Aseptic operations for post DHMR processing of MOMA Mass Spectrometer

Erin Lalime, SGT/KBR Wyle, GSFC,
Radford Perry, NASA, GSFC,
John Canham, Northrop Grumman, GSFC

Abstract

Mars Organic Molecule Analyzer – Mass Spectrometer (MOMA-MS) is an instrument in the MOMA instrument suite for the European Space Agency (ESA) ExoMars 2020 Rover. The rover is Planetary Protection Mission Category IVb, the first IVb mission since the Viking missions in the 1970s. Within the sample path of the MOMA instrument suite, hardware surfaces of the must be sanitized to a level of <0.03 spore/m^2. To meet this requirement, the MS sample path was subjected to Dry Heat Microbial Reduction (DHMR) to decrease the number of viable spores by 4 orders of magnitude from a measured 88 spores/m^2 to 0.009 spores/m^2. Before DHMR, the hardware is handled using standard cleanroom practices. After DHMR, planetary protection filters protect the sample path for most of integration, but when sample path exposure is required, aseptic operations are instituted and exposure times are kept to an absolute minimum. The surface area of exposure is also taken into account to determine safe exposure times. Before work begins, the ISO class 5 aseptic workspace is cleaned and tested for surface and airborne bioburden, and all tools that will contact or be used near sample path surfaces are sterilized. During the exposure activity, sterile garments are worn, sterile gloves are changed as often as necessary, and the environment is monitored with active and passive fallout for bioburden and real time airborne particle counts. Sterile tools are handled by a two person team so that the operator touches only the tool and not the exterior surfaces of the sterilization pouch, and a sterile operating field is established as a safe place to organize tools or parts during the aseptic operations. In cases where aseptic operations are not feasible, localized DHMR is used after exposure. Any breach in the planetary protection cleanliness would necessitate repeating instrument level DHMR, which not only has significant cost and schedule implications, it also becomes a risk to hardware that is not rated for repeated long exposures to high temperatures.

ExoMars 2020

Mars Organic Molecule Analyzer- Mass Spectrometer

ExoMars 2020 is a European Space Agency Mars Rover to be launched with Roscosmos in 2020. One of the instrument suites on ExoMars 2020 is the Mars Organic Molecule Analyzer (MOMA). MOMA is a life detection instrument that is capable of analyzing a wide range of organic molecules by laser desorption mass spectrometry and gas chromatograph-mass spectrometer. The MOMA- Mass Spectrometer (MS) is a linear ion trap MS, built by NASA at Goddard Space Flight Center.

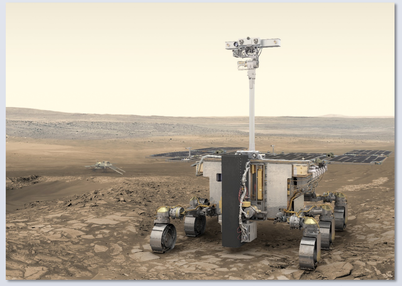
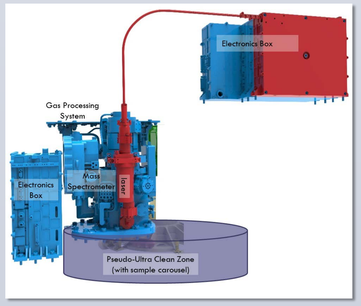


Image: European Space Agency (ESA)

In order to meet Planetary Protection (PP) requirements for a Mars life detection instrument, the surfaces of the MOMA sample path, must have <0.03 heat resistant spores/m^2. To reach this, the MS, associated plumbing, and pseudo-ultra clean zone (pUCZ) was baked for 60 hours at 110°C to achieve a 4 order of magnitude reduction. From the measured 88 spores/m^2 before DHMR, this achieved a bioburden density of 0.0088 spores/m^2. The non-sample path (external) surfaces of the hardware must meet <1000 spores/m^2.



All parts of the MOMA-MS sample path are compatible with DHMR, but components of MOMA, particularly the electronics boxes and laser are not compatible. Because of this, DHMR was conducted only on the compatible components prior to the main integration and test campaign. This leads to unavoidable exposure of the sample path at later steps of integration. In order to avoid needing to repeat DHMR or re-contaminating the microbially reduced surfaces, the sample path was only opened in an aseptic ISO 5 environment.

Preparation and aseptic verification

The cleanrooms used by the MOMA-MS project are maintained clean and monitored for bioburden, but not at levels sufficient for aseptic operations. Prior to aseptic operations, the room is cleaned thoroughly with 70% isopropanol and 7% hydrogen peroxide. When possible, ultra-violet C lamps are also used. The hardware is wiped with 70% isopropanol, and where feasible, a sterile bag or drape is used to isolate the non-sterile exterior of the hardware from the area to be exposed.

Multiple locations around the work area and on the hardware are sampled for bioburden using both ATP (adenosine triphosphate) rapid bioassay in Relative Light Units (RLU) and the ECSS swab bioassay for colony forming units (CFU). Any high ATP readings require immediate re-cleaning before swab bioassays.

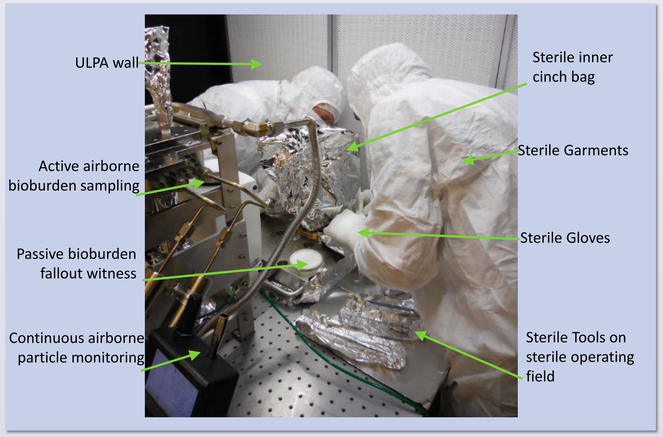
After cleaning and bioassay, the cleanroom is closed to all entry for 72 hours until the results from swab bioassays are finalized.

All tools used during an aseptic operation are cleaned and sterilized. Tools such as torque wrenches that cannot be sterilized are cleaned and wrapped in sterile foil before being handled, and the sockets that interact directly with the hardware are sterile.

Cleanroom monitoring ATP to CFU bins

RLU Range	# Samples	# w/ CFU	% Positive
0-5	146	5	3.42
6-100	130	30	23.08
101-500	20	5	25.00
501-1000	4	3	75.00
1000-5000	16	16	100.00

Sample path exposure activities



During aseptic activities, sterile garments, sterile gloves, and sterile tools are used in the aseptic ISO 5 environment. Because the hardware surfaces outside of the sample path are not sterile, double gloves are used, and are changed any time contact with non sterile surfaces is required. Each exposure is kept to an absolute minimum, and is timed to ensure that the likelihood of viable particle falling into the exposed sample path is low.

Environment monitoring during sample path exposure

In addition to sampling the aseptic work area 3 days before any aseptic activities, the immediate environment was monitored for airborne particles and bioburden during the aseptic activity.

Particle levels in the air were continuously monitored using a laser particle counter, with an audible alarm if the particle levels exceeded ISO class 5. If the particle count exceeded ISO class 5, all activity would be stopped and personnel would step away from the hardware, and wait for particle count to come back down. During the aseptic activities at GSFC, particle levels never exceeded ISO class 5 while the sample path was exposed.

Single colony of Staphylococcus epidermidis detected during biomonitoring of an aseptic operation

Active airborne microbe sampling occurred from before the sample path was opened until after the sample path was closed. One cubic meter of air is pulled through a gelatin filter which captures bacteria. The filter is later transferred to a pre-poured R2A plate for incubation. Passive microbe fallout was monitored using the same gelatin filters exposed during the duration of sample path exposure.

Risk analysis before aseptic activities

Both NASA and ESA PP standards require that any operations involving sterile hardware occur in an aseptic environment, but do not specify the amount of time that hardware can be exposed during an aseptic operation.

Acceptable exposure time was determined by calculating the probability of a viable microorganism falling into the exposed sample path. The probability was calculated using the surface area of the sample path, established fall out rates of various particle sizes in an ISO class 5 environment, and room monitoring data from active air sampling for microorganisms. The active air sampling trend data allowed an approximation of the CFU/particle in MOMA clean rooms. The probability of contamination has to remain below the bioburden requirement (0.03 CFU/m^2) multiplied by the exposed sample path surface area.

Calculated acceptable exposure

	Surface area (m^2)	Acceptable Probability (CFU)	Time (min)
Example	2	0.06	0.02
Base of MS	0.0212	6E-4	3
Plumbing	1.4e-5	4E-7	2000
Manifold 1	1e-4	3E-6	200

Cumulative sample path exposure during aseptic operations

Operation	Date	Exposed sample path	Total Exposure time
P0/P5 plumbing connections	9/19/17	0.28 cm^2	1 minute
MS from pUCZ to Vibe Plate	9/29/17	106 cm^2	17 seconds
R6G Sample addition	10/23/17	106 cm^2	36 seconds
MS from Vibe Plate to pUCZ	10/27/17	106 cm^2	18 seconds
P0/P5 plumbing connections	10/27/17	0.28 cm^2	4 minutes 45 seconds
Manifold 1 Swap	12/21/17	1 cm^2	25 minutes*
GC installation	12/27/17	0.14 cm^2	1min 21 seconds

* Cumulative exposure time for 8 plumbing locations, resulting in 16 sites that were opened, capped, uncapped, and remated.

Risk analysis after aseptic activity

Parameters	Expected	Actual	
Settling Rate (0.5 µm) =	8.80E-05	8.80E-05	m/s
ISO Class	5	4	
Particles (0.3µm)#/m3	10176	1018	particles /m^2
Particles/cfu	1.76E+04	2.04E+02	particles/cfu
Active Sample Volume	0.2	0.2	m^3
Particles in sampled volume	2035	204	particles
Viable particles in sampled volume	0.116	1	viable particles
Exposure Time	30	17	Seconds
Critical Surface Area	0.0106	0.0106	m^2
Particles in settled volume	2.85E-1	1.61E-2	particles
Viable fallout	1.62E-5	7.91E-05	viable particles
Acceptable limit	6E-4	6E-4	viable particles

In six of the seven aseptic operations, no colonies were detected on the active or passive monitors. In one operation, a single colony was detected from the active air sampling plate.

During this operation the particle counts stayed in the ISO class 4 range, 2 fallout witnesses were exposed for 7 minutes (both clean) and the active sampler sampled for 4 minutes (0.2 m^3) (1 colony). The sample path was open for 17 seconds during this time.

To determine the risk to the sample path a similar analysis to the pre-aseptic activity exposure time was conducted. In the initial analysis, a 30 second exposure in ISO class 5 conditions held a risk of 1.62E-5 for a viable microorganism to enter the sample path. In the actual operation where the colony was detected, the risk increased to 7.91E-05, which is still well below the acceptable limit, indicating that it is unlikely that the planetary protection levels of the sample path were compromised.

Conclusion

Exposure of sample path surfaces after terminal DHMR is a high risk activity that can be managed by working in aseptic ISO class 5 conditions, and limiting the amount of time that the sample path is exposed. The allowable exposure time can be calculated using established particle fallout rates, and approximations for the percentage of particles likely to be viable. Each aseptic event is closely monitored with real time particle counting along with active and passive bioburden monitoring. In case of detection of bioburden during an aseptic operation. Further risk analysis is conducted to determine the likelihood of compromising the planetary protection requirements. The analysis here demonstrates that the recorded exposure times maintained planetary protection requirements for all aseptic activities even when a colony was detected on an active witness plate.

References

NASA-HDBK-6022 (NASA), ECSS-Q-ST-70-58C (ESA), ECSS-Q-ST-70-55C (ESA), ISO 14644

Radford L. Perry, "A dynamic approach to monitoring particle fallout in a cleanroom environment", Proceedings of SPIE Vol. 7794, 77940K (2010) SPIE Digital Library