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Implementing bioburden reduction and control on the deliquescent hydrogel of the HABIT/ExoMars 2022 instrument

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ABSTRACT

The HabitAbility: Brines, Irradiation and Temperature (HABIT) instrument will be part of the ExoMars 2022 mission (ESA/Roscosmos) and will be the first European In-situ Resource Utilization (ISRU) instrument capable of producing liquid water on Mars. HABIT is composed by two modules: Environmental Package (EnvPack) and Brine Observation Transition To Liquid Experiment (BOTTLE). EnvPack will help to study the current habitability conditions on Mars investigating the air and surface thermal ranges and Ultraviolet (UV) irradiance; and BOTTLE is a container with four independent vessels housing deliquescent salts, which are known to be present on Mars, where the liquid water will be produced after deliquescence. In order to prevent capillarity of deliquescent or hydrated salts, a mixture of deliquescent salts with Super Absorbent Polymer (SAP) based on polyacrylamide is utilized. This mixture has deliquescent and hydrogel properties and can be reused by applying a thermal cycle, complying thus with the purpose of the instrument. A High Efficiency Particulate Air (HEPA) grade filter made of polytetrafluroethylene (PTFE) porous membrane sandwiched between spunbounded nonwoven fabric stands as a physical barrier allowing interaction between the gaseous molecules of the Martian atmosphere and the salt mixtures, and at the same time preventing the passage of any potential biological contamination from the cells to the outside or vice-versa. In addition to the physical barrier, a strict bioburden reduction and analysis procedure is applied to the hardware and the contained salt mixtures adhering to the European Cooperation for Space Standardization protocol of microbial examination of flight hardware (ECSS-Q-ST-70-55C). The deliquescent salts and the SAP products need to be properly treated independently to adhere to the planetary protection protocols. In this manuscript, we describe the bioburden reduction process utilized to sterilize the salt mixtures in BOTTLE and the assays adopted to validate the sterilization. We also describe the construction of a low-cost, portable ISO 7 cleanroom tent, exclusively designed for planetary protection tests. The sterilization process involves Dry Heat Microbial Reduction (DHMR) of the deliquescent salts and the SAP mixtures. The performance of SAP after DHMR is validated to ensure its working efficiency after sterilization. A slightly modified version of the standard swab assay is used in the validation process and a comparison is made between samples exposed to a thermal shock treatment and those without thermal shock, to determine the best assay to be applied for future space hardware utilizing such salt mixtures for planetary investigation and In-Situ Resource Utilization (ISRU). The demonstration of the compatibility of these products with the processes commonly required for space applications has implications for the future exploration of Mars.

1. Introduction

The HABIT (HabitAbility: Brines, Irradiation and Temperature) instrument will be a part of the ExoMars 2022 mission (ESA/Roscosmos). It will be the first Swedish Instrument to land on the surface of Mars and the first European In-Situ Resource Utilization (ISRU) instrument capable of producing liquid water on Mars. For that it will extract water vapor from the atmosphere and will produce liquid brines by

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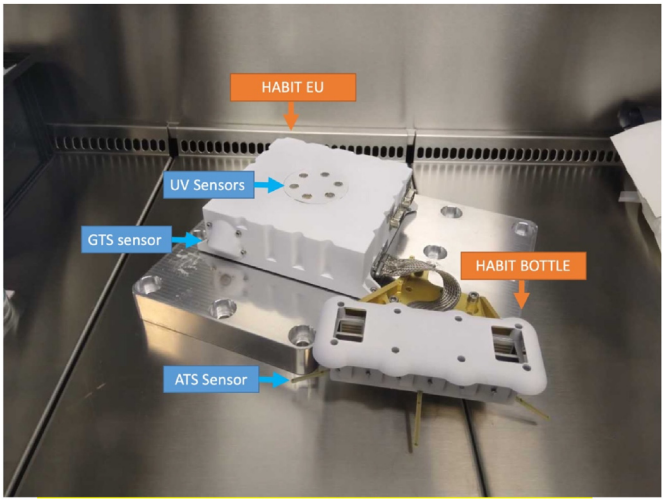


Fig. 1. HABIT Flight Model. The physical units and sensors are labelled.

deliquencesce of the salts stored in the Brine Observation Transition To Liquid Experiment (BOTTLE) container. HABIT also will study the current habitability conditions on Mars investigating the air and surface thermal ranges and the Ultraviolet (UV) irradiance. The HABIT instrument consists of two physical parts, namely (1) the Electronic Unit (EU) that houses the electronics, the Ground Temperature Sensor (GTS) and Ultraviolet (UV) photodiodes to measure the UV irradiance on the surface and (2) the Container Unit (CU) which consists of three Air Temperature Sensors (ATS) and the BOTTLE unit. The BOTTLE is the container element of HABIT with four independent vessels housing deliquescent salts, namely calcium chloride, ferric sulphate, magnesium perchlorate, and sodium perchlorate, which are known to be present on Mars, that will be exposed to the Martian atmosphere. The deliquescence process will be monitored by observing the changes in electrical conductivity (EC) in each container [1]. Fig. 1 shows the flight model of the HABIT instrument.

The HABIT instrument is mounted on the Russian lander of the ExoMars 2022 payload, also called “Kazachok”. The landing site chosen for the ExoMars 2022 mission is Oxia Planum. Oxia Planum preserves a rich geological record of the planet's wetter past, with approximately 4 billion-year-old sedimentary clay deposits. This region is marked by ancient highland cratered terrains highly eroded towards the highland–lowland boundary, and falls in a wide basin at the outlet of the Cogoon Vallis System between Mawrth Vallis and Ares Vallis [2]. The geomorphological setting of the region and the composition of clay sediments indicate a possibility of them being lagoon or deltaic deposits near an ancient Martian ocean, and hence serves as an ideal location to search for clues that may reveal the presence of past life on Mars [3]. ExoMars, being a two-part astrobiology mission, has the goal to search for the presence of past life on Mars. The ExoMars 2022 mission relies on the use of a lander and a rover system to investigate the presence of past life, and demands a strict bioburden control to prevent forward contamination by the spacecraft with earth borne microbes that may compromise the results of the experiment, and also contaminate the Martian environment.

The Committee on Space Research (COSPAR) Planetary Protection (PP) policy places lander missions to Mars under Category IV [4], which requires stringent bioburden reduction. Furthermore, because the primary goal of ExoMars is the search for the presence of past life on Mars, the mission is assigned Category IVb. The Category IVb demands a strict bioburden requirement such that the entire landed system is restricted to a surface bioburden level of $\leq$ 0.03 spores per m^2, or to levels of bioburden reduction driven by the nature and sensitivity of the particular life-detection experiments [5]. Any subsystem of the lander system that is involved in the mission must also be subjected to a bioburden control of $\leq$ 0.03 spores per m^2. The contents of the BOTTLE unit of HABIT (and of course the full hardware of the HABIT instrument) must also meet these requirements. To ensure the compliance with the required standards, the bioburden control and analysis is made based on the European Cooperation for Space Standardization norms (ECSS). ECSS is a cooperative effort of the European Space Agency, national space agencies and European industry associations for the purpose of developing and maintaining common standards. This manuscript discusses the bioburden reduction and control process implemented on the BOTTLE contents of the HABIT instrument along with the development of a low-cost portable cleanroom facility in which the planetary protection procedure was carried out.

2. Material and methods

2.1. Planetary protection standards used

Our approach to comply with the Planetary Protection standards for the BOTTLE contents is based on past mission strategies coupled with a portable clean-area solution to conform to the planetary protection standards recommended by the COSPAR Planetary Protection Policy [5,6]. Table 1 mentions the ECSS standards used in the BOTTLE Bioburden reduction and control of the HABIT instrument.

2.2. HABIT BOTTLE contents

The HABIT BOTTLE has a High Efficient Particulate Air (HEPA) filter, with pore size 0.3 μm, as shown in Fig. 2. In general, in space applications, HEPA filters are used for contamination control, preventing any molecular and particulate contamination that could be detrimental to the required operation, reliability or performance of a part, component, subsystem or system, including science instruments looking for life processes [7]. In the configuration of HABIT, the HEPA filter isolates the contents of the BOTTLE from getting into contact with the external environment and also prevents any inward contamination to the BOTTLE products, which can produce liquid water on Mars. This provides the first layer of planetary protection thereby preventing any forward contamination of the BOTTLE contents to the Martian environment. In addition to this, the BOTTLE has also been sterilized and controlled.

The BOTTLE contents of HABIT are a mixture of four deliquescent salts with Super Absorbent Polymer (SAP). The four deliquescent salts, namely calcium chloride, ferric sulphate, magnesium perchlorate, and sodium perchlorate, have been chosen for BOTTLE because they have been found on Mars, and also their phase diagrams allow to absorb atmospheric water and eventually hold liquid conditions under the expected temperature range of Oxia Planum. For each salt, the eutectic temperature, the lowest possible melting temperature over all of the mixing ratios of salt and water is indicated in Table 2. Additionally,

Table 1
ECSS standards used in the bioburden reduction of BOTTLE contents.

Standard	Title	Date of Publication
ECSS-Q-ST-70-55C	Microbial examination of flight hardware and cleanrooms	November 15, 2008
ECSS-Q-ST-70-57C	Dry heat bioburden reduction for flight hardware	August 30, 2013
ECSS-Q-ST-70-58C	Bioburden control of cleanrooms	November 15, 2008

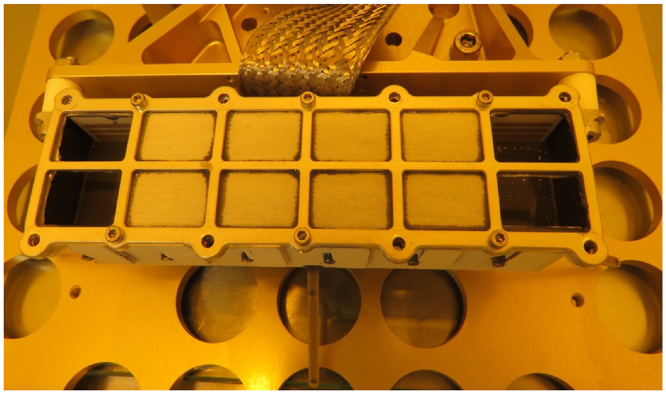


Fig. 2. HEPA Filter Assembly of HABIT Flight Model glued and screwed to the BOTTLE structure using six M2 bolts.

Table 2
Eutectic temperature and humidity of HABIT Salts.

Salt	Te (K)	RH (%)	Reference
$NaClO_4$	236	53	Hennings et al. (2013a,b) [8]
$CaCl_2$	226	60	Toner et al., 2014 [9]
$Mg(ClO_4)_2$	206	52	Stillman and Grimm (2011) [10]
$Fe_2(SO_4)_3$	246.35	40	Hennings et al., 2013a [11]

these salts have been chosen because their response to the Earth environment is such that it allows for long storage under clean room conditions without initiating deliquescence into liquid.

In order to maintain the liquid brine in a semisolid state within the container, a mixture of the salt with SAP was used. Each salt mixture in HABIT BOTTLE constitutes 1.5 g of the respective salt and 0.75 g SAP, mixed in a weight ratio of 2:1. Table 3 lists the final contents and ratios of products in the HABIT BOTTLE unit. Hydrogel derived matrices of the hygroscopic salts using sodium alginate [12] were tested, but they did not have the desired properties of reusability through mild temperature cycles including the frozen state (not shown). SAP was tested instead, and its swelling ratio was measured after dry heat sterilization at 125 °C and also under depressurized conditions. The SAP did not suffer any degradation in swelling ratio performance and was therefore selected as hydrogel agent for BOTTLE. The SAP used in the HABIT BOTTLE is based on Poly (acrylamide-*co*-acrylic acid). The SAP has a grain size ranging from 200 to 1000 μM.

2.3. Bioburden reduction technique

The use of such salt mixtures in HABIT BOTTLE is the first of its kind for an instrument payload and adhering to the bioburden requirements for planetary protection without compromising the efficiency of the water absorbing capability of the salt mixture is a challenge. There exists a wide range of strategies for bioburden control [13], but the preferred strategy adopted to achieve compliance with the bioburden requirement for the salt mixture is Dry Heat Microbial Reduction (DHMR). Wet heat sterilization cannot be adopted owing to the presence of deliquescent salts and the effect of UV sterilization or gamma sterilization on the behaviour of the SAP has not been validated. Hydrogen Peroxide sterilization has been discarded for the same reason. The oven temperature is monitored continuously to ensure that the temperature stays within the DHMR limit of 125 °C. The humidity in the oven is also monitored such that it remains constant and low. Fig. 3 illustrates the DHMR process carried out. The entire procedure is carried out in a portable cleanroom tent that we have designed with an ISO 7 cleanliness level. Fig. 4 shows the interior of the portable cleanroom tent constructed with a commercial grade gazebo and with a HEPA filter based air purifier.

Table 3
Final contents in HABIT BOTTLE.

Cell #	Product formula	Product name	Dry weight (g) (salt + SAP)
1	Open container to collect Martian dust		
2	$CaCl_2$: $C_6H_8KNO_3$	Calcium chloride + SAP	2.25 = (1.5 + 0.75)
3	$Fe_2(SO_4)_3$: $C_6H_8KNO_3$	Ferric sulphate + SAP	2.25 = (1.5 + 0.75)
4	$Mg(ClO_4)_4$: $C_6H_8KNO_3$	Magnesium perchlorate + SAP	2.25 = (1.5 + 0.75)
5	$NaClO_4$: $C_6H_8KNO_3$	Sodium perchlorate + SAP	2.25 = (1.5 + 0.75)
6	Open container to collect Martian dust		

2.4. Portable cleanroom tent construction

The tent is constructed with a double walled enclosure made of 90% polyester, 10% polyurethane to minimize the influx of particles from getting into the interior. Polyester is normally used in the manufacture of cleanroom wipes owing to low particle generation. Polyurethane adds strength to the tent walls making it more durable and robust. A third layer of plastic covering is spread over the tent to further prevent particle influx. In order to have an optimal ventilation to the cleanroom space, a netted space is left in bottom close to which we have installed a commercial air purifier with a HEPA filter. The commercial air purifier has a dual filter that ensures greater cleaning ability with a combination of HEPA filter and an activated carbon filter. The 40 W power rated air purifier has the design capability to clean an area of approximately 35 m^2 which is much greater than the tent area of 9 m^2. To ensure the cleanliness of the cleanroom tent, a strict housekeeping with 45% isopropanol based disinfectant (DAX Surface Disinfection Plus from CCS Healthcare AB) is used. Though the 70% isopropanol based disinfectant has the maximal disinfectant capability, and is recommended in the standard, due to commercial limitation on the time of delivery of this product at the time of these tests, we used 45% isopropanol instead as disinfectant. As our tests demonstrated, this concentration, together with all other precautions taken, was sufficient to achieve the desired level of disinfection. Once the interior of the cleanroom tent is cleaned with the isopropanol based disinfectant, the air purifier is switched on.

To guarantee the adequate cleanliness level, the area is pre-cleaned with the air filter during a period of 48 h before each experiment. The qualitative reduction in number of particulates was easily detected with the cleanroom particle meter. Further cleaning of the tent interior with isopropyl alcohol based disinfectant is done every morning before beginning the experiments to have a very low microbial concentration in the air. This was verified by sampling the air inside the tent using a Sartorious air sampler with a gelatine substrate to capture the cultivable microbes in the ambient air. 1000 L of air were sampled at 50 L/minute. The gelatine substrate was then cultivated on a Petri dish with 20 ml R2A agar in an incubator maintained at 32 °C, proceeding with microbial colony counts noted after 24, 48 and 72 h respectively. As it can be observed in Fig. 5, the daily cleaning of the tent with isopropyl alcohol based disinfectant had a great impact on the microbial concentration in the air. The number of colony forming unit rises when cleaning is only applied on alternate days.

For control, we include the studies of the area outside the tent. This serves to illustrate what is the existing natural background of microorganisms in the ambient air of the laboratory. The comparison with the interior of the tent, when cleaning is applied on a daily basis, shows that this solution produces a very dramatic improvement in a semi-enclosed environment. Cleanroom garment is also mandatory to enter into the cleanroom space. ISO 5-certified, one-time-use, cleanroom garment was chosen for our application. Fig. 6 shows the full cleanroom garment suit worn prior to entering the cleanroom tent. To maintain the

Fig. 3. Sequence of DHMR process carried out on the HABIT BOTTLE mixture. 1).Weighing of salts and SAP in proportion of 2:1; 2). Wrapping in aluminium foil to distribute heat evenly; 3). Samples ready for DHMR; and 4). DHMR process carried at 125 °C for 10 h.

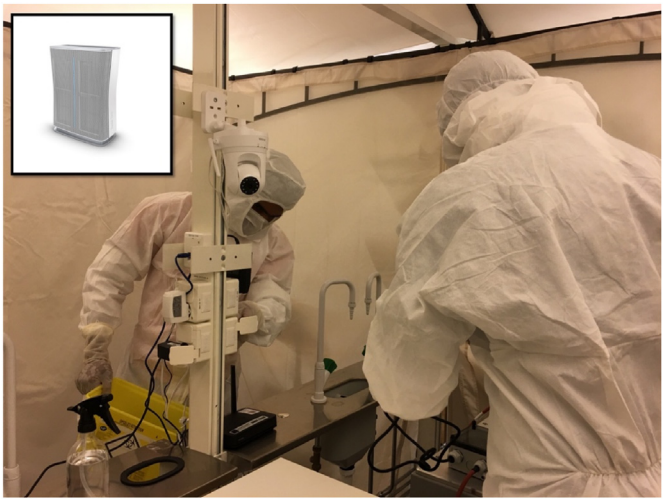


Fig. 4. Interior of the cleanroom tent constructed with a commercial gazebo. The inset image shows the HEPA filter based air purifier that is kept inside the cleanroom tent.

cleanroom standard, top to bottom cleanroom dressing with double gloves, sleeves, facial mask, headgear and cleanroom boots was necessitated. A portion of the laboratory where the cleanroom tent was installed was cordoned off, cleaned and designated as the gowning area. A combination of strict garmenting and housekeeping along with the commercial grade tent and air purifier had provided us a ISO 7 cleanroom workspace as per the 14644-1 standard.

2.5. Portable cleanroom tent validation

The ISO rating has been verified using the METONE HHPC 6+ hand held cleanroom monitor instrument with reference to the ISO. The cleanroom tent with an area of 9 m^2 was sampled at three different locations inside the tent. The METONE HHPC 6+ instrument has 6 channels to measure particles with dimensions from 0.3 to 10 μm. A target cleanliness level of ISO 5 was chosen and 0.5 μm sized particle was taken as the reference. Air volume of 5.66 L was sampled in 2 min based on the requirement of ISO 14644-1 standard which necessities minimum sampling of 2 L for at least 1 min in a single location. The same process was repeated for the region outside the cleanroom tent.

Fig. 7 shows the particle count analysis outside (top) and inside (bottom) of the cleanroom. It could be demonstrated that the exterior of the cleanroom tent was ISO class 8. This portable approach allows to achieve a ISO reduction of one class in cleanroom standards within a normal laboratory environment. Based on the ECSS-Q-ST-70-58C, we are well within the ISO level required to perform the bioburden reduction and the bioburden assay validation.

2.6. Bioburden assay procedure

The bioburden monitoring and validation for the HABIT BOTTLE salt mixtures was done by following the protocol of the ECSS-Q-ST-70-55C standards. The bioburden assay validation was also performed inside the portable cleanroom tent. All the materials used in the bioburden assay validation have been procured from Sigma Aldrich and VWR Sweden. A modified version of the standard swab assay procedure was followed for the bioburden assay validation, where the swab is skimmed over the top surface of the salt mixture in the jelly state. ECSS-Q-ST-70-55C standard necessitates swabbing an area of 0.0025 m^2 as required for Category IVb missions, but due to the limited amount of salt mixtures (2.25 g) needed in the HABIT BOTTLE cells, the salt mixtures could only be spread evenly over an area of 0.00125 m^2, which is half the swabbing area mentioned in the ECSS-Q-ST-70-55C standard. Nevertheless the full volume of the salt mixtures used in the HABIT BOTTLE cells was swabbed.The sterilized SAP salt mixture in the aluminium container is skimmed with a flocked sterile Nylon swab (FLOQSwabs® from Copan) dipped in an Eppendorf tube containing 1 ml of sterile water. The swabbing was performed at an angle of 30° to the surface to be swabbed. The process was repeated two times, each time changing the direction of the swabbing motion by 90°. And by also rotating the head of the swab each time when the swabbing motion is changed. The swab is then broken at the breaking point which is 80 mm away from the tip of the flocked swab and is then suspended into a 15 ml sterile centrifuge tube which is filled with 2.5 ml of sterile Phosphate Buffer Saline (PBS + 0.02 v/v % Tween 80, pH 7.2). The sample must be analyzed within 24 h and needs to be maintained at a temperature between 4 and 8 °C. Four samples were taken following the described process.

As a double verification strategy, the bioburden assay validation was done in parallel with two samples each sent to the Institute of Aerospace Medicine, DLR, Germany. The samples were shipped in a

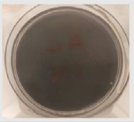
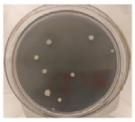
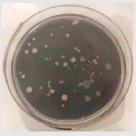
Sample	24hr count	48hr count	72hr count	Picture after 72hrs
Inside Tent – Everyday Cleaning	0	0	0	
Inside Tent- Alternate day Cleaning	3	5	8	
Outside Tent	9	16	32	

Fig. 5. Microbial colony forming units detection in the 1000 L air samples taken from inside and outside the tent. The picture on the right shows the colonies observed after 72 h incubation.

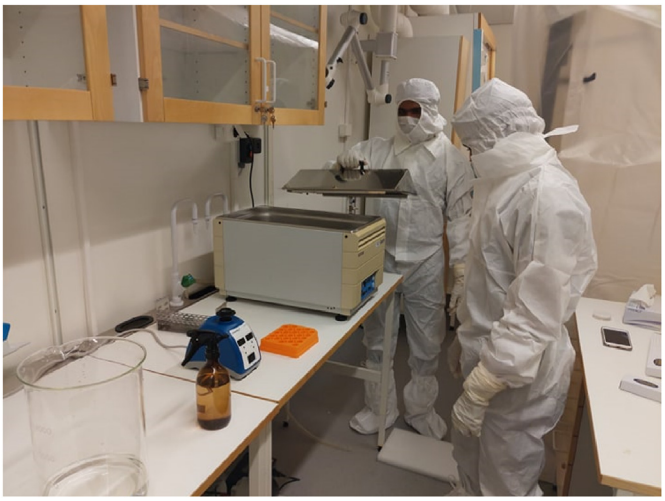


Fig. 6. A very strict garmenting is followed inside the cleanroom tent using ISO 5-certified one-time use bunny suits with double gloves tapped over the sleeves to avoid gapping and contamination release.

temperature-controlled box with temperature maintained between 4 and 8 °C and a delivery time within 24 h. The centrifuge tubes containing the buffer and the swab is then mixed on a vortex mixer at maximum power for 5–6 s. A heat shock procedure is imparted to target the mesophilic aerobic spores and bacteria that are able to survive the heat treatment for 15 min at 80 °C. The vortexed centrifuge tubes are subjected to a heat shock by immersing the centrifuge tubes in the hot water bath at 80 ± 2 °C for 15 min. Care is taken to ensure that the level of the buffer in the centrifuge tubes lies below the water level in the water bath. The centrifuge tubes are then taken out of the hot water bath and cooled rapidly in an ice bath such that the temperature of the buffer is around 30–35 °C. Prior to plating the suspension on the agar plates, the centrifuge tubes are again vortexed for 5–6 s. Then 0.5 ml of the suspension is aseptically pipetted from the centrifuge tube on to the surface of the R2A agar plate using the EHP (Ergonomic High Performance) Micropipette tool with sterile pipette tips. A L-shaped sterile spreader is used to spread the buffer evenly onto the surface of the ready to use R2A agar plate. Care is taken to ensure that the spreader does not pierce into the agar medium. The incubation is carried out aerobically at 32 °C in an incubator with the agar plates kept inverted to prevent moisture from condensing onto the agar medium and interfering with the growth of microbial colonies. The plates are checked every 24 and 48 h and later examined for colony counts at

Outside Tent	Number of Locations - 6						
Counts/m3	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6	Average
sample1	1203534	1107244	1004947	954947	906890	1070671	
sample2	1173498	1081802	1001060	926325	978622	1060424	
Location average	1188516	1094523	1003004	940636	942756	1065548	
Square of errors	451062627	323642594	7553996	409606580	2572704046	52504603	1039164
Square root of (sum of squared errors / Number of locations -1)							43687
							1082851
						95% CFI	1028708
						Limit for ISO8	3520000

Cleanroom Tent	Number of Locations 3			
Counts/m3	Location 1	Location 2	Location 3	Average
sample1	196820	178975	148057	
sample2	192580	162191	153534	
Location average	194700	170583	150795	
Square of errors	8990072	140859720	14999312	172026
Square root of (sum of squared errors / Number of locations -1)				9079
				181105
			95% CFI	172050
			Limit for ISO7	352000

Fig. 7. (Top) ISO-8 level of the workspace outside the cleanroom tent. (Bottom) ISO-7 validation of the cleanroom standard inside the cleanroom tent.

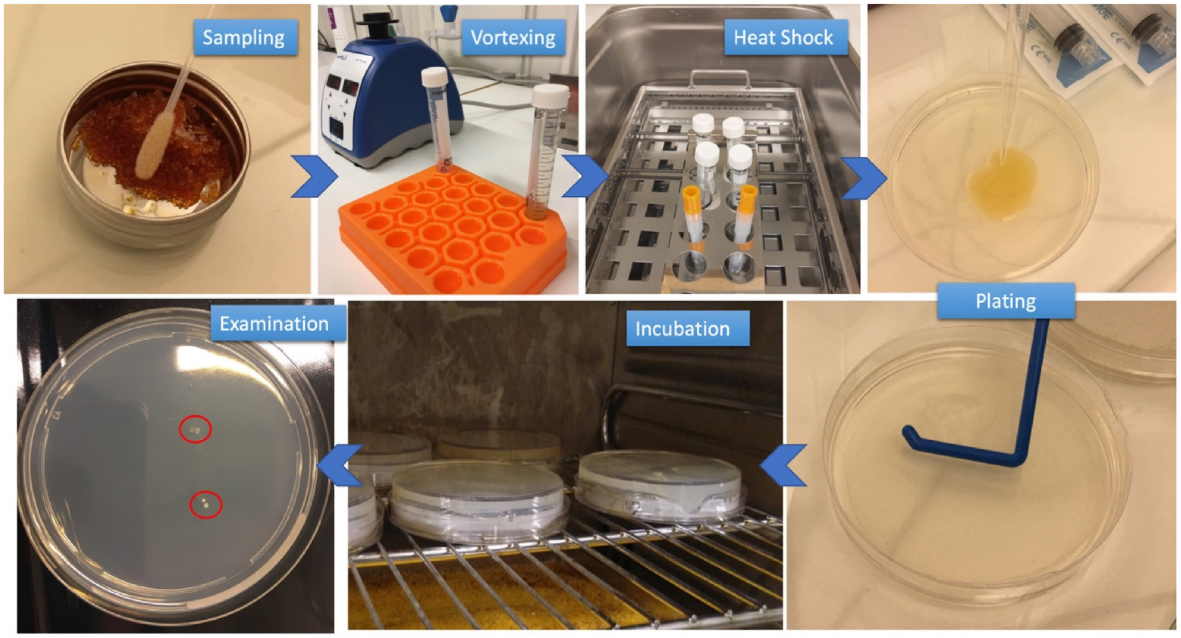


Fig. 8. Bioburden assay validation steps followed for HABIT BOTTLE Planetary Protection requirement. The Petri dish on the bottom-left shows a microbial colony of 2 CFU, corresponding to one of the lab negative controls obtained from swabbing a 5 × 5 cm^2 area of a table outside the cleanroom tent (the reflection from the lid of the Petri dish in inverted position is seen as a false duplicate colony nearby the observed true colonies).

72 h. It should be ensured that the Petri dish cover is not opened until the 72nd hour. The Petri dish is examined for the number of colony forming units (CFU) counts observed. Having no CFU in the sample area swabbed, passes the planetary protection sterilization tests.

In case of higher colony counts observed, the sterilization and cleaning procedures need to be repeated with even better aseptic handling. During the standard swab assay, controls are also needed to be tested by undergoing the same procedure indicated above over the non-DHMR salt + SAP mixtures. Furthermore, two lab negative controls are also performed by swabbing a surface area 5 × 5 cm^2 area of the worktable and the walls of the clean room tent. Fig. 8 shows the entire procedure followed right from sample preparation, assay and validation. The disposal of the bacterial colony filled agar Petri dish is very crucial. Proper care should be taken to dispose the cultured agar petri dishes in biohazard containers.

3. Results and discussion

The results of the number of CFU after plating of the cultivable microorganisms from the samples show no CFU in the HABIT BOTTLE salt mixture that has been exposed to DHMR even after 72 h of incubation. In order to determine the presence of aerobic mesophiles in the sample, a non-thermal shock version of the bioburden assay was also carried. For this version, the samples were not subjected to thermal shock. After vortexing the swabs in PBS, the sample was directly pipetted out and plated on the Petri dish and incubated. Fig. 9 shows the consolidated test results obtained with the thermal shock assay and non-thermal shock assay. Two samples for each assay was processed and validated (not shown). In both the thermal shock and the non-thermal shock version, the presence of aerobic mesophiles or aerobic mesophilic bacteria or spores could not be detected in the samples. However, in the sample taken from outside tent, the non-thermal shock assay indicated the presence of microbial colony forming units. This is consistent with the observations of colonies in the direct agar plating from the Sartorious air sampler, that was shown in Fig. 5.

The results from the Institute of Aerospace Medicine, DLR, Germany, to where the samples were sent also confirm the absence of colonies in the HABIT BOTTLE swabs. The complete absence of colony forming units in both the samples subjected to DHMR and the control (not subjected to DHMR) indicate that the salt itself as delivered by the provider, Sigma Aldrich, as dry fine powder in a closed container is not contaminated. Not surprisingly, these pure salts (i.e. 100% concentration and no water) in turn inhibit the growth of microbes. Different salt concentrations influence the ability of microbes to grow depending on the bacterial species and the salt. E.g. the bacterium *E. coli* (*Escherichia coli*) can grow in the presence of 2.5% (~0.25 M) sodium perchlorate whereas *Staphylococcus aureus* (*S. aureus*) exhibit slow growth in the presence of up to 7.5% (~0.75 M), but no growth has been observed when the concentration increases to 10% (~1.0 M) of sodium perchlorate.

A study on haloarchaea suggests that the haloarchaeon *Haloarcula argentinensis* can grow in the presence of 5% (~0.5 M) perchlorate and even in medium containing about 15% (~3.0 M) of NaCl [14]. Another study indicates strong growth of halotolerant *Haloarcula*, *Haloferax* and *Halomonas* in the presence of 4% (~0.4 M) Sodium perchlorate and a weak growth of Haloferax at 6% (~0.6 M) [15]. Methanogenesis has been demonstrated to proceed in the presence of 1% (~0.1 M) perchlorate, but not in higher concentrations for strains of *Methanobacterium*, *Methanosarcina* and *Methanothermobacter* [16]. However, when adapted to higher concentrations of perchlorate salts, these methanogens appeared to metabolize despite the presence of up to 0.5 M (~5%) of perchlorate concentration but did not metabolize at 1.0 M (~10%). Microbes can also use perchlorate as a terminal electron acceptor for anaerobic respiration [17–22], however in concentrations below 1 mM. This is of great astrobiology relevance owing to the deliquescent nature of the salts which may induce the formation of brines [23] and its stability in the liquid state [24], however any putative life form would still need to be tolerant to very high concentrations of salts [25]. The studies above, describe substantial microbial growth in lower concentrations of perchlorate. However, in the case of HABIT, a set of experiments have been performed under simulated Martian conditions for the expected environmental conditions at the ExoMars landing site at Oxia Planum, with these products mixtures and in the same amount. The experiments allowed to calculate the amount of water that can be absorbed from the atmosphere by 1.5 g of these salts. These tests show that the expected salt concentration in a brine formed by hydration and

Sample	Sampling area [m²]	24hr cfu count [TS]	48hr cfu count [TS]	72hr cfu count [TS]	24hr cfu count [Non-TS]	48hr cfu count [Non-TS]	72hr cfu count [Non-TS]
Calcium Chloride – 10HR DHMR	0.00125	0	0	0	0	0	0
Ferric Sulphate – 10 HR DHMR	0.00125	0	0	0	0	0	0
Magnesium Perchlorate – 10 HR DHMR	0.00125	0	0	0	0	0	0
Sodium Perchlorate – 10 HR DHMR	0.00125	0	0	0	0	0	0
Calcium Chloride – Control	0.00125	0	0	0	0	0	0
Ferric Sulphate – Control	0.00125	0	0	0	0	0	0
Magnesium Perchlorate – Control	0.00125	0	0	0	0	0	0
Sodium Perchlorate – Control	0.00125	0	0	0	0	0	0
Inside Tent - Workbench	0.0025	0	0	0	0	0	0
Outside Tent - Table	0.0025	0	0	0	1	2	4
* [TS] – Thermal Shock Assay ; [Non-TS] – Non-Thermal Shock Assay							

Fig. 9. Consolidated bioburden results obtained through thermal shock and non-thermal shock assays with colony count monitoring every 24 h.

deliquescence on Mars would be for Calcium chloride ($CaCl_2$), 58% w.t. = 1.5/(1.5 + 1.06); for Ferric sulphate $Fe_2(SO_4)_3$, 68% w.t. = 1.5/(1.5 + 0.71); for Magnesium perchlorate Mg $(ClO_4)_2$, 62% w.t. = 1.5/(1.5 + 0.93) and for Sodium-perchlorate ($NaClO_4$), 64% w.t. = 1.5/(1.5 + 0.83). These values are very high, and therefore the low water activity of these solutions should inhibit the growth of terrestrial-like microbial life [26].

The absence of microbes in the lab negative controls from swabbing the region outside the tent in the thermal shock version and the presence of very few colony forming units in the non-thermal shock version indicate the low microbial population of aerobic mesophiles in the environment where the LTU campus is located, near the Arctic circle. This can be corroborated with a study in the Arctic indicating a very low microbial dispersal and its implications to study planetary protection for human mars missions [27].

Avoiding re-hydration of the HABIT BOTTLE salt mixtures during storage and transfer phase is crucial. An aluminium lid with a custom-made silicone gasket has been designed to seat over the HEPA filter of the BOTTLE. The aluminium lid with the gasket forms a hermetic seal which shall be replaced with the original HABIT lid prior to launch. Fig. 10 (left) shows the aluminium lid with the HEPA filter mounted on the HABIT BOTTLE, for ground-configuration (during transport, storage and integration), and the flight ready configuration of HABIT with the original lid (right). The first measurements of HABIT, after delivery to IKI and integration in the Surface Platform, confirm the absolute hermeticity of this sealing with the conductivity indicating zero (not shown) demonstrating that the salts have not been exposed to laboratory air.

4. Conclusion

We conclude that salt + SAP mixture in the BOTTLE container of HABIT is compliant with the ExoMars planetary protection Category IVb. requirements. The low cost portable clean room tent design has also been validated and has demonstrated its potential to adapt a laboratory to have a temporary clean room area where products or hardware can be manipulated, sterilized and tested. Such low-cost portable cleanroom tents with aseptic manipulation with proper cleanroom gear can be of interest for small sized payloads or components as it requires a minimal investment of time and resources. The procedures that should accompany any of these activities have been described here: 1) cleaning; 2) particulate monitoring; 3) bioburden monitoring; and 4) sterilization according to the DHMR standards with adequate training of the personnel.

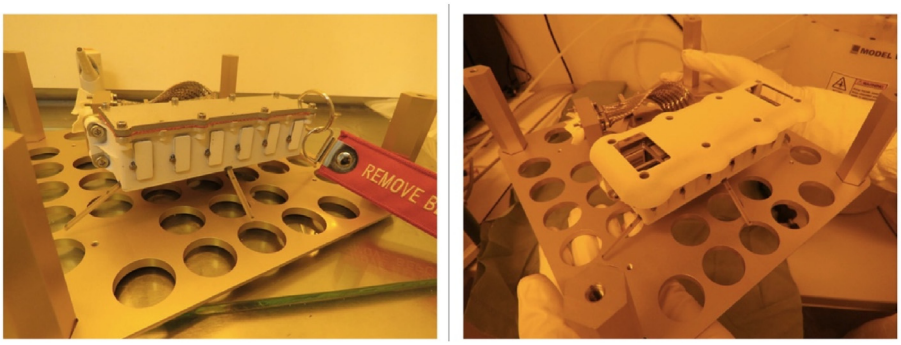


Fig. 10. (left) HABIT BOTTLE fitted with the solid aluminium lid and HT870 gasket during the storage phase; (right) HABIT BOTTLE fitted with the roof, which is the flight ready configuration of the HABIT BOTTLE.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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