

# Establishing and Monitoring an Aseptic Workspace for Building the MOMA Mass Spectrometer

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## ABSTRACT

Mars Organic Molecule Analyzer (MOMA) is an instrument suite on the European Space Agency (ESA) ExoMars 2020 Rover, and the Mass Spectrometer (MOMA-MS) is being built at Goddard Space Flight Center (GSFC). MOMA-MS is a life-detection instrument and thus falls in the most stringent category of Planetary Protection (PP) biological cleanliness requirements. Less than 0.03 spore/m<sup>2</sup> are allowed in the instrument sample path. In order to meet these PP requirements, MOMA-MS must be built and maintained in a low bioburden environment. The MOMA-MS project at GSFC maintains three clean rooms with varying levels of bioburden control. The Aseptic Assembly Clean room has the highest level of control, applying three different bioburden reducing methods: 70% Isopropyl Alcohol (IPA), 7.5% Hydrogen Peroxide, and Ultra-Violet C (UVC) light. The three methods are used in rotation and each kills microorganisms by a different mechanism, reducing the likelihood of microorganisms developing resistance to all three. The Integration and Mars Chamber Clean rooms use less biocidal cleaning, with the option to deploy extra techniques as necessary. To support the monitoring of clean rooms and verification that MOMA-MS hardware meets PP requirements, a new Planetary Protection lab was established that currently has the capabilities of standard growth assays for spore or vegetative bacteria, rapid bioburden analysis that detects Adenosine Triphosphate (ATP), plus autoclave and Dry Heat microbial Reduction (DHMR) verification. The clean rooms are monitored for vegetative microorganisms and by rapid ATP assay, and a clear difference in bioburden is observed between the aseptic and other clean room.

Keywords: Planetary protection, Contamination, ExoMars, Aseptic, Mars, Rover, Clean room

## 1. INTRODUCTION

The ExoMars Rover is a European Space Agency (ESA) mission that will send a rover to Mars in 2020. The largest instrument on the rover will be the Mars Organic Molecule Analyzer (MOMA). The MOMA instrument is an international collaboration between NASA Goddard Space Flight Center (GSFC), which is providing the Mass Spectrometer (MOMA-MS) and electronic boxes, Germany's Max Plank Institute and Laser Zentrum, which are providing the oven, tapping station, and laser, and France's LATMOS (Laboratoire Atmosphères, Milieux, Observations Spatiales) and LISA (Laboratoire Interuniversitaire des Systèmes Atmosphériques) providing the gas chromatograph. MOMA will be able to detect a wide range of organic molecules, and examine the biotic or abiotic origin of the molecules using chirality.

As MOMA is designed to detect organic molecules with biotic origin, it is classified as a "life-detection" instrument. This classification has implications for both the science and the Planetary Protection (PP) engineering requirements for the mission. Planetary Protection is a requirement levied on any mission visiting "the Moon or other celestial bodies" in the 1967 "Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies", which is often referred to as "The Outer Space Treaty". In Article IX of the treaty, it is specified that any mission visiting or landing on a celestial body other than Earth has to demonstrate that it will "avoid harmful contamination" and avoid "potentially harmful interference with activities of other States Parties"<sup>1</sup>.

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The Office of Planetary Protection at NASA Headquarters is responsible for ensuring that every NASA mission meets these treaty obligations and sets requirements for either the specific level of biological contamination permitted on each mission, or the acceptable risk level of a mission to contaminate a planetary body. For example, the Viking landers were required to represent less than  $1 \times 10^{-4}$ , or 1 in 10,000 risk of harmful contamination to Mars<sup>2</sup>, while the Mars Science Laboratory with Curiosity rover was required to meet a specific bioburden load of no more than 500,000 bacterial spores total and no more than 300 spores per square meter on exposed surfaces<sup>3</sup>. The specific engineering, science, and documentation requirements are constantly evolving to reflect the most recent understanding of the environments on other planets or moons. The current requirements for NASA missions are found in the NASA Procedural Requirements documents NPD 8020.7G “Biological Contamination Control for Outbound and Inbound Planetary Spacecraft”<sup>4</sup> and NPR 8020.12D “Planetary Protection Provisions for Robotic Extraterrestrial Missions”<sup>5</sup>, which defines the mission PP category is based on mission type and destination(s). ExoMars 2020 is a life detection mission due to the capabilities of the MOMA-MS, it has a mission category of IVb, making the first class IVb mission attempted since the Viking landers in 1975<sup>6</sup>. Class IVb missions must ensure that “the subsystems which are involved in the acquisition, delivery, and analysis of samples used for life detection [are restricted to a surface biological burden level of 30 spores].”<sup>5</sup>

When the Viking landers were built, the entire lander inside its aeroshell was processed for bioburden reduction by Dry Heat Microbial Reduction (DHMR) inside a bioshield. This allowed the entire spacecraft to meet the most stringent bioburden requirements. Recent Mars lander missions have not been compatible with full system DHMR as many modern components and subsystems cannot tolerate the higher temperatures. Instead different components of the spacecraft are cleaned to different biological cleanliness requirements. For the ExoMars mission, the exterior of the rover needs to be clean to less than 300 CFU/m<sup>2</sup> and anything on the sample acquisition and processing path that goes into the mass spectrometer must meet the most stringent bioburden level of 0.03 bacterial spores per square meter. Bacterial spores (also referred to as bacterial endospores) are the hardy dormant form of many bacterial species which have been demonstrated to survive exposure to the temperatures and pressure of outer space<sup>7</sup> and are therefore considered the most likely organisms to survive an interplanetary voyage aboard a spacecraft.

Preparing the Viking lander for lander level DHMR presented major design challenge which required extensive research, testing, and often redesign to ensure that various components and subsystems could withstand the lengthy high temperature bake. Even with extensive development and testing, some problems still arose that were traced to the terminal sterilization process<sup>2</sup>. As materials and instruments have progressed since 1975, sterilization has not gotten easier, which means that most Mars missions now sterilize at the component or subsystem level, rather than the entire spacecraft. While Viking lander terminal sterilization happened after all final closeouts, ExoMars instruments will go through separate sterilization processes, and will be assembled in aseptic environments. For the MOMA-MS, this means that after the instrument is built, and goes through the instrument level DHMR, the sample path will need to be opened at least twice for further integration with the MOMA suite of instruments and eventually the rover. To accommodate this without re-contaminating the sterilized surface, aseptic assembly environments had to be developed and verified.

## **2. ASEPTIC CLEAN ROOM MAINTINANCE**

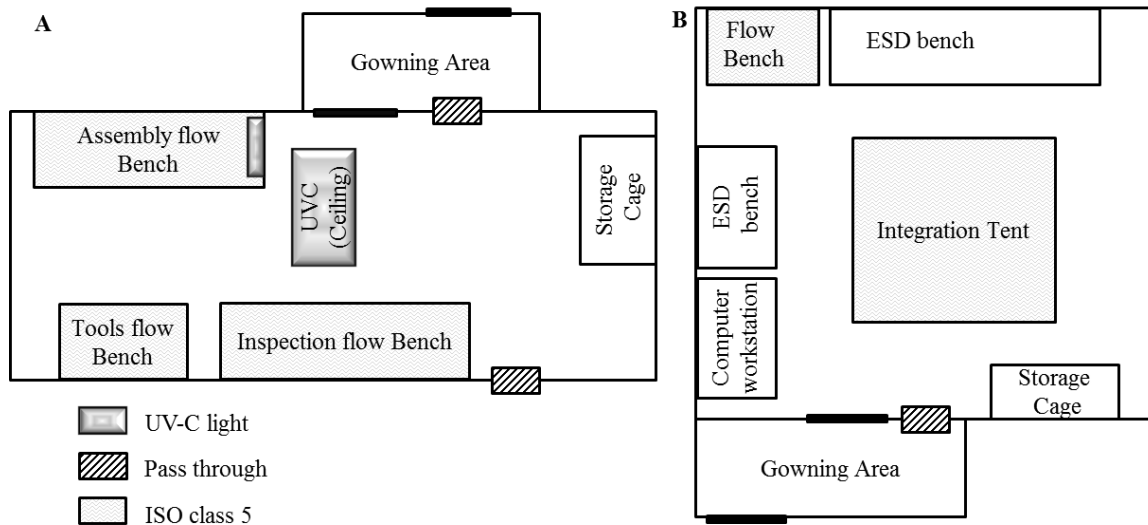
### **2.1 MOMA-MS clean rooms**

MOMA-MS assembly, integration, and testing at Goddard Space Flight Center occurs in three primary clean spaces, which will be referred to as the aseptic assembly clean room, the integration clean room, and the testing chamber/tent. To meet the MOMA-MS PP requirements, any work that exposes the sample path must happen in an aseptic ISO class 5 (ISO 14644-1)<sup>8</sup> environment using aseptic technique. Aseptic technique includes sterile garments, sterile tools, and a heightened awareness of potential contact transfer while working in a clean low particle environment.

### **2.2 Aseptic assembly clean room**

The aseptic assembly clean room layout is shown in Figure 1.A, and includes a gowning room, two pass-throughs, two large and one small laminar flow work benches, an electrically grounded ESD (ElectroStatic Discharge) bench, and various staging and storage areas. The room is set up as an ISO class 7 clean room, and the laminar flow benches provide work spaces that are ISO class 5. Clean room garments for non-critical activities are standard clean room

coveralls, hoods, and boots, which are changed twice a week, and single use gloves and surgical masks. For critical aseptic activities, single use sterile coveralls, hoods and gloves are used. To maintain biological cleanliness of the aseptic clean room, a rigorous cleaning schedule using three different biocidal treatments is implemented. Every day the room is vacuumed, mopped with one of two biocidal solutions, and critical work surfaces are wiped with pre-wet sterile 70% isopropyl alcohol (IPA) saturated wipes. The mopping solutions, 70% IPA and 7.5% Hydrogen Peroxide ( $H_2O_2$ ), are used in alternating weeks, with a week of IPA mopping followed by a week of  $H_2O_2$  mopping. The third biocidal treatment is ceiling and flow bench mounted Ultra Violet-C (UVC) lamps that are run twice a week. The three biocidal treatments, 70% IPA, 7.5%  $H_2O_2$ , and UVC, light were chosen specifically to have different modes of antimicrobial activity to decrease selection for extremophiles that are resistant to the cleaning treatments<sup>9</sup>.



**Figure 1:** Layout of MOMA-MS Clean rooms. **A:** Aseptic Assembly Clean room is maintained at ISO class 7, with two ISO class 5 flow benches. **B:** Integration Clean room is maintained at ISO class 7, with an ISO class 5 flow bench and ISO class 5 integration tent.

A common disinfectant in biological labs and the pharmaceutical industry is 70% isopropyl or ethyl alcohol with 30% water by volume. IPA and ethyl alcohol have similar biocidal properties, but because IPA is the preferred cleaning solvent in NASA cleanrooms, it will be the focus in this paper. IPA kill microorganisms by causing the organism's proteins to unfold and misfold, a process known as "denaturing". 70% IPA is a superior disinfectant to one hundred percent IPA. 100% IPA will denature proteins, but so quickly that only the outermost proteins in a cell become denatured, creating an impenetrable layer which prevents the alcohol from getting deeper into the cell and causing further damage. Once the pressure of the 100% IPA is removed through evaporation, the undamaged core of the cell is able to produce and replace the damaged outer proteins. On the other hand, 70% IPA is able to penetrate into the cell causing denaturation of surface and interior proteins, destroying the cells ability to produce proteins that could repair the damage thus killing it. 70% IPA also evaporates slower than 100% IPA, increasing the amount of time that the chemical is in contact with a cell and able to affect it<sup>10</sup>. The high volatility of 70% IPA is an advantage in a clean room setting because it does not leave a chemical residue on the treated surfaces. As the MOMA-MS sample path has stringent molecular contamination requirements, it is important not to introduce potential contaminants via cleaning procedures. One drawback of the disinfection properties of 70% IPA is that it is ineffective against bacterial spores<sup>10</sup>, which are the microorganisms most likely to survive the extreme temperatures, pressures, and radiation environments in space and on the surface of another planet. Wiping with 70% IPA will mechanically remove bacterial spores, and may kill vegetative microorganisms that could become spores. In the MOMA-MS aseptic clean room, sterile wipes pre-wet with 70% IPA are used to clean critical work surfaces like the laminar flow hoods every day, and a 70% IPA solution is used for daily mopping on alternating weeks with 7.5%  $H_2O_2$ .

The use of 7.5%  $H_2O_2$  partially addresses the major weakness of 70% IPA in a spacecraft clean room by being effective against bacterial spores<sup>10</sup>.  $H_2O_2$  has been shown effective against microorganisms isolated from spacecraft surfaces<sup>11</sup> and

also inactivates microorganisms via a different mechanism than 70% IPA, which should help prevent the selection of extremophiles.  $\text{H}_2\text{O}_2$  kills bacteria by producing hydroxyl free radicals which will react with a wide range of biomolecules that leads to degradation of the cell wall and an organism's DNA<sup>10,12</sup>. Some microorganisms produce catalase enzymes that will breakdown hydrogen peroxide into water and oxygen, leading to some level of resistance to its biocidal effects, but the concentrations found in cleaning solutions are usually high enough to overcome this resistance<sup>10</sup>. Other concentrations of  $\text{H}_2\text{O}_2$  with or without other additives are available for cleaning and sanitizing, particularly  $\text{H}_2\text{O}_2$  with peracetic acid, which has a higher sporicidal activity than  $\text{H}_2\text{O}_2$  alone, but the solution without additives was selected for MOMA-MS because it would evaporate without leaving a chemical residue.

The third biocidal approach to decreasing bioburden in the aseptic clean room is the use of UVC light, which kills bacteria through a different mechanism from IPA and  $\text{H}_2\text{O}_2$ . UVC is a short wave ultraviolet light, in the wavelength range of 200 to 280 nanometers (nm), with wavelengths around 250 having the most germicidal activity. Unsaturated organic compounds, particularly the nucleic acid thymidine, absorb UVC light and through a photochemical hydration reaction, thymine bases lose the hydrogen bond to the adenine across the DNA helix and end up covalently linked to each other. This thymine dimer interferes with DNA replication, leading to mutations, and with enough mutations, an organism is no longer able to replicate<sup>13</sup>. The dose of UVC, measured in  $\text{mJ}/\text{cm}^2$ , that is required to inactivate specific microorganisms has been established experimentally, and there is a wide range of susceptibility to UVC irradiation from  $<3 \text{ mJ}/\text{cm}^2$  to over  $300 \text{ mJ}/\text{cm}^2$  for highly resistant molds<sup>14</sup>. For the MOMA-MS aseptic clean room, mercury vapor bulbs were installed in the ceiling and in the primary assembly laminar flow bench, along with a safety system that includes a warning light outside the clean room and a motion sensor that automatically turns off the UVC lights if any movement is detected in the room. The intensity of the UVC light was measured at the floor of the lab, and exposure time was adjusted to reach a dose of  $22 \text{ mJ}/\text{cm}^2$ , which should give a two order of magnitude, or 99% reduction of most bacteria and bacterial spores on exposed surfaces. To decrease bioburden by UVC, the lights are run twice a week for 15 minutes. This sanitization step is limited to surfaces in direct line of sight with the UV bulbs, so is not sufficient as a sole form of sanitization.

### **2.3 Integration clean room and testing chamber**

The integration clean room (Figure 1.B) and testing chamber/tent are not regularly maintained at aseptic levels, but the cleaning methods can be implemented as necessary. The aseptic workspace implementation is only required when the mass spectrometer sample path is exposed after terminal sterilization by DHMR, or if a volume that had been verified for PP cleanliness before final closeout that to be reopened. In the non-aseptic clean rooms, the floor is mopped with 5% IPA, and horizontal surfaces are wiped with 100% IPA twice a week. The garments used in the integration clean room are standard coveralls, hoods, boots, with disposable facemasks and gloves, and are changed twice a week. The testing chamber and tent is not in regular use, however it is cleaned before use, and follows a cleaning and garment schedule similar to the integration clean room.

## **3. PLANETARY PROTECTION LAB CAPABILITIES**

### **3.1 Lab establishment**

To support the stringent planetary protection requirements for MOMA-MS, a planetary protection microbiology lab has been established at GSFC. The lab needs to be able to support standardized bioburden assay protocols provided by ESA<sup>15</sup> and NASA<sup>16</sup>, though because MOMA-MS is part of an ESA mission, the focus has been on ESA protocols. The initial lab setup started in July 2014, and the first MOMA-MS hardware samples, from the MOMA-MS Structural Thermal Model, were processed in November 2014. Initial laboratory capabilities include microbial growth assays, sterilization verification, and rapid bioburden analysis by ATP detection.

### **3.2 Microbial growth assays**

The first protocol established in the laboratory was the standard ESA swab assay<sup>15</sup>, which uses flocked nylon swabs to sample up to  $25 \text{ cm}^2$  of surface area. The swabs are transported in 2.5 ml of water, and the microorganisms picked up by the swab are extracted by agitation on a vortex mixer and in an ultrasonic water bath. If the assay is quantitating spore

forming microorganisms, the extracted solution is then treated by heating to 80°C for 15 minutes, called a “heat shock”. The heat shock kills off microorganisms that are in a vegetative state, which means they are actively growing, while the inactive bacterial spores survive. A sample that is not heat shocked will grow both vegetative microorganisms and spores. The extracted solution is transferred onto four petri dishes filled with nutrient agar, with 500 µl of sample spread onto each dish. The petri dishes are incubated for 72 hours and the total number of colony forming units observed is used to calculate the original density of bioburden on the sampled surface.

Another way to monitor for viable organisms in the environment is air sampling. Air sampling can be performed via passive or active methods. Passive sampling involves the use of a settling plate which is an agar petri dish is set out and exposed to air for a defined period of time. Rather than directly measuring the density of viable microorganisms in the air, passive sampling measures the fallout rate of microorganisms onto a surface<sup>17</sup>. In MOMA-MS clean rooms, passive sampling is not used due to concerns about off-gassing from the nutrients in the agar petri dishes being a potential source of molecular contamination. Instead, active air sampling is used. For active sampling, a measured volume of air is pulled through a gelatin filter, which captures bacteria from the air. The filter is then transferred to an agar petri dish. The gelatin filter dissolves into the agar, and any captured microorganisms grow on the surface of the agar. The filter can also be dissolved in water, and processed through the heat shock protocol to select for spores, but thus far, only viable monitoring of airborne samples has been used for the MOMA project.

### **3.3 Sterilization verification**

Another type of biological assay supported by the GSFC PP lab is sterilization verification. The two most common forms of sterilization for the MOMA-MS are DHMR and autoclave sterilization. DHMR is used to sterilize flight hardware as well as select ground support equipment (GSE) that comes in contact with flight hardware. Autoclave sterilization is used primarily to sterilize PP lab equipment and a subset of GSE tools that are compatible. A biological verification system involves including a measured amount of resistant microorganisms into a sterilization cycle, generally in a contained strip or solution, and verifying by growth analysis whether any of the microorganisms survived the sterilization. Products used in sterilization verification need to meet ISO-11138 standards<sup>18</sup>. Both dry heat and autoclave biological sterilization verification supplies are stocked in the GSFC PP lab, but only the autoclave verification is used regularly due to the risk of including any additional materials that could introduce molecular or particulate contamination into a DHMR cycle with flight hardware.

### **3.4 Rapid bioburden analysis**

The gold standard of detecting bioburden for planetary protection is to verify the presence of viable microorganisms by direct observation of growth. The major drawbacks of measuring bioburden by growth is that a colony growth test is only able to grow roughly 1% of the bacterial or fungal cells that are in a sample. A viable organism might not grow in a laboratory setting due to the lack of unknown nutrients, wrong incubation temperature, not enough humidity, or other environmental factors that have not been fully characterized or cannot be replicated in laboratory settings<sup>19</sup>. Molecular assays can detect microorganisms that will not grow in laboratory conditions, but are not directly quantitative for viable (alive and able to replicate) microorganisms.

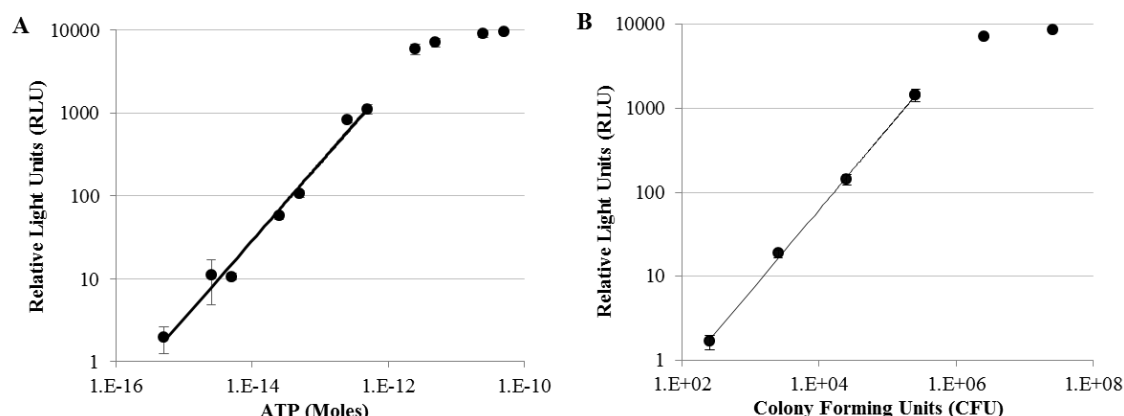
One molecular method for verifying bioburden cleanliness that is used by the MOMA-MS project is a variation of the NASA approved assay that quantitates the total adenosine triphosphate (T-ATP) in a sample<sup>16</sup>. The T-ATP assay is used to pre-screen hardware and to gain an overall idea of the level of bioburden. Results from this method can be obtained within minutes, unlike a typical swab and plating assay which takes days. The T-ATP process is centered on the same chemical reaction that causes a firefly to glow. A firefly’s glow is produced when the chemical luciferin reacts with ATP, in the presence of the enzyme luciferase, to yield light<sup>20</sup>.

The NASA approved assay method uses a sterile polyester swab to sample a surface, then the bioburden is extracted from the swab and processed using an ATP Releasing Agent, and a Luciferin-Luciferase Reagent. The sample is analyzed using a Kikkoman™ Lumitester™. Unfortunately, the specified equipment and reagents are no longer made, thus an alternate apparatus and supplies are being used. The current assay that is in implementation for the MOMA-MS uses the Hygiena SuperSnap™ swab kits along with a Hygiena Ensure luminometer. SuperSnap swabs greatly simplify sample processing relative to the NASA approved assay, as the swab already has the Luciferin-Luciferase reagent within an attached bulb. Sampling and analysis of a surface can take less than five minutes. To sample a surface, the pre-wet

swab is removed from the swab tube and used to sample a surface area. To process the sample, the snap valve in the bulb is broken and the liquid in the bulb is expelled into the tube with the swab. The swab tube is shaken for 5-6 seconds and placed into the Luminometer. Analysis of the sample takes roughly 15 seconds and the results are displayed in relative light units (RLU).

The Hygiene Ensure Luminometer and SuperSnap swab system allows for detection down to 0.1 femtomoles of ATP, which is comparable to the NASA approved method using the Kikkoman Lumitester<sup>16</sup>. The ATP system will detect most microorganisms and even dead cells, as long as there is still ATP present within them<sup>21</sup>. However, the ATP system does not detect bacterial spores. Spores tend to be relatively dormant by nature, and as a result they do not contain a sufficient amount of ATP to be detected by the ATP system<sup>22</sup>. Another disadvantage of the commercial SuperSnap swabs is that they are pre-wetted with a solution containing chlorhexidine digluconate that leaves a residue which makes it incompatible for use on hardware that is sensitive to molecular contaminations.

The unit “RLU” is dependent on the sensitivity of the system and does not directly represent the amount of ATP or the number of microorganisms, a calibration curve for ATP to RLU as well as Colony Forming Units (CFU) to RLU was established. These correlations were made by pipetting specified concentrations of ATP or actively growing *Bacillus atrophaeus* onto the SuperSnap swabs and analyzing them using the Hygiene Luminometer. The system exhibits a power relationship between ATP and RLU that remains linear to around 7000 RLU, and then forms an asymptote to a maximum reading of 9999 as the instrument detector saturate as shown in Figure 2.A. The relationship between CFU and RLU is also in the form of a power curve, as shown in Figure 2.B. The direct relationship between CFU and RLU is limited to a species and metabolic state of microorganisms. Any variations of species, metabolic state, and size can lead to different amounts of ATP per microorganism. This variability limits the usefulness of ATP detection as a direct correlate to the number of organisms, but it can be used to generally determine the overall biological cleanliness of a surface.



**Figure 2:** Calibration curves for the Hygiene EnSure luminometer with Supersnap swabs against an ATP standard (A) and laboratory grown *Bacillus atrophaeus* (B).

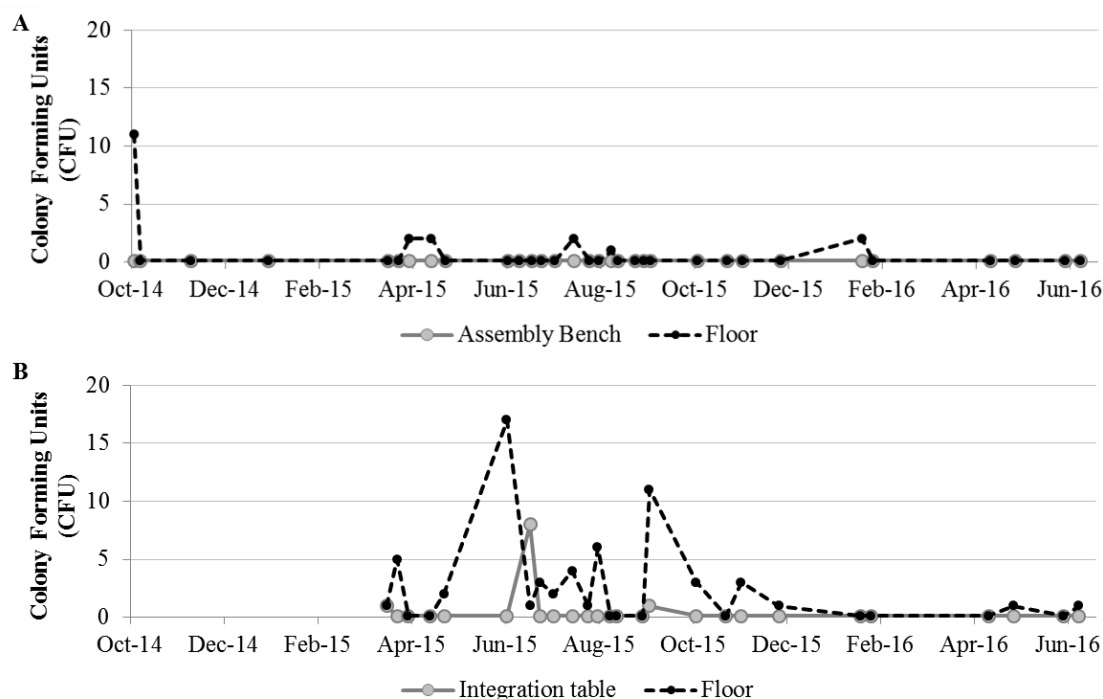
## 4. CLEAN ROOM BIOBURDEN MONITORING

### 4.1 Clean room monitoring methods

To ensure that the cleanliness of the MOMA-MS clean rooms is maintained, and that the biocidal cleaning methods in the aseptic assembly clean room are effective, the rooms are being monitored by three types of bioassay. Surfaces are monitored for colony forming units using the viable colony forming units assay and rapid bioburden analysis for levels of ATP contamination. Airborne microorganisms are monitored by drawing 1m<sup>3</sup> of air through a filter which is transferred to a petri dish to monitor growth. Due to the very low particulate level in clean room air, almost no colonies were observed, and the data is not presented here.

## 4.2 Clean room culturable surface microorganisms

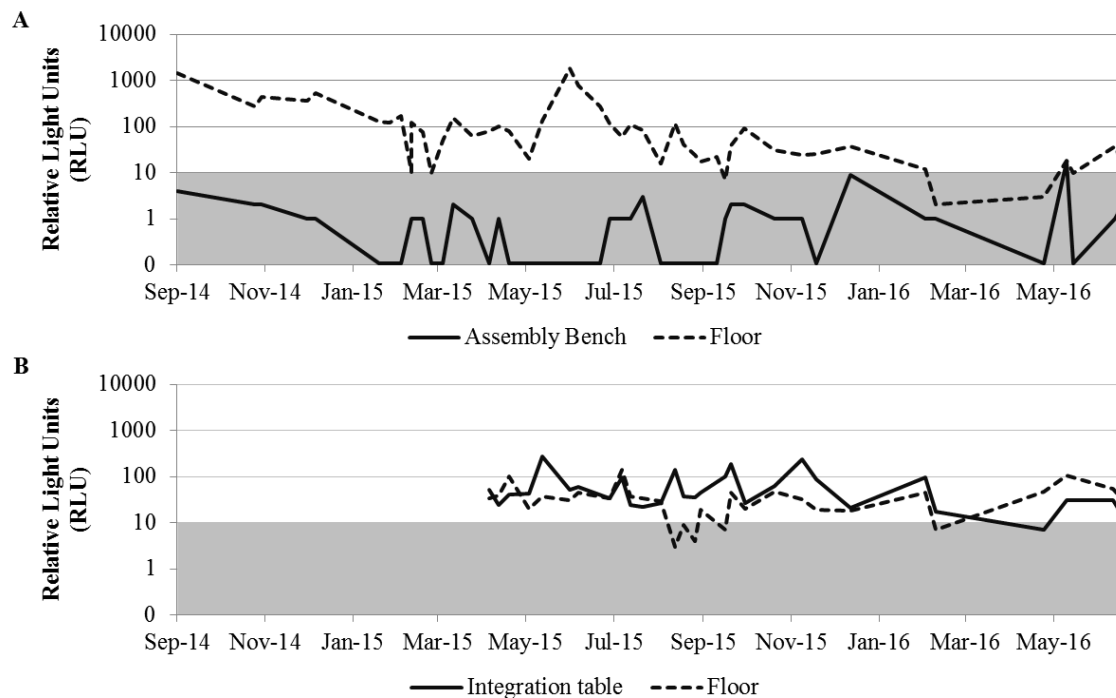
To monitor for culturable microorganisms in the clean room, samples were processed for vegetative microbes without the heat shock step, which should result in more CFU than the spore specific protocol, as both spore and non-spore bacteria can grow. Flight hardware bioburden monitoring and verification samples, which are not reported in this study, undergo the additional heat shock step to select for the spores that are most likely to survive in outer space. A more conservative accounting for clean room microorganisms is to count not only the hardy spores, but also the actively growing microbes that could become spores. Over the course of 20 months of monitoring the aseptic clean room, no viable microbes have been detected in the assembly bench samples, and very few microbes have been detected from the floor (Figure 3.A). This does not prove that the assembly bench is a sterile environment, as the sample area is small, and many environmental microbes cannot be grown in laboratory settings<sup>19</sup>, but it is an indicator of overall low surface bioburden. By comparison, higher levels of microbes are found on both the floor and the integration table in the integration clean room (Figure 3.B), which does not undergo the additional bioburden reducing cleaning steps but has the same ISO clean room classification of ISO class 7 for the room and ISO class 5 for the main work surfaces.



**Figure 3:** Viable bioburden expressed as colony forming units per sampled area. Each sample covered 25cm<sup>2</sup> of the indicated surface. **A:** Aseptic assembly clean room with ISO class 5 assembly bench and clean room floor (ISO class 7). **B:** Integration and Test clean room, not aseptically maintained with ISO class 5 Integration table and clean room floor (ISO class 7).

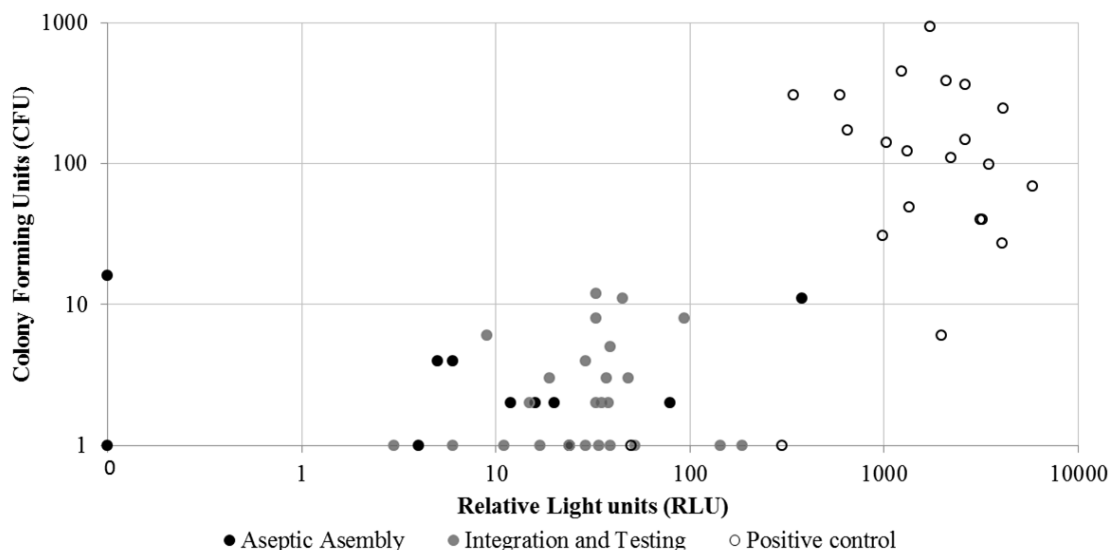
### 4.3 Clean room rapid ATP bioassay

The rapid bioburden assay monitoring of the clean rooms also showed a clear difference in the workspaces between the aseptic assembly clean room and the integration clean room. The ATP bioassay is not approved by NASA or ESA for planetary protection verification, so there is not a standard pass/fail requirement for ATP levels. The manufacturer default for the Ensure Luminometer for a passing cleanliness is 10 RLU for the food and beverage industry<sup>23</sup>. In Figure 4.A, the graph shows that the assembly bench in the aseptic assembly clean room stayed at 10 RLU or below with the exception of one measurement in May 2016. This higher reading was traced to specific activities at the assembly bench, which was then re-cleaned, and the next day the levels of ATP detected had returned to 0 RLU. By comparison, the Integration and testing clean room ATP bioassay monitoring data shown in Figure 4.B, both the floor and primary work surface at the integration table consistently return values above 10 RLU. This data serves as a clear demonstration that the ATP surface test is more sensitive at detecting biological contamination than growth assays, but the limitations of microbe quantitation remains. As the project moves toward and past final instrument level DHMR, bioburden reducing cleaning steps and sterile gowning practices will be instituted in the integration clean room, and it is expected that the level of bioburden measured by both viable CFU and ATP RLU will decrease.



**Figure 4:** Clean room monitoring with rapid bioburden assay expressed as relative light units (RLU) per sampled area. Grey shaded region indicates “pass” bioburden levels according to manufacturer’s specifications. Each sample covered 25cm<sup>2</sup> of the indicated surface. **A:** Aseptic assembly clean room with ISO 5 assembly bench and clean room floor (ISO 7). **B:** Integration and test clean room, not aseptically maintained with ISO 5 integration table and clean room floor.





**Figure 5:** Correlation between colony growth (CFU) and detected ATP (RLU) for all samples with countable colonies. Samples with no colonies or too many colonies to count are excluded from the graph.

#### 4.4 Correlation between culturable microorganisms and ATP levels

While Figure 2.B shows a close correlation between the amount of bacterial growth (CFU) and levels of ATP (RLU) detected in a controlled laboratory experiment, environmental ATP readings do not closely correlate with the number of detected colonies, as shown in Figure 5. The ATP detected on a surface could come from multiple sources in addition to the culturable microorganisms, such as shed human cells, non-viable (dead or dying) microbial cells, and non-cultivable microorganisms<sup>19,21</sup>. Of a total of 316 environmental surface sample sets (ATP and CFU on a surface), only 59 samples were positive for any colony growth, and only 36 of these were from the clean rooms. Due to the high number of samples with no colony growth over a wide range of RLU, the data does not allow direct prediction of how many colonies might be expected based on ATP level. To address the lack of linearity, and provide a tool to decrease risk for the project, the ATP data has been binned in Table 1 to indicate the likelihood of a surface with ATP readings in a given range to result in positive colony forming units with the three day growth assay. Using the manufacturer provided “pass” criteria of 10 RLU or less, less than 5% of the samples had any growth. When the ATP readings are above 500 RLU, 95% of the samples were positive. The intermediate range from 10-500 RLU have about a 25% likelihood of colony formation. Using this binned data, the project can confidently proceed with further integration steps when the ATP readings are low without waiting for three to four days for the colony growth data.

**Table 1:** Percentage of samples that are positive for any colony forming units by range of ATP relative light units.

RLU Range	Total Samples	Samples with CFU	Percent Positive
0-10	170	8	4.71
6-100	106	27	25.47
101-500	20	5	25.00
501-1000	4	3	75.00
1000-5000	16	16	100.00

## 5. CONCLUSION

Building a life detection instrument for a planetary mission presents the scientists and engineers at GSFC with a set of challenges that has not been asked of a NASA team since the mid-70s with the Viking missions. Meeting these challenges requires a multifaceted approach. New mitigations to decrease bioburden with a stringent cleaning schedule were added to existing cleanrooms to provide an aseptic workspace. To verify that these mitigating steps produce a biologically clean work space and instrument, a planetary protection lab was established as a new capability at GSFC. The bioburden monitoring of aseptic and non-aseptic cleanrooms demonstrated that cleanrooms were the additional bioburden reducing cleaning schedule had lower levels of surface bioburden. To expedite the process of bioburden sampling, the rapid bioburden analysis by ATP was explored to establish risk categories that will allow the project to proceed with final integration steps without waiting for the three day colony growth tests.

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