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

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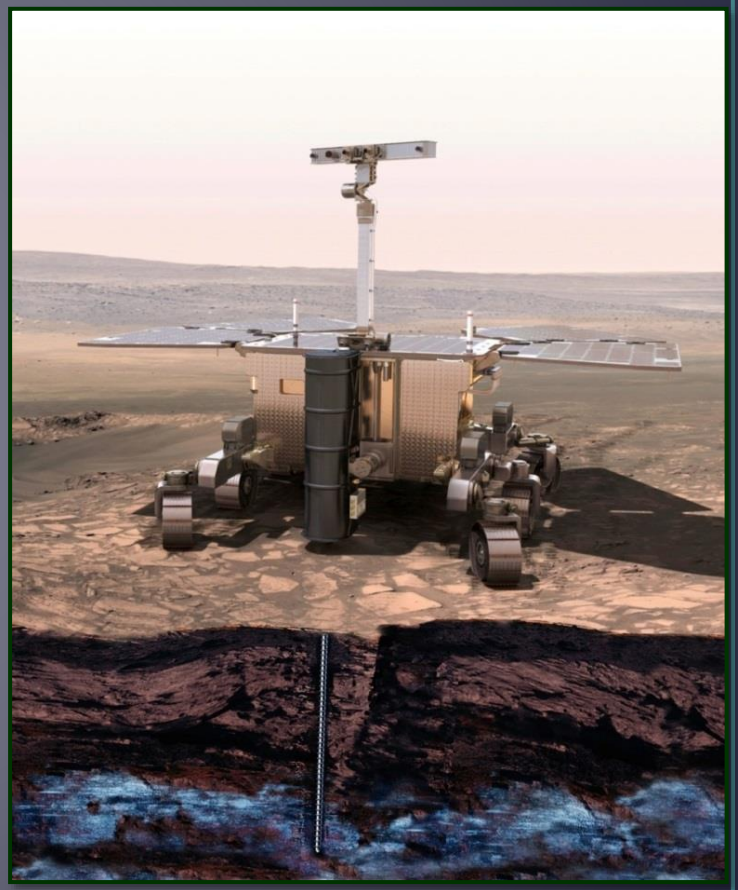
Exomars 2020 & Mars Organic Molecule Analyzer (MOMA):

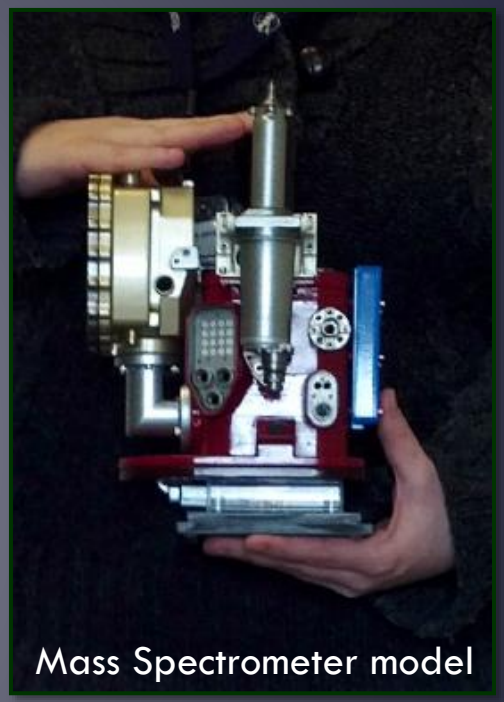
- Exomars 2020- an ESA lander and rover:
  - Scheduled Launch Date: July 2020
  - **Life detection mission**
  - Samples will be collected up to 2m below the surface by a drill
- Mars Organic Molecule Analyzer (MOMA) is an instrument suite on rover
  - **Mass Spectrometer (MS) – NASA/GSFC**
  - Sample Ovens – MPS
  - Gas Chromatograph (GC) – LISA and LATMOS
  - Laser Desorption (LD) - LZH



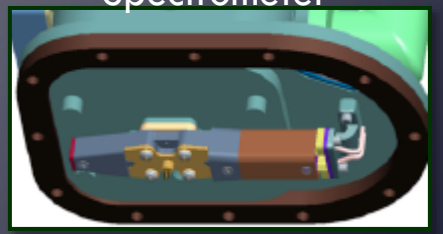
The ExoMars rover. *Credit: ESA*

MOMA Hardware bioburden requirements

- Sample path (Ultra Clean Zone): **<0.03 spores/m^2**
  - Accessible areas:
    - Base of MS
    - Internal surface of pseudo-Ultra Clean Zone (pUCZ)
  - Inaccessible areas:
    - Internal surfaces of Mass Spectrometer (MS)
    - Internal surfaces of Wide Range Pump (WRP)
    - Internal surfaces of Gas Processing System (GPS)
- Surfaces not in contact with sample path: **300-1000 spores/m^2**
  - Exterior of MS, pUCZ, WRP, GPS,
  - Internal and external surfaces of electronics boxes


Mass Spectrometer model

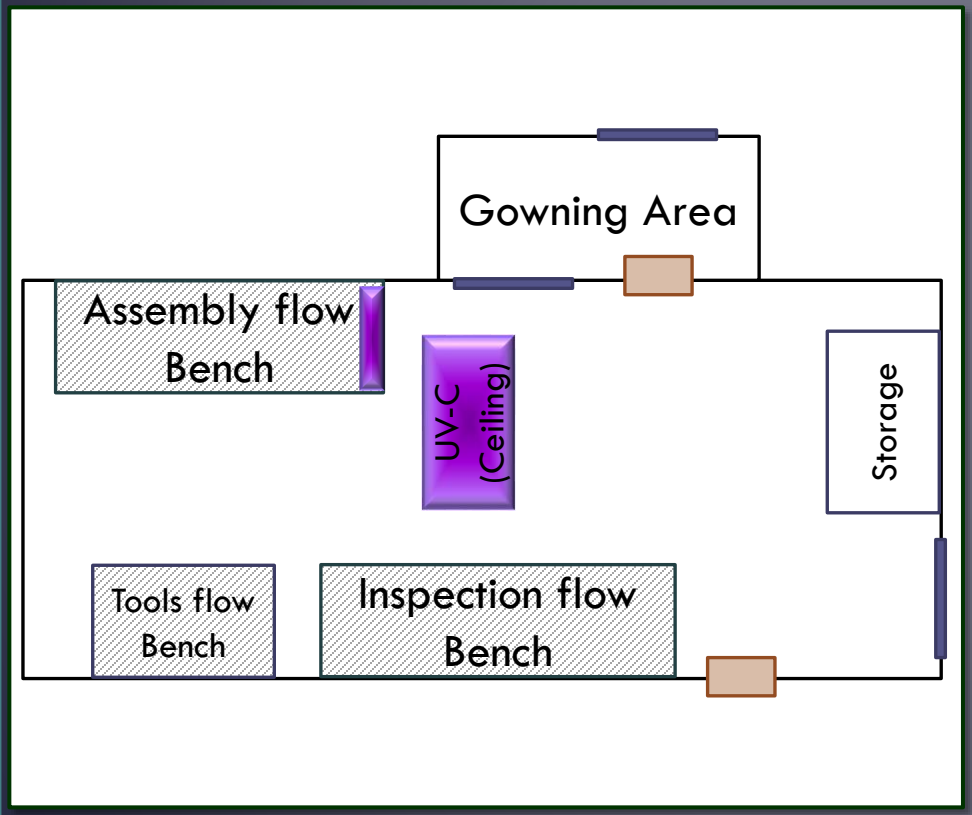
Base of Mass Spectrometer



Establishing clean working space and handling for MOMA-MS

- Three cleanrooms used during build, integration, and testing
  - Aseptic Assembly Cleanroom:
    - Smallest cleanroom
    - Highest and continual bioburden control
  - Integration and Test Cleanroom:
    - Largest MOMA cleanroom, additional ULPA filter tent for sensitive integration steps
    - Bioburden control to be added as needed
  - Vacuum chamber with clean tent: and Mars environment testing:
    - Custom vacuum chamber for Mars environmental testing
    - Bioburden control to be added as needed

Aseptic Assembly Cleanroom

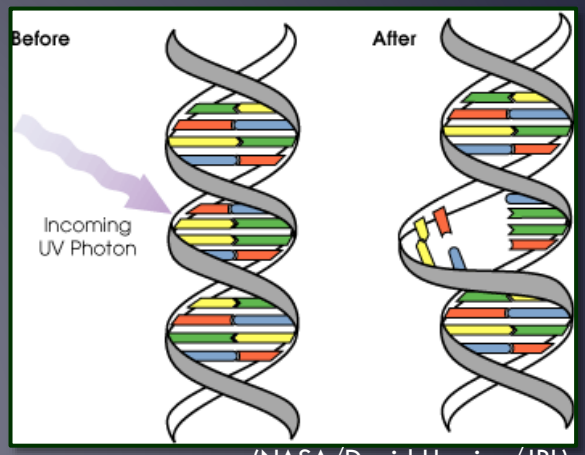


- Certified ISO class 7
- Maintains close to ISO 5

- Daily
  - Mop with weekly alternations between 70% IPA and 7.5% H_2O_2
  - Wipe critical surfaces with sterile 70% IPA
- Twice a week:
  - Wipe horizontal surfaces with 100% IPA
  - Replace all garments
  - Run UV-C lamps

Ultraviolet Light treatment of MOMA assembly cleanroom

- Ultraviolet-C (UV-C 100-290nm), 250-260nm is germicidal
  - Kills by crosslinking DNA, which prevents the organisms from faithfully replicating its DNA
- 22,000 μWs/cm^2 is a sufficient energy dose to kill 99% of most common bacteria and bacterial spores on an exposed surface
- UV-C lamps (253nm) installed in cleanroom ceiling and on wall of assembly clean bench
- UV-C intensity at the floor of the cleanroom was measured at 30 μW/cm^2, 15 min exposure to reach 22,000 μWs/cm^2

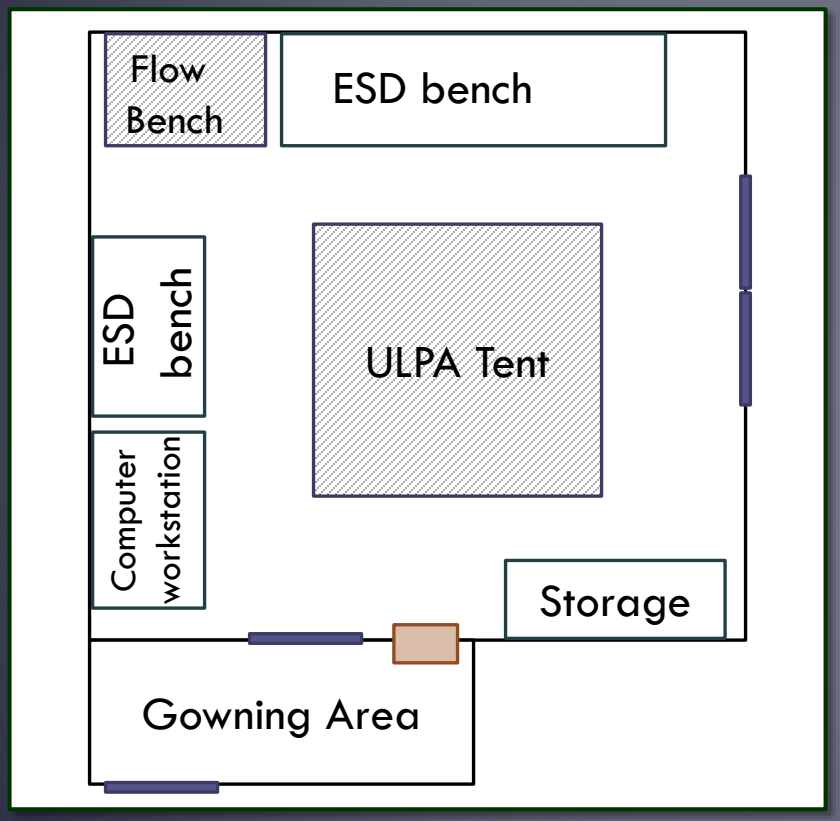


(NASA/David Herring/JPL)

Biocidal mopping

- Cleanroom mopped daily (M-F) with either 70% IPA or 7.5% H_2O_2
  - Alternate between IPA and H_2O_2 weekly
  - Selected for biocidal action without leaving a residue
- Different biocidal mechanisms to prevent selecting for resistant organisms
  - 70% IPA denatures proteins
    - 70% IPA is a more effective biocide than 100% IPA
  - 7.5% H_2O_2 disinfects by oxygen radical damage to DNA and proteins

Integration and Testing Cleanroom



- Daily:
  - Vacuum
- Twice a week:
  - Mop with 5% IPA
  - Wipe horizontal surfaces with 100% IPA
  - Replace all garments
- Bioburden control to be instituted as necessary:
  - During sample path exposure post DHMR

- Room certified ISO Class 7
- ULPA tent: ISO Class 4 >99% of the time.

Bioburden Monitoring of Cleanrooms and Hardware

- MOMA Planetary Protection Lab
  - New capability at Goddard Space Flight Center to support MOMA-MS
  - On-site planetary protection assay support allows closer monitoring and faster results
- Lab Development
  - Initial lab setup from July 2014, first MOMA-MS hardware samples processed November 2014
  - "***All operations involving the manipulation of sterile items and sample processing shall be performed in laminar flow environments meeting at least Class 100 air cleanliness requirements***" -NASA-HDBK-6022
  - Biological safety Cabinet class II type A2
    - Meets ISO Class 5/ Class 100 conditions
    - Provides both product and personnel protection
    - 70% air recirculation, HEPA filtration for cabinet and exhaust

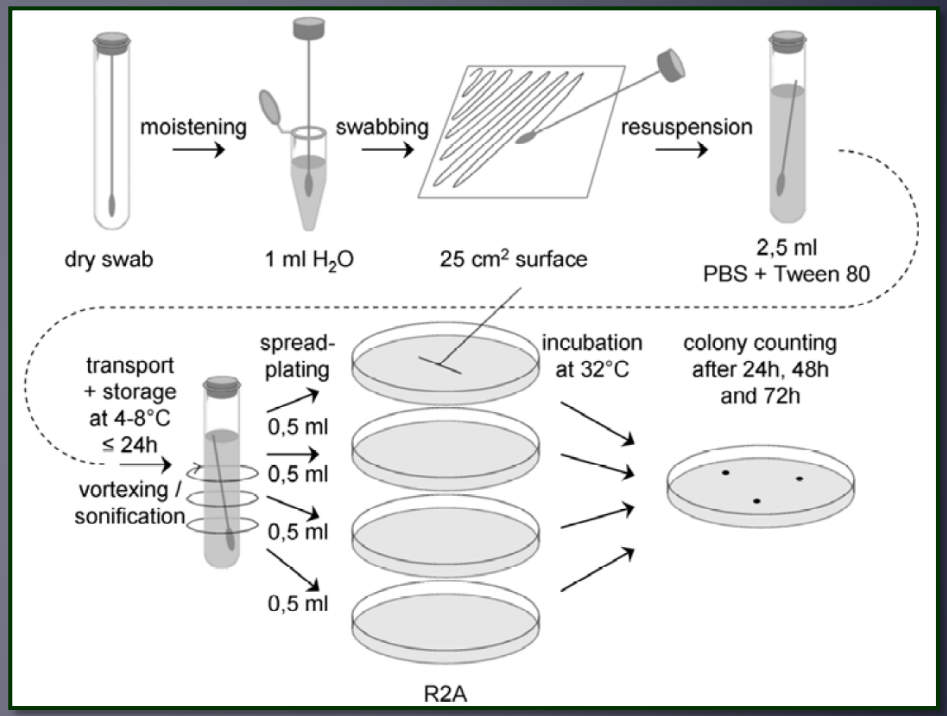
(http://www.labconco.com/)

MOMA Planetary Protection Lab

- Planetary Protection functionalities:
  - Rapid assay (ATP) (5min)
  - Testing airborne microbes (4 days)
  - Standard swab assay (4 days)
  - Active air sampling (4 days)
  - Autoclave sterility verification (2 days)
- Short term capacity expansion:
  - Wipe assay for larger surface areas
  - DHMR (Dry Heat Microbial Reduction) verification
  - Biodiversity testing

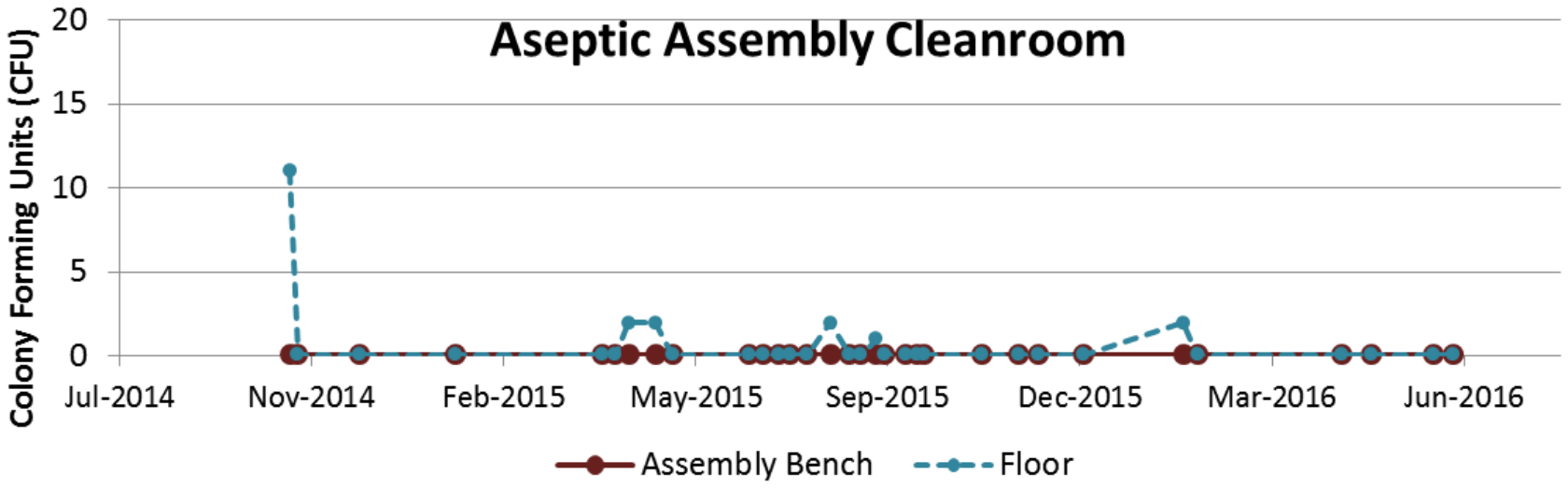
Facility bioburden monitoring

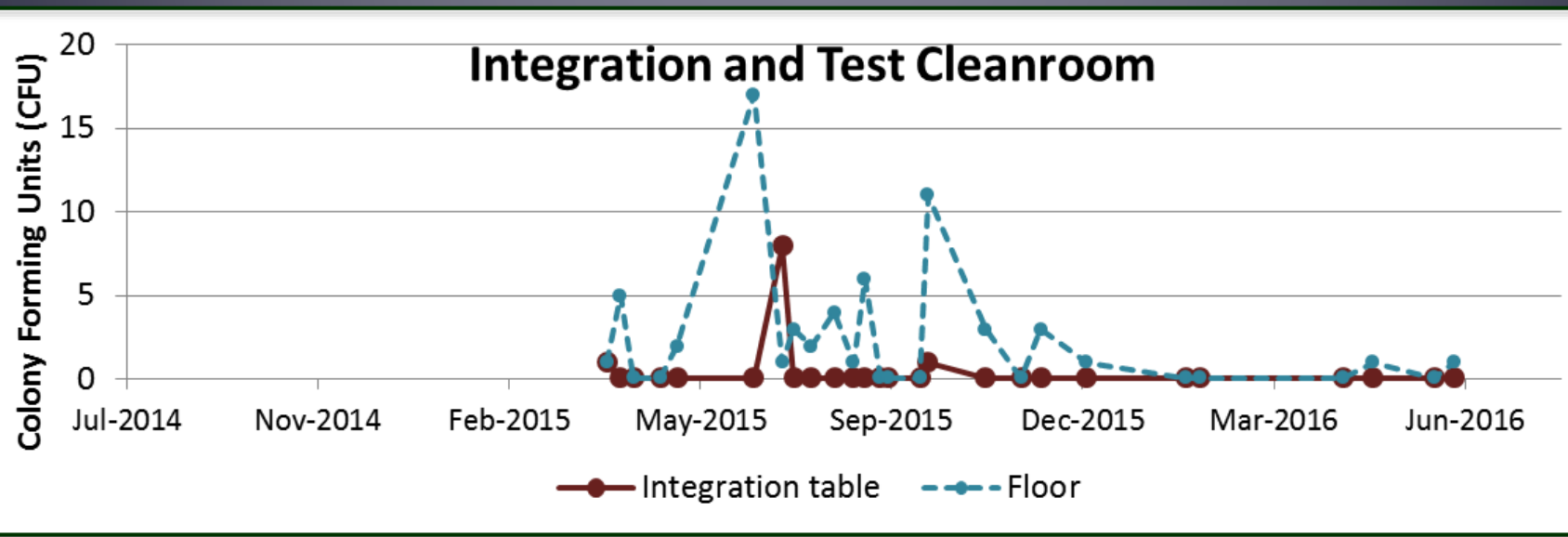
- Bioburden swabs in assembly and I&T cleanrooms
  - General viable microbe screen (not spore specific)
  - Swab a 25cm^2 area on work surface with a damp flocked nylon swab
  - Sample transported in 2.5ml sterile water
  - Processed by ESA protocol: ECSS-Q-ST-70-55C



(ECSS-Q-ST-70-55C, D.2.1)

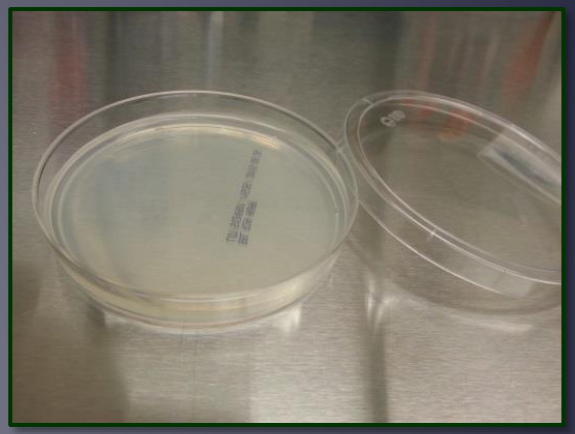
Consistently low viable microbe counts





Airborne microbial monitoring

- Passive monitoring: Allowing airborne microbes to settle onto a plate surface
  - Requires review in NASA cleanrooms because of high volatile content of plates
- Active monitoring: pulling air through a filter which is later transferred to a plate
  - Used in MOMA cleanrooms:
    - **Almost no growth seen in weekly cleanroom samples**
  - Used to monitor immediate environment during highly sensitive activities

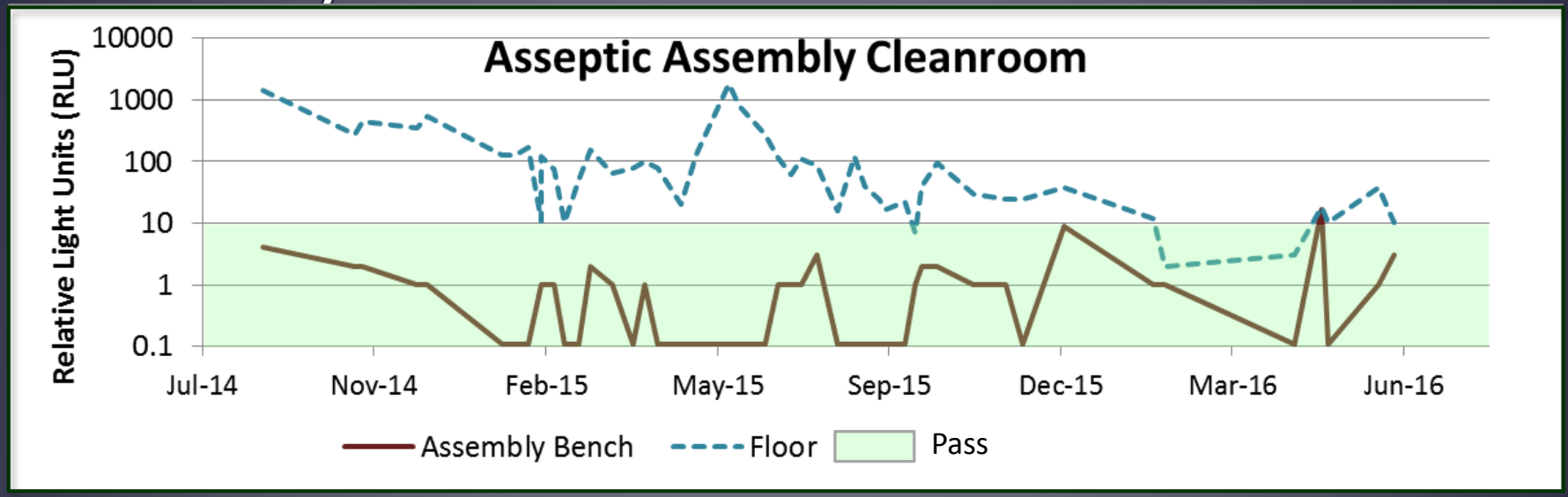


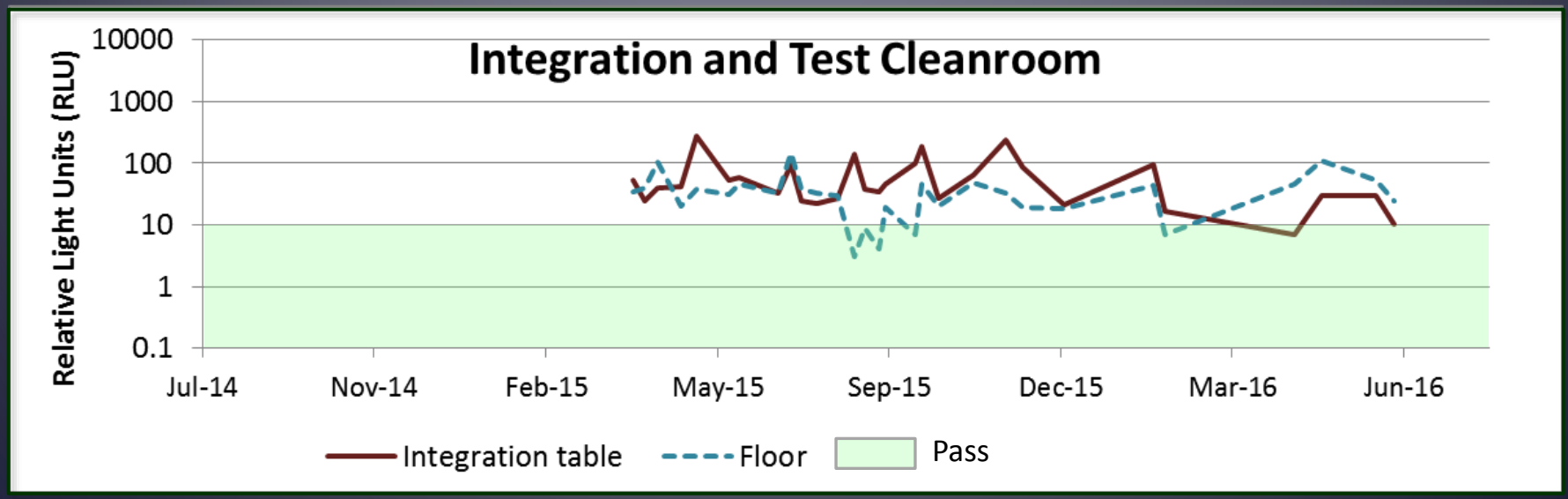
ATP rapid Bioburden Assessment


Everystockphoto.com/Clearly Ambiguous

- Pre-wet swab is used to sample a surface, swished in the reactant buffer
  - ATP is the energy carrying molecule in all cell types
  - ATP in the sample will react with the luciferase and luciferin in the buffer and produce light
  - Less than 5 minutes to sample
- Pre-wet swab contains *Chlorhexidine digluconate*
  - Not to be used on sensitive hardware
  - Removable by 70% IPA wiping

Critical work surfaces in assembly cleanroom have very low ATP bioburden

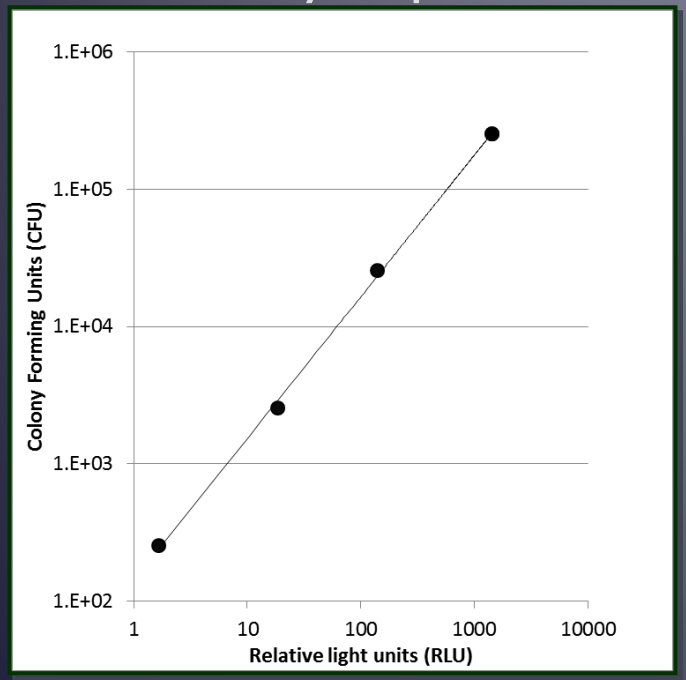




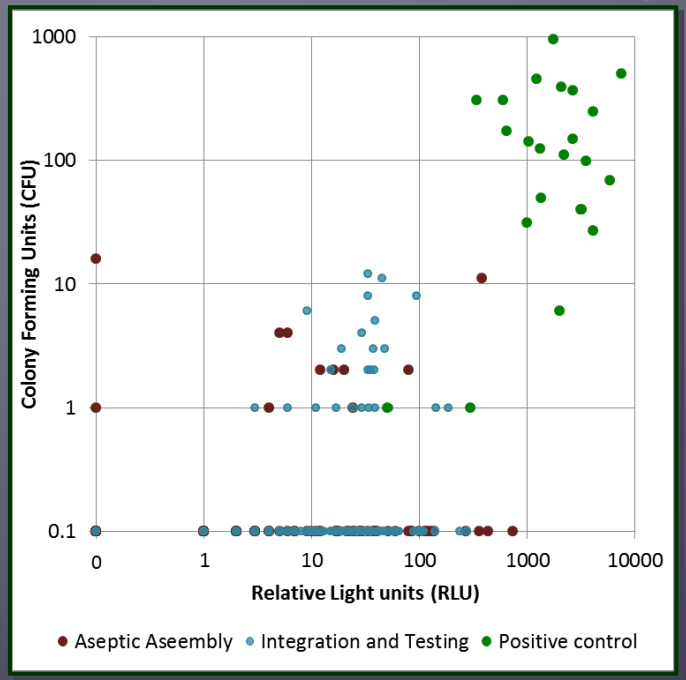
Determining risk from ATP readings

- Most cleanroom and hardware samples do not have any CFU
  - 99% of environmental microorganisms do not grow in a laboratory setting
  - <15% of cleanroom samples had CFU within 72h
  - RLU and CFU does not directly correlate in environmental samples

Laboratory Experiment



Environmental Monitoring



RLU Range	# Samples	# with 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

Monitoring and maintaining MOMA-MS hardware cleanliness

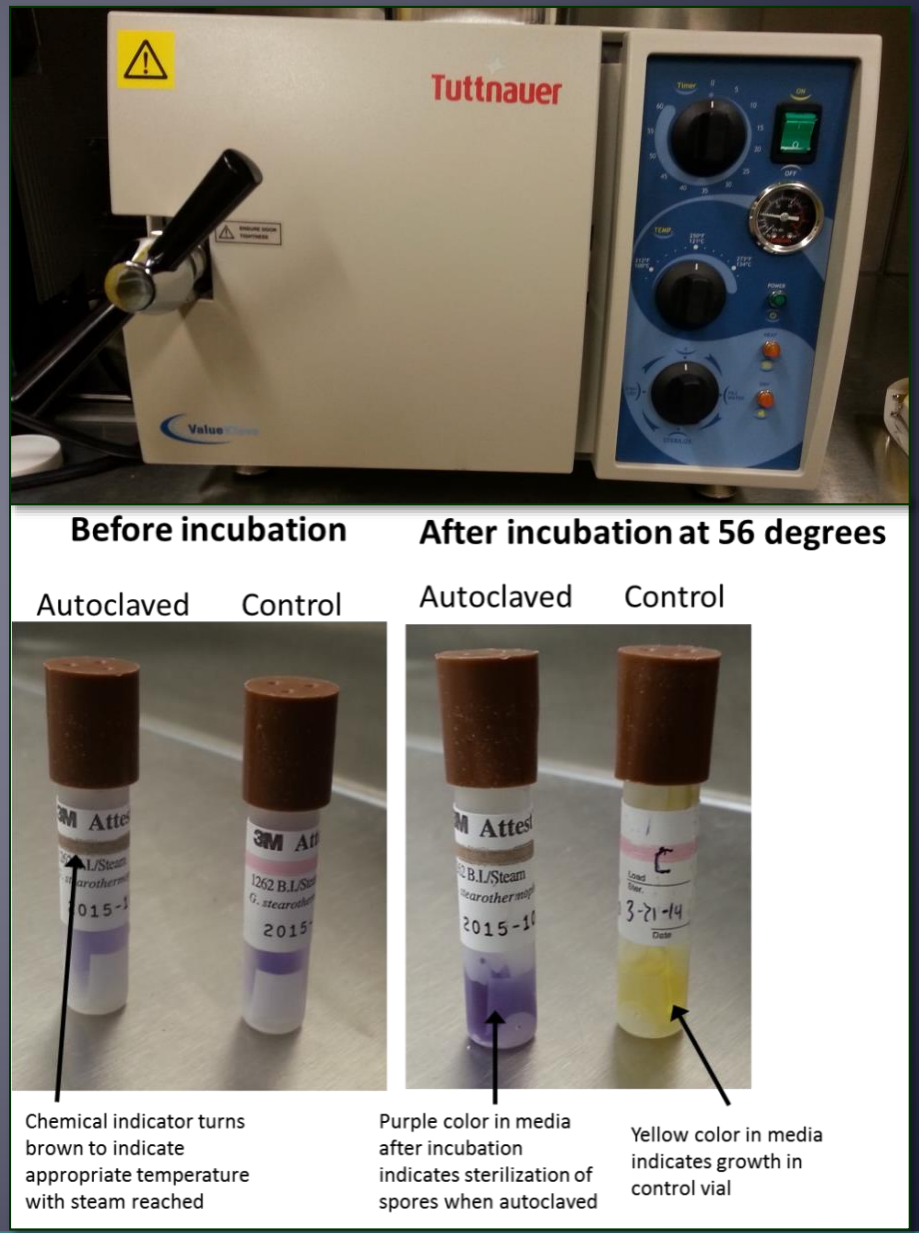
- Three cleanliness categories:
  - Sample path- <0.03 CFU/m^2
  - External non sample path surfaces- <300 CFU/m2
  - Surfaces in closed non-sample path volumes- <1000 CFU/m2
- Non sample path surfaces:
  - Sampled for heat resistant spores using standard swab assay before last access
    - External non sample path surfaces- sampled before shipping
    - Surfaces in closed non sample path volumes- sampled before final closeout
- Sample path
  - Sampled for heat resistant spores using standard swab assay before sterilization
  - Terminal sterilization by Dry Heat Microbial Reduction (DHMR)
    - 110°C bake for 60 hours
  - Any and all post DHMR handling must occur in an aseptic ISO Class 5 workspace

Post DHMR handling and cleanroom maintenance

- All sample path bioburden testing occurs prior to final access **before** DHMR
  - Post DHMR testing risks recontamination of the surface, and bioburden will be below limit of detection
- Any access to sample path post DHMR must occur in an aseptic ISO Class 5 environment
  - Train all team members interacting with the sample path in aseptic processing
  - Sterile garments, gloves, and tools required
  - Workspace cleaned and tested for bioburden before work, actively monitored with air bioburden sampler

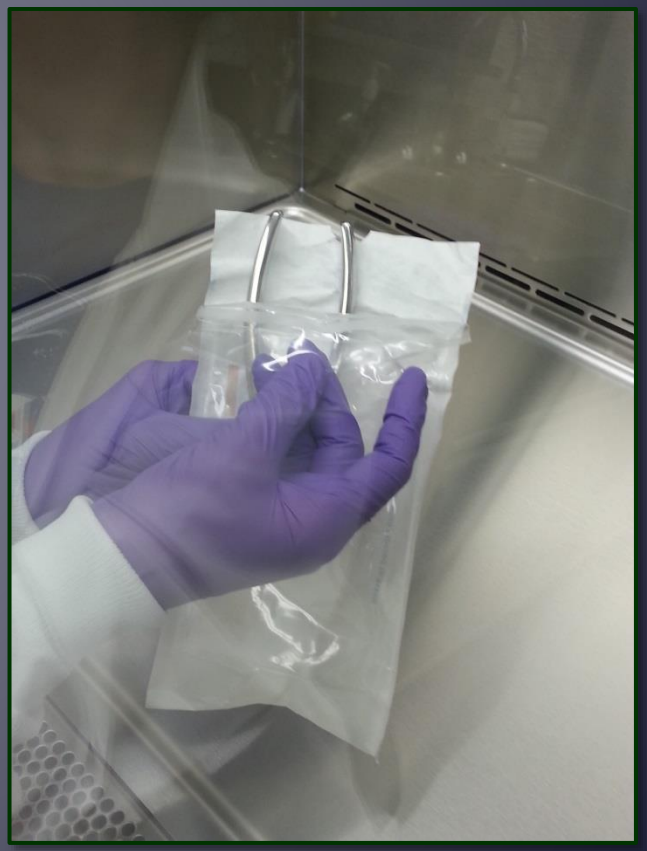
Post DHMR Tool Sterilization

- After precision cleaning and white light inspection, compatible tools will be sterilized
  - Autoclave sterilization: 20 min 121°C, 100 kPa
  - DHMR: 60 min, 165 °C
- Biological indicators used to ensure sterilization
- Tools not compatible with sterilization will not be used in direct contact with sample path surfaces post DHMR



Post DHMR Sterile Tool Handling

- Must only be exposed to ISO Class 5 or cleaner aseptic conditions
- Must be handled wearing sterile gowning
- Only wiped with sterile wipes
- Must only be set on sterile surfaces, sterile fields
- Must be opened by an assistant who is not handling sterile items
- Packages of foil will be sterilized for sterile fields (working surfaces)
- Sterile fields are single use and only for the continuous working session



Post DHMR Biological Contamination Prevention - Personnel

- Personnel management
  - One day Planetary Protection/ aseptic processing training for all personnel working directly with flight hardware
  - Single use sterile cleanroom coveralls, hood, and gloves
  - Two person system to manage sterile tools (pass sterile tool into workspace as needed)
- Sample path work only in an aseptic ISO Class 5 environment that has been verified by bioassay
- No tools that have not been sterilized in contact with Sample path

Summary

- MOMA-MS planetary protection requirements require the establishment of aseptic work spaces during assembly, integration, and testing
  - Three cleanrooms will be used at GSFC
  - Aseptic Assembly cleanroom is currently maintained with additional bioburden control steps: very low level of biological contamination
  - Integration and Testing cleanroom has not had additional bioburden control steps instituted: higher level of biological contamination
- Planetary Protection laboratory at GSFC developed to support onsite bioassay processing
- After DHMR exposures of sample path will be limited
  - Open only in a monitored aseptic work space
  - Handled only with sterile garments, sterile tools

Acknowledgements

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- GSFC Code 541 (Materials Engineering)