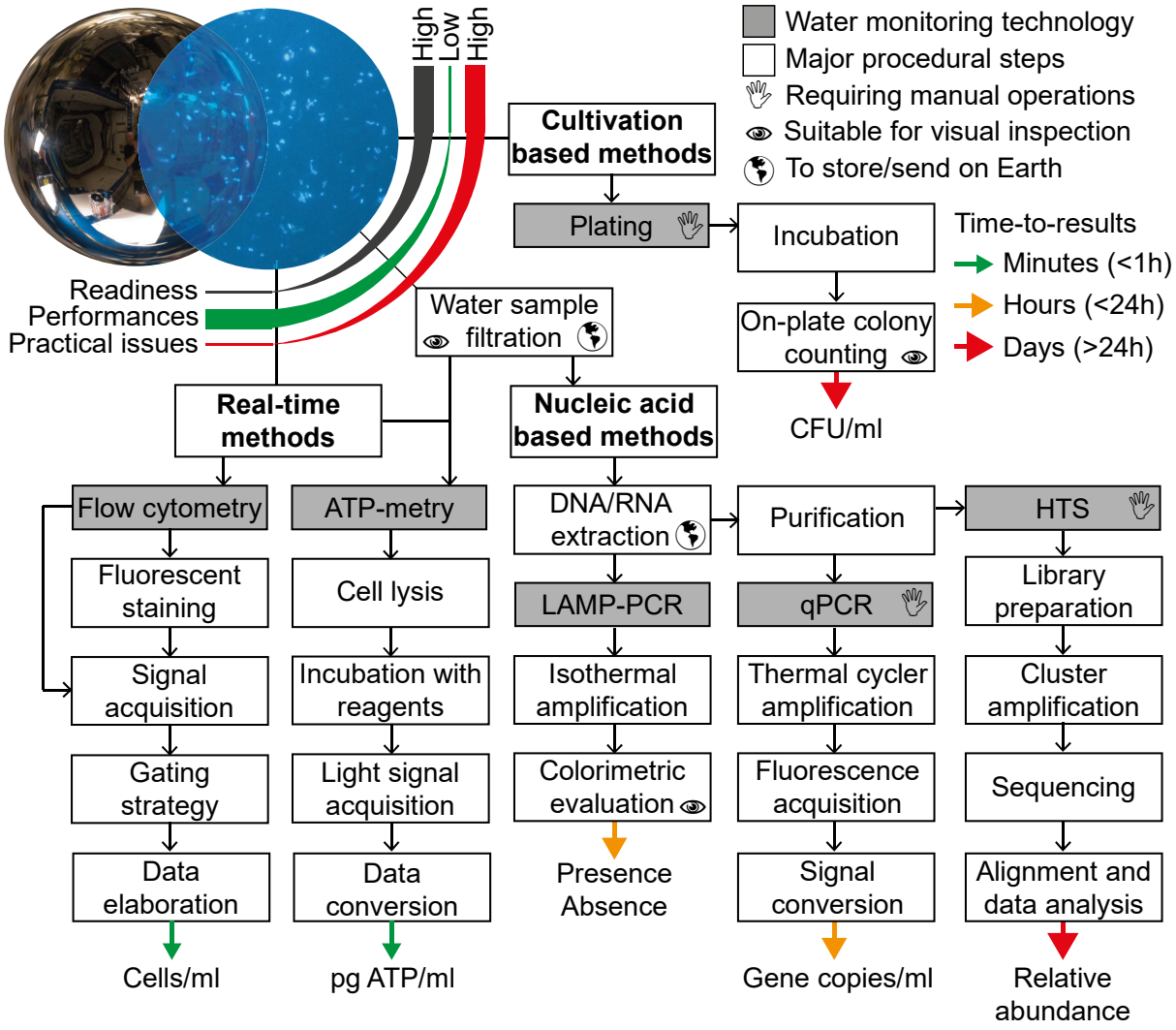


Figure 3. Integrated breadboard of the ATP-metry system, developed within the European H2020 project BIOWYSE. The hardware was designed (upper left photo) and built (upper right photo) to fly within the European Drawer Rack Mark 2. The photo below shows the Canadian astronaut Chris Hadfield holding the flow cytometry platform Microflow1, successfully deployed and tested on the ISS (Credits: BIOWYSE project consortium and NASA).

5 Comparative analysis of space-relevant technological features

The procedural workflows of each of the mature technologies, herein entitled for water microbial monitoring in space, comprise major steps and provide different types of results. The overall time-to-results can vary from minutes to days, largely relying on user-dependent manual operations and technological solutions for automation (figure 4).



572

573 **Figure 4**. Workflows of mature technologies entitled for microbial water monitoring during
574 space missions. Cultivation-, nucleic acid-based, and real-time methods are suitable to
575 identify and quantify the waterborne microorganisms, ideally present in a drop of ISS water
576 and visualized by epifluorescence microscopy on a filtration membrane (upper left pictures).
577 Comparative levels of readiness, analytical performances, and practical issues were reported
578 along with major procedural steps, timing, and types of achievable results.

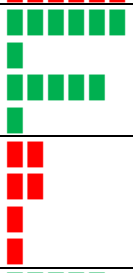
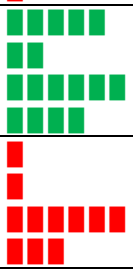
579

580 Both current and future applications in space will be necessarily bounded by the definition of
581 novel standards of microbial quality (i.e., other than those applied on Earth by cultivation-

582 dependent approaches) (Amalfitano et al., 2018b), but also by a compromise between the best
583 analytical performances and detrimental practical issues to cope with during spaceflights.
584 The implementation of successful on-board workflows will critically rely on the overall water
585 cycle management (Pickett et al., 2020), while fundamental analytical aspects can be
586 constrained by the minimal amount of water available for routine monitoring activities. For
587 example, technological accuracy and result reproducibility are directly linked to workable
588 water volumes and the constitutive occurrence of microbial targets in the sample (e.g.,
589 microbial cell abundance, per-cell gene copies, cell viability). Volumes required for accurate
590 and reproducible analyses will reasonably range between few tens of microliters (e.g., for
591 flow cytometric assessments) and hundreds of milliliters (e.g., for cultivation- and nucleic
592 acid-based methods), as also reported in terrestrial studies (Safford and Bischel, 2019).
593 Notably, sample filtration, a major pre-treatment step for ATP-metry and nucleic acid-based
594 methods, can selectively concentrate the microbial biomass and target microorganisms, while
595 minimizing the water loss for analytical needs. The filtering surfaces are also suitable for
596 visual inspection (e.g., by microscopy), storage, and comparative analysis (e.g., on Earth).
597 However, filtration can influence the composition of the dissolved organic matter of the
598 permeate water, possibly triggering microbial regrowth in the downstream distribution
599 system (Park et al., 2018), and it will also necessitate additional crew time for manual
600 operations (e.g., filter substitution, regeneration, disposal).
601 Sufficient data are not yet available from real space conditions to implement protocol details
602 within the procedural workflows. Although the selected technologies were demonstratively
603 applied under either simulated or real microgravity conditions, their own critical advantages
604 and limitations will require full reconsideration for flight-like and space applications.
605 From the one hand, analytical benefits will necessarily include the time needed to achieve
606 results (i.e., speed to results), the accuracy and flexibility in detecting specifically-selected

607 microbial targets (i.e., identification depth) with reproducible consistent results (i.e.,
608 reproducibility), and the number and multiple type of achievable results (i.e., herein named
609 multiparametricity). On the other hand, the operating conditions can be particularly stringent
610 and limiting the direct applicability in space, unless addressing critical requirements such as
611 reagent usage, waste production, operator skills, and crew time (table 1).
612 Using a simplistic pairwise comparison, we sought to emphasize that few selected
613 technological features have to be consciously retained from the methodological proof-of-
614 concept level up to the device deployment, instrumental demonstration, and routine use in the
615 on-board housekeeping program. Although the comparative scores were assigned
616 subjectively (table S1), it is likely evident that the stringent requirements of microbial water
617 monitoring in space cannot be met by a single technological solution.
618
619 **Table 1**. Advantages and limitations of the most promising approaches for microbial
620 monitoring in space settings. The selected technologies are flexible (i.e., applicable to
621 different microbial targets in samples of various origin), suitable for miniaturization and
622 automation with limited maintenance, and already tested in flight-like conditions. A
623 comparative score was arbitrarily assigned through a pairwise comparison matrix for each of
624 selected space-relevant technological features, including major analytical performances (i.e.,
625 speed to results, identification depth, reproducibility, multiparametricity – green marks) and
626 practical issues to cope with in space (i.e., reagent usage, waste production, operator skills,
627 crew time – red marks).
628

629

Technology	Advantages	Limitations	Analytical performances/ Practical issues	Comparative Scores	Applications in space
Plate Cultivation	– No pretreatment – Detection limit 1 cell/100 ml – Low equipment requirements	– No direct quantification – Risks of contamination – Reliant on cultivation conditions	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		La Duc et al., 2004 Morrison et al., 2017 Pierson et al., 2013
LAMP	– Detection limit ~10^2 gene copies/ml – Unaffected by template conc. – No effects of inhibitory compounds	– No direct quantification – Risks of contamination	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		Ott et al., 2014
qPCR	– Target-specific and quantitative – Detection limit ~10^3 gene copies/ml	– Pretreatment processing – Affected by template and inhibitory compounds – Reliant on PCR related issues	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		Boguraev et al., 2017 Parra et al., 2017
High Throughput Sequencing	– In-depth phylogenetic resolution – Specific for unknown non targeted microorganisms	– Pretreatment processing – Reliant on PCR related issues – Complex data interpretation	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		Castro-Wallace et al., 2017 McIntyre et al., 2016
ATP-metry	– Real-time data (< 5 min) – Detection limit ~0.1 pg/ml	– Unspecific detection – Destructive analysis – Risks of contamination	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		Guarnieri et al., 1997 La Duc et al., 2004
Flow cytometry	– No pretreatment – Quantitative and near-real time data (< 20 min) – Detection limit ~10^2 cells/ml	– Complex data interpretation – Clogging issues	Speed to results Identification depth Reproducibility Multiparametricity Reagent usage Waste production Operator skills Crew time		Dubeau-Laramée et al., 2014 Phipps et al., 2014

630

6 System miniaturization and future challenges

An important point is that the monitoring technologies can be mission-dependent, but only those instruments that minimize crew involvement in their end-to-end operation are likely to be applicable on the long-term (Karouia et al., 2017). Overall, the selected devices have to be compact, suitable for automation, low power-consuming, and virtually invisible except when needed (Limero and Wallace, 2017). The ground-based counterparts have already been miniaturized using microfluidics, but the deployment of monitoring-effective tools onboard spacecrafts will also require substantial reengineering and instrumental customization. The instruments deployed so far have not been yet permanently used in spaceflight water monitoring, yet numerous examples of successful international projects and commercial partnerships dedicated to the human space exploration let argue that critical space water-related tasks can be operatively accomplished at reasonable costs and times (< 5 years) (Karouia et al., 2017).

Considerable progress has been made in miniature the onboard instrumentation to assess the water microbiological contamination. This also includes microbial cultivation systems, such as the AquaPad developed by the French CNES space agency (Augelli, 2018). The microfluidic chips that allow cell isolation and incubation have been developed for ground-based applications, and appear suitable for space uses. For example, the encapsulation of single cells from a mixed microbial community into small droplet compartments of a water-in-oil emulsion can offer further opportunities for physiological studies and viability assays (e.g., metabolic by-products diffusion, resistance to toxicants, enzymatic activities) on clonal populations isolated into plugs or micro-Petri dishes (Boedicker et al., 2008; Boitard et al., 2015).

Considering the ability of microfluidic systems to efficiently conduct measurements on small volumes of complex fluids without the need for a skilled operator, lab-on-a-chip technologies

656 and portable diagnostic devices have gained increased popularity for sensing a wide range of
657 water parameters and microbial pathogens even in the most remote settings (Mairhofer et al.,
658 2009). More recently, new qPCR platforms based on microfluidic technologies have been
659 developed allowing the simultaneous analysis of numerous genes and samples in volume
660 chambers of few nanoliters, placed at high density on a chip (Ricchi et al., 2017). Being less
661 sensitive to inhibitors than qPCR, the digital PCR (dPCR) is mainly applied to monitor gene
662 transcriptions in microbial cells without the need of a standard curve for gene copy
663 quantification (Devonshire et al., 2016).
664 The system miniaturization was also considered the most appealing trait for space
665 applications of the nanopore DNA sequencer MinION (Castro-Wallace et al., 2017). By
666 assembling miniaturized and lab-on-a-chip solutions, the Water Monitoring Suite developed
667 by NASA represents so far the best performing custom-built device applied successfully on
668 the ISS to monitor different water quality properties. Along with the HACH colorimeter and
669 the Organic Water Module, respectively used for inorganic and organic chemical
670 assessments, the hardware suite also includes the PCR-based instrument RAZOR EX, with
671 customized sample pouch kits containing all pre-loaded reagents necessary for sampling,
672 sample preparation, and real-time PCR
673 (https://www.nasa.gov/mission_pages/station/research/experiments/explorer/Investigation.ht
674 ml?#id=1847). However, as space exploration progresses toward extended missions to cis-
675 lunar space and Mars, PCR-based and multi-omics instruments onboard spacecraft should not
676 be considered in separation from other technologies needed for the in-flight microbiological
677 research (Karouia et al., 2017).
678 In particular, microfluidic platforms developed for the direct cell detection showed promising
679 perspectives because of the potential for precise and easy-to-use analytical procedures. While
680 enhancing analytical performances, the system miniaturization presents also the advantages

of reduced consumption of reagents and the ability to integrate monitoring and isolation procedures within a single device (Auroux et al., 2002). A simple microfluidic system was successfully tested for rapid and semi-automated bacterial enumeration in freshwaters and promising outcomes suggested its applicability to drinking waters under both ground and space conditions (Yamaguchi et al., 2014). Moreover, both sensitivity and specificity of ATP-metry and FCM can be also improved by miniaturizing the core systems down to scales closer to the ones of microorganisms. A micro-fluorescence-activated cell sorting (μFACS) was used to sort out cells of interest by changing the flow direction after cell detection (Fu et al., 1999). An integrated platform that combines two different force fields in a single microfluidic device (Dielectrophoretic–Magnetic Activated Cell Sorter - iDMACS) was applied for simultaneous sorting of multiple bacterial targets (Kim and Soh, 2009). More recently, a high-throughput Raman flow cytometer was developed on a microfluidic chip for the label-free molecular fingerprinting at the single-cell level (Hiramatsu et al., 2019).

The onboard laboratory miniaturization included also the fluidic components (e.g., pumps, valves, electronics), thus paving the way to the use of advanced biosensors for screening food safety and water quality in space (Roda et al., 2018). Following the proofs of concept and wearable technologies suited to monitor astronauts' health, the biosensing diagnostic instrumentation, most reasonably based on electrochemical and optical detectors, was argued as a secondary future option for in-flight water biochemical analyses (Choi et al., 2018; Limero and Wallace, 2017; Nelson, 2011). In particular, the amperometric biosensors were proven sensitive to monitor different water analytes, chemical contaminants (e.g., pesticides, organophosphates, carbamates), and numerous microbial biomarkers successfully targeted to detect the major microbiological agents, food- and water-borne pathogens (e.g., *E. coli, Salmonella, L. monocytogenes, C. jejuni, B. cereus, M. smegmatis*) (Grieshaber et al., 2008; Velusamy et al., 2010). However, despite providing concrete benefits for health services and

706 our life on Earth and beyond, the spectroscopic and biosensing devices are still at a low level
707 of technological readiness for in-flight applications (García-Descalzo et al., 2019; Own et al.,
708 2019; Roda et al., 2018), thus falling out of the scopes of this review.
709 In general, a critical aspect of all microfluidic and lab-on-a-chip solutions is bound to the
710 very limited system reusability. Most miniaturized devices for terrestrial applications are of
711 single-use and, in many cases, based on disposable cartridges that cannot be safely stowed
712 onboard during long-term space missions. When cells are delivered to different system
713 compartments for collection, there is no clear approach for removal the analyzed samples
714 without compromising the system functioning and risking contamination. Clogging issues
715 may also interfere with the analyses due to the processing of large sample volumes. Reusable
716 systems have been tested, but their applications in space are likely constrained by the risk of
717 sample carryover, reduced analytical performance upon extended reuse, and the resources
718 required for cleaning and reactivation procedures. In this regard, the technological
719 development is highly demanding and still far from being accomplished in a near future.
720

721 **7 Conclusions**

722 • Current technologies for water microbial monitoring can satisfy the needs of long-term
723 space exploration missions at reasonable costs and times, although substantial
724 instrumental reengineering has to be considered.
725 • The suitable methodological applications at a high technology readiness level (i.e., at
726 least validated in a flight-like environment) will require reduced space to be allocated and
727 can potentially provide rapid and specific responses regarding the in-flight occurrence of
728 the microbiological contamination.
729 • The advanced biomolecular characterization of water samples from the ISS is promoting
730 a better understanding of the onboard levels and patterns of microbial contamination, thus

contributing to the development of space bound technologies for the rapid and specific identification of microorganisms of health concerns.

- As space exploration progresses toward longer missions, PCR-based and multi-omics approaches can be complemented by real-time technologies needed for the in-flight microbiological research and suitable for the early-warning microbial monitoring of space waters.

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