Aseptic handling of the MOMA Mass Spectrometer after Dry Heat Microbial Reduction

Contamination, Coatings, Materials, & Planetary Protection Workshop
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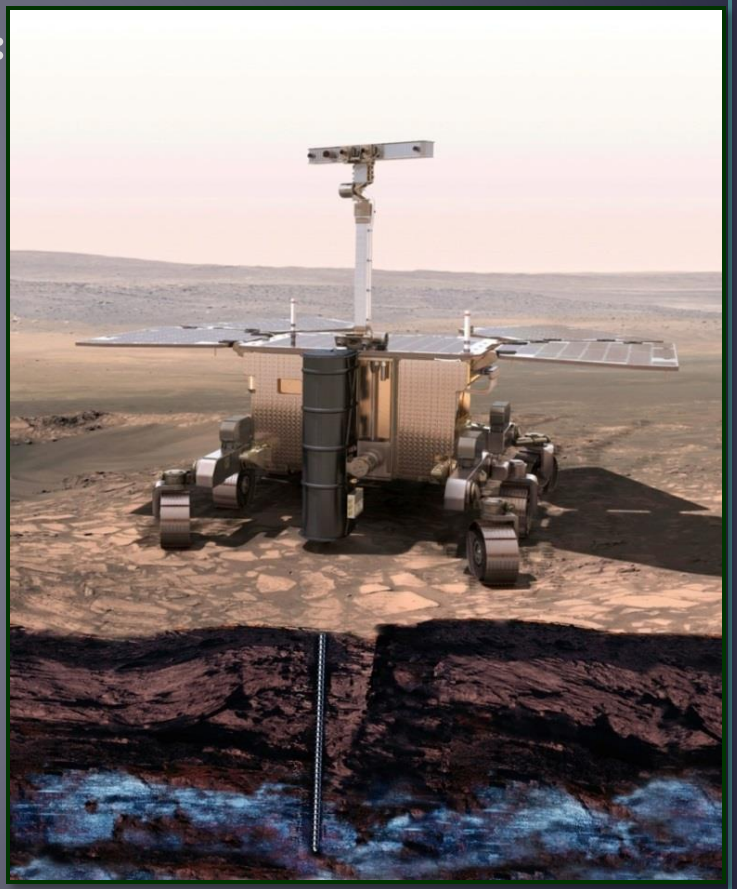
Dr. Erin Lalime, SGT/GSFC
Code 546 Contamination and Coatings Engineering Branch

ExoMars 2020
Mars Organic Molecule Analyzer (MOMA):

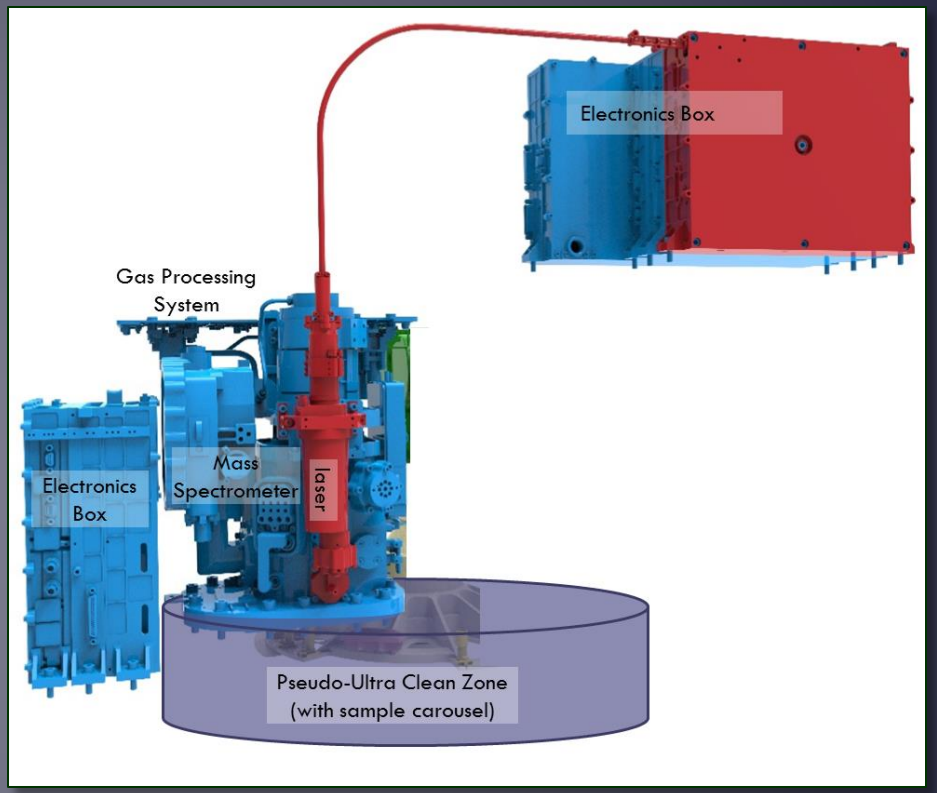
- ExoMars 2020- an ESA lander and rover:
  - Scheduled Launch Date: July 2020
  - **Life detection mission**
  - Samples will be collected up to 2m below the surface by a drill
- Mars Organic Molecule Analyzer (MOMA) is an instrument suite on the rover:
  - **Mass Spectrometer (MS) – NASA/GSFC**
  -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



The ExoMars rover. *Credit: ESA*

Verifying MOMA-MS Bioburden Requirements

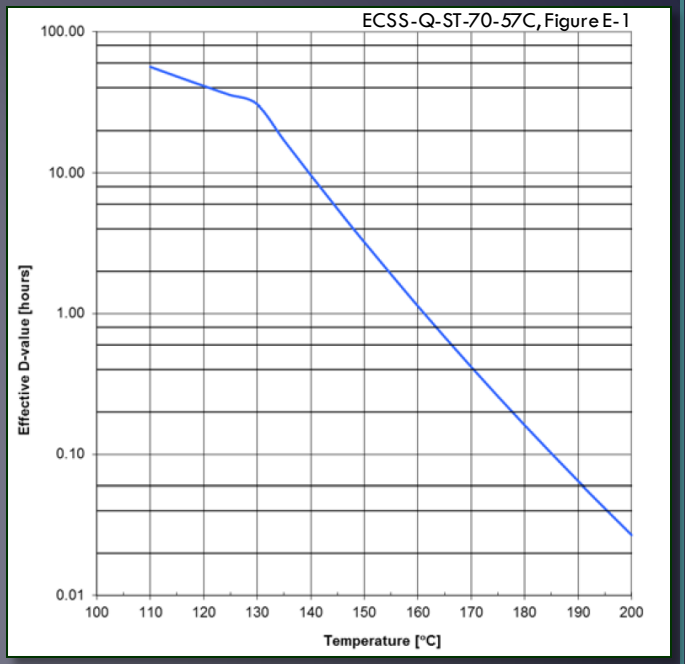
- Sample path: **<0.03 spores/m²**
  - Accessible areas:
    - Bioassay to 300 spores/m² at final access before 4 order of magnitude bioburden reduction with Dry Heat Microbial Reduction (DHMR)
  - Inaccessible areas:
    - Bioassay surfaces with similar handling, calculate bioburden reduction credit from (DHMR)

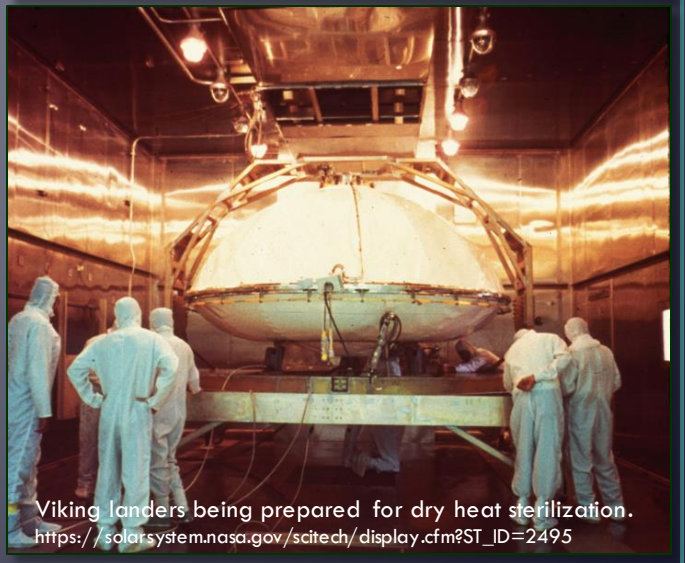


- Surfaces not in contact with sample path: **300-1000 spores/m²**
  - Internal volumes of electronics boxes: Inspect and bioassay before final assembly
  - Exterior surfaces: Inspect and bioassay before shipment and delivery to ESA.

DHMR: Dry Heat Microbial Reduction

- Standard approved method of bioburden reduction on flight hardware
  - Exposing hardware to temperatures of at least 110°C with controlled humidity
  - 4 orders of magnitude decrease in viable bioburden
  - Higher temperatures= shorter bake, but many components are not compatible with high temperatures
- Viking: DHMR entire lander
- Today, subcomponents are usually treated
- Alternates to DHMR have to be analyzed, proven, and approved by PPO





Viking landers being prepared for dry heat sterilization.
https://solarsystem.nasa.gov/scitech/display.cfm?ST_ID=2495

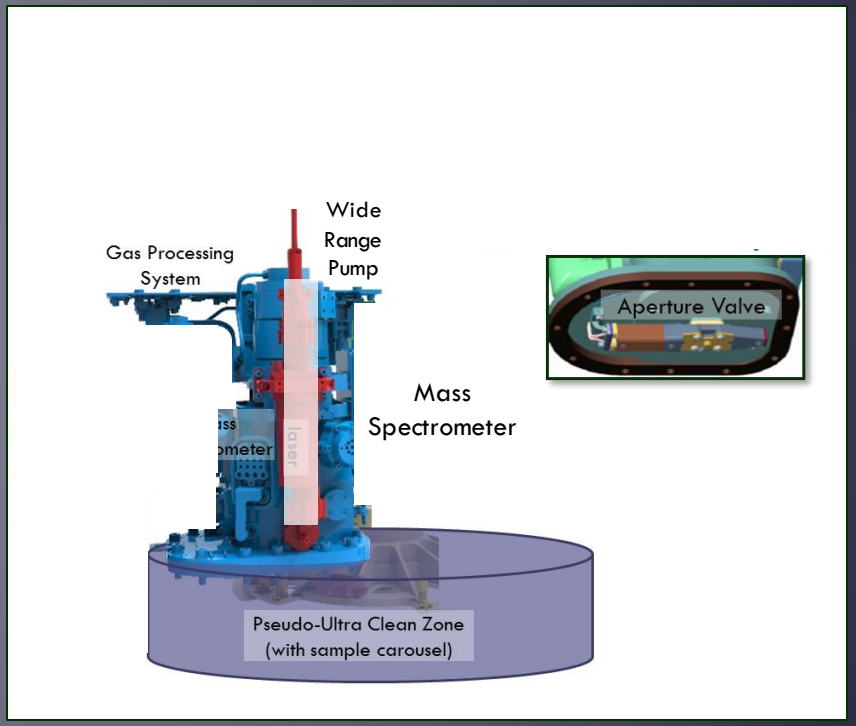
Pre-DHMR Handling


Image: Erin Lalime, Credit: Rich Hoffman

- Clean all hardware and tools before entering the cleanroom:
  - Multi step solvent cleaning process (detergent, water, acetone, hexane or toluene, isopropyl alcohol)
  - Inspect to VCHS+UV
  - Bake for dryness (GSE) or contamination bake out (flight hardware or critical GSE).
    - Contamination bakeout can double as bioburden reduction bakeout at the right times and temperatures.
- Clean (laundered) non-sterile garments (change 2x/week), with surgical masks and non-sterile gloves
- Handle hardware in an ISO class 7 clean rooms, mostly in an ISO class 5 tent or ISO class 5 flow bench
- Reclean/reinspect before and after any major move (between cleanrooms)

MOMA-MS bioburden reduction DHMR

- Only sample path is included in DHMR
  - Electronic components and Laser are not compatible with DHMR temperatures
- 4 orders of magnitude reduction of viable microorganisms for surface contamination only.
  - 60 hours at 110 °C
- All post-DHMR sample path exposure must be conducted using aseptic operations

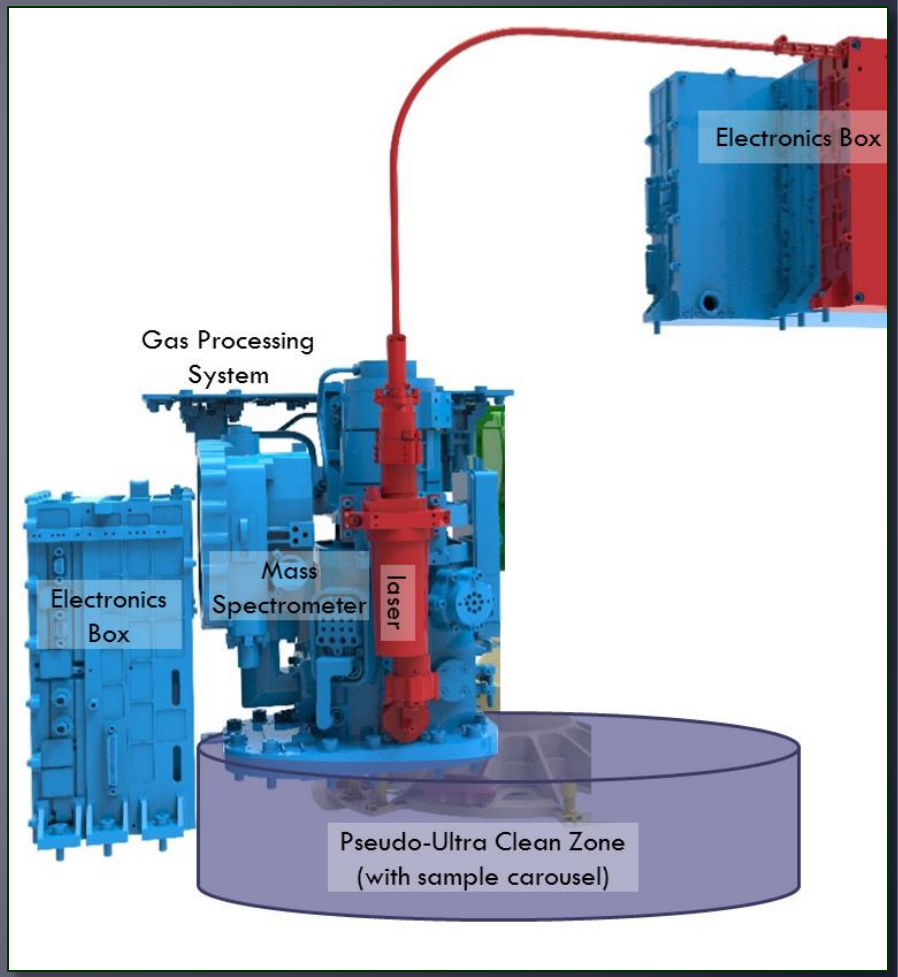


Post DHMR Overview

- Maintain hardware in biologically controlled (but not sterile) cleanroom
  - Increase cleaning frequency, add biocidal solvents, increase bioburden monitoring
- Non sample-path portions of the hardware do not need special handling post DHMR except for increased bioburden control in the cleanroom
- Minimize sample path exposure as much as possible
  - Implement aseptic handling when sample path must be exposed
  - Environmental monitoring for bioburden and particulate during sample path exposure

Activities that Require Aseptic Protocols

- Sample path surfaces include:
  - Gas processing system
  - Mass Spectrometer interior
    - WRP
    - Laser window
  - Pseudo-Ultra Clean Zone (pUCZ)) with sample carousel
    - Aperture Valve interface with Mass Spectrometer (MS)
- Planned Aseptic Activities
  - Connections/disconnections at the gas processing system
  - Move MS from pUCZ to vibration plate
  - Move MS from vibration plate to pUCZ
  - Replace laser window



Aseptic Handling Training

- Half day training in addition to full day PP training
- Review expected aseptic activities
- Sterile gowning overview with demo of sterile gloves and single use sterile garments
- Tool preparation: sterilization or isolation
- Tool handling: careful attention to sterile and non sterile surface, contact transfer awareness, multi person handling to ensure no contact between sterile and non sterile surfaces
  - Sterile tools may only touch sterile surfaces (gloves, hardware, work surface)
- If sterility is compromised through contact transfer, change out whatever was contacted (gloves, garment, tools, sterile work field, etc.)

Kimtech Pure* A5 Sterile Cleanroom Apparel Gowning Procedure

Before Gowning

Step 1
(Pre-Entry) Don Hair Net and Shoe Covers after removing all jewelry and cosmetics.

Step 2
(Gowning) Wash hands and gown first pair of sterile gloves. Sanitize gloves after gowning each article if required.

Step 3
Apply mask and hood assuring a snug fit.

Step 4
Open vacuumed-packed apparel. Tear at notched edge.

Step 5
Grasp the blue line. Located on the inside middle back.

Step 6
Gently unfold coverall utilizing blue indicator line. Arms and legs are pre-drawn and snapped in place. Garment is already folded inside-out and unzipped.

Snaps allow gathered-up arms and legs to expand during gowning.

Begin Gowning

Step 7
Hold garment at waist.

Step 8
Put one leg in and point toe through opening until snap releases.

Step 9
Do the same with the other leg.

Step 10
Insert one arm and extend until snap releases.

Step 11
Do the same with the other arm.

Step 12
Slip thumbs through thumb loops.

Final Step
Cross legs and zip up coverall.

After A5 Sterile Cleanroom Apparel is gowned

- Add boot covers.
- Complete gowning by adding goggles and a second pair of sterile gloves.

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Kimtech Pure* G3 Sterile Sterling* Nitrile Gloves Donning Procedure

Before starting the donning procedure, wash hands thoroughly and dry.

Step 1

Peel open sterile pouch and unfold glove wallet (DO NOT touch the exterior surface of gloves). Pinch the sides of wallet to open.

Step 2

Apply first glove to hand by sliding palm up into glove (thumb facing outward). Bend thumb toward center of palm and slide into glove while pulling up on the cuff. Leave the cuff rolled up.

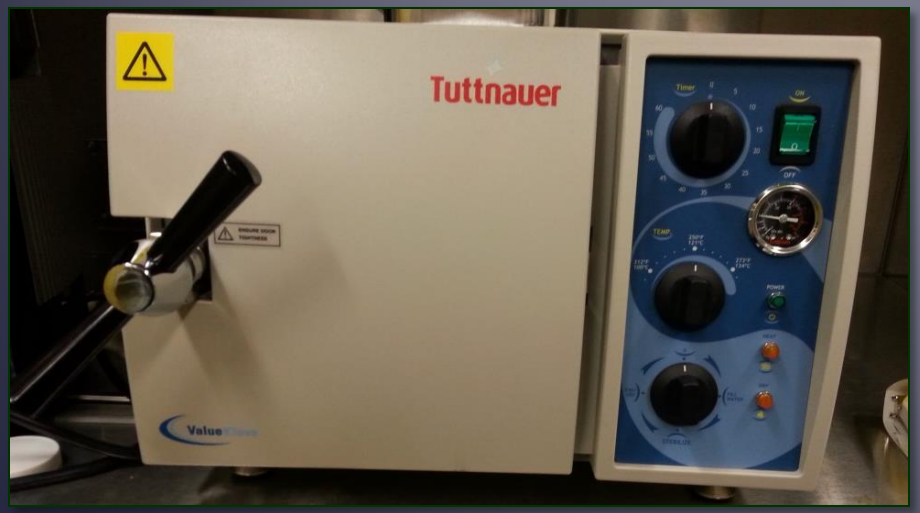
Step 3

Apply second glove to hand by sliding the four gloved fingers into cuff of the second glove. Slide ungloved palm (thumb facing outward) into glove. Bend thumb toward center of palm and slide into glove while pulling up with fingers of gloved hand.

Step 4

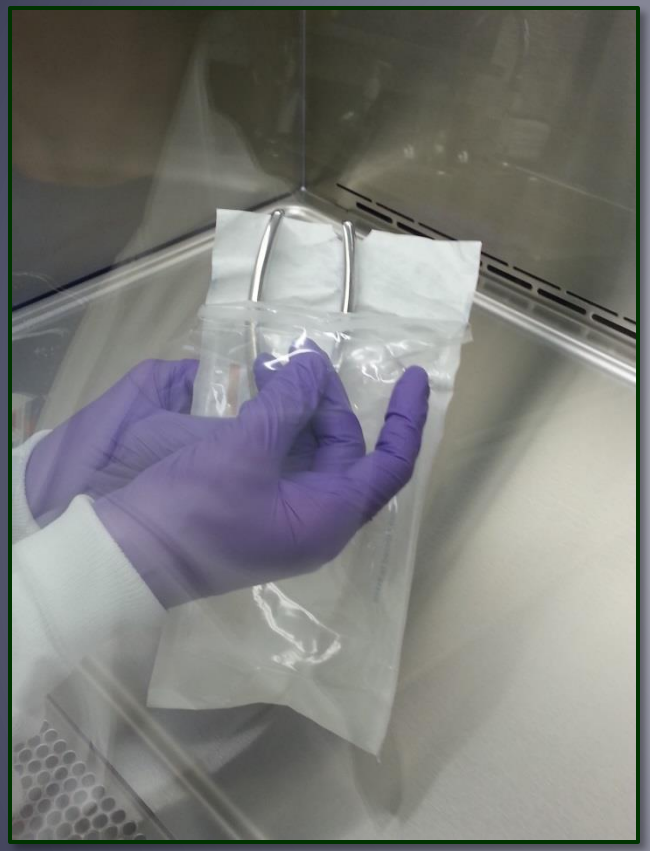
Complete donning the gloves by pulling up the cuff of the first glove with the fingers of the second hand[1].

Preparing Sterile Tools



- Tools compatible with sterilization:
  - DHMR: 60 min, 165 °C
  - Autoclave sterilization: 20 min 121°C, 100 kPa (15psi)
- Tools not compatible with sterilization
  - Not be used in direct contact with sample path surfaces post DHMR
  - May be used during aseptic process if non sterile components are wrapped in sterile foil or handled by non-sterile operator
- Prepare extra tools in case sterility is compromised

Aseptic Process Sterile Tool Handling



- Only be exposed to aseptic ISO Class 5 or cleaner conditions
- Must be handled wearing sterile gloves and garments
- Sterile pouches or foil opened by an assistant who is not handling sterile items
- Tools only be set on sterile surfaces
  - Sheets of foil will be sterilized to establish sterile working surfaces
  - Single use and only for the continuous working session

Preparing an aseptic work space

- Four days prior to aseptic procedure:
  - Mop with 7% hydrogen peroxide, then 70% IPA
  - Wipe horizontal and vertical surfaces in work area with sterile 70% IPA
  - Sample work surfaces and air for bioburden using ATP (rapid test for risk reduction) and CFU (3 day test, gold standard)
- Day of aseptic procedure
  - Confirm low bioburden from earlier surface samples
  - Clean again as above
  - Check work surfaces for bioburden using ATP
  - Ensure sterile garmenting/gloves are stocked
  - Prepare aseptic monitoring kit
  - Establish a sterile work field with sterile foil as a place to set tools or parts

Biocidal cleaning solutions:

- Selected for biocidal action without leaving a residue
- Use multiple cleaning solutions with different biocidal mechanisms to prevent selecting for resistant organisms
- 70% Isopropyl Alcohol (IPA) / 30% deionized water
  - Denatures proteins and damages cell membrane
  - 70% IPA is a more effective biocide than 100% IPA
  - Effective against vegetative microorganisms, but not spores. Some effectiveness against spores in mechanical removal.
- 7.5% H_2O_2 in deionized water
  - Disinfects by oxygen radical damage to DNA and proteins
  - Effective against spores at long exposure times

ATP rapid Bioburden Assessment

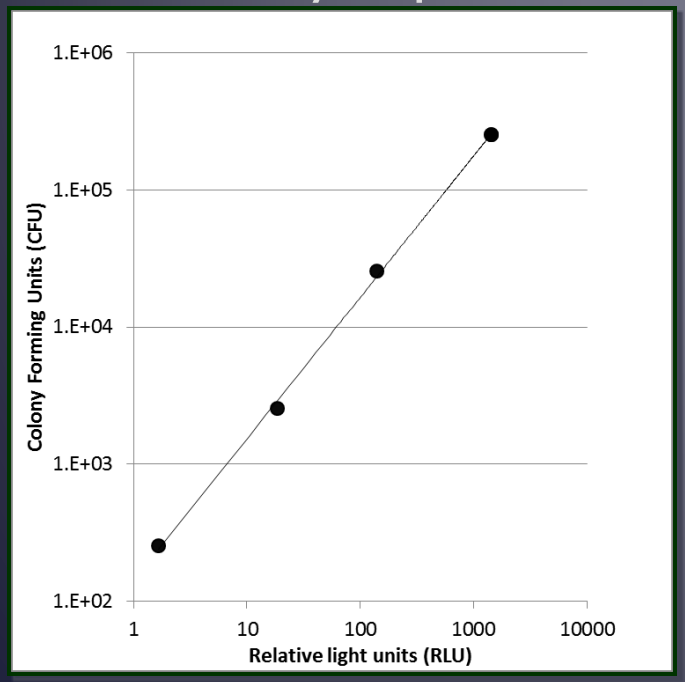

Everystockphoto.com/Clearly Ambiguous

- Pre-wet swab is used to sample a surface, swished in the reactant buffer
  - Adenosine Triphosphate (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*
  - Used to sample environmental surfaces or GSE
  - Not to be used on sensitive hardware
  - Residue removed by 70% IPA wiping

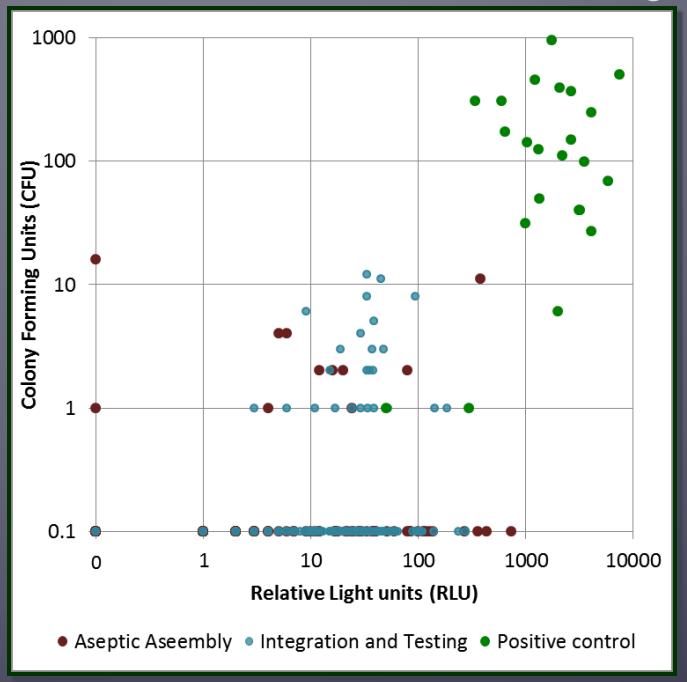
Determining risk from ATP readings

- 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

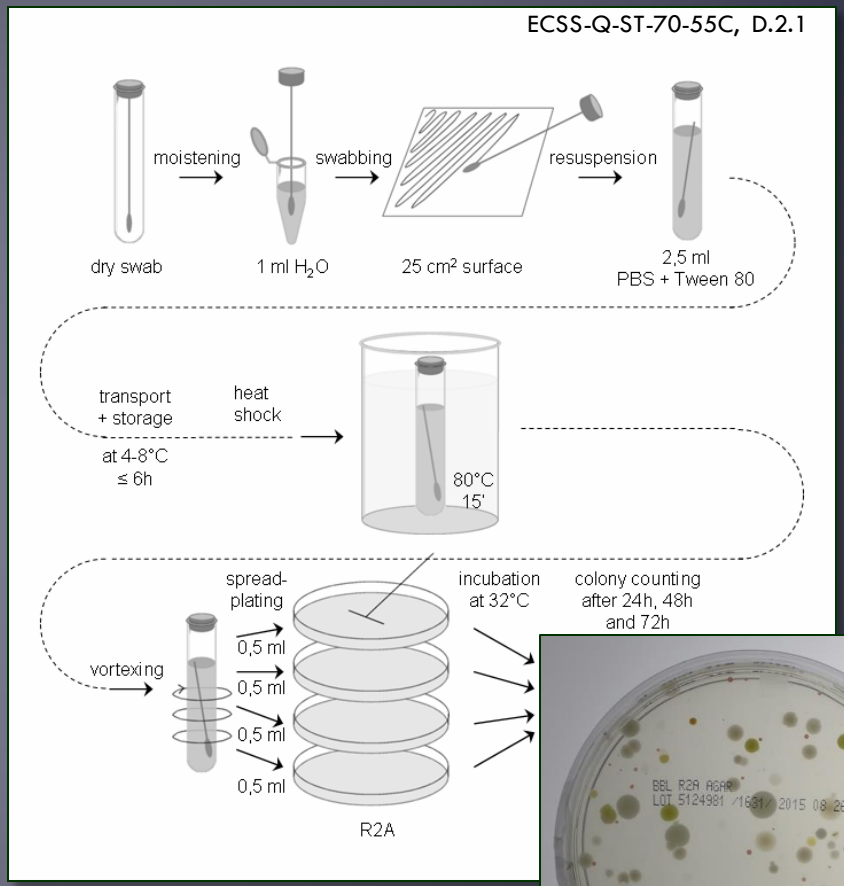


RLU Range	# Samples	# with CFU	% Positive
0-5	146	5	3.42
6-100	130	30	23.08
101-500	20	5	25.00
501-1000	4	3	75.00
1000-5000	16	16	100.00

Surface bioburden monitoring (swab)

Processed according to ESA protocol ECSS-Q-ST-70-55C

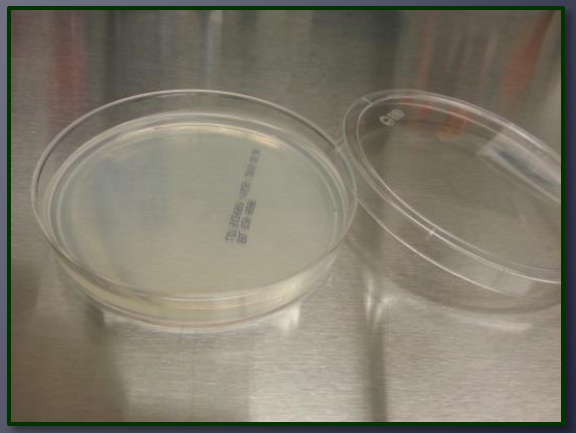
- Spore specific or general viable microbe screen (with or without heat shock)
- Swab a 25cm^2 area on work surface with a damp swab
- Plated onto multiple pre-poured agar plates
- 72 hour incubation to count colonies (reported CFU/m^2)




Bacterial colonies growing on a plate

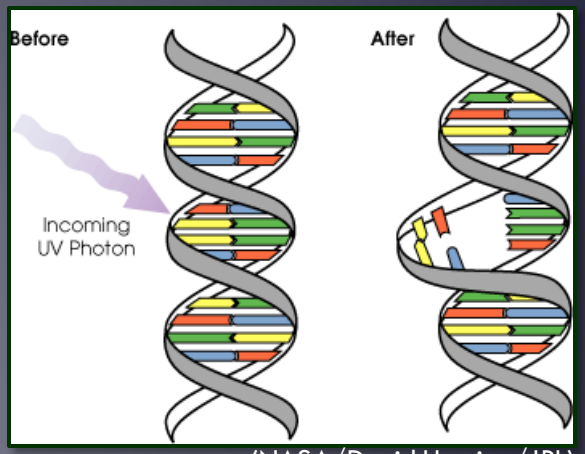
Airborne microbial monitoring

- Active monitoring: pulling air through a filter which is later transferred to a plate
  - Almost no growth seen in weekly cleanroom samples (20 min, $1m^3$)
  - Most aseptic activities should be <20 min exposure time
- Passive monitoring: Allowing airborne microbes to settle onto a surface
  - Standard: Use agar plate to capture settling microorganisms. Not used in GSFC cleanrooms because of high volatile content of plates
  - Alternate: Use dry gelatin filters as fallout witnesses to transfer to an agar plate.



Ultraviolet Light to Sanitize work surfaces

- Ultraviolet-C (UV-C 100-290nm), 250-260nm is germicidal
  - Kills by crosslinking DNA, which prevents the organisms from faithfully replicating its DNA
  - Line of sight limited
- UV-C lamps (253nm) **to be implemented in clean tent if bioburden levels are high**
  - UV-C intensity at work surface to be measured to determine appropriate exposure time



(NASA/David Herring/JPL)

Monitoring aseptic processing

- Dedicated Contamination Control personnel to monitor:
  - Sterile gowning
  - Any contact between sterile gloves and non sterile surface (change gloves!)
  - Handling and passage of sterile tools
  - Tracking sterile and non sterile GSE/tool components
- Active and passive bioburden air sampling during the duration of sample path exposure
- Active particle air sampling to ensure ISO class 5 is maintained
- Compromise of the sample path can result in substantial project impact in schedule, money, and risk to the hardware.

Summary

- As part of a life detection Mars Rover mission, MOMA-MS planetary protection requirements necessitate DHMR to reach 0.03 spores/m^2
- After DHMR, any sample path exposure must be handled in an aseptic ISO class 5 work spaces
  - Sample path exposure is kept to a minimum, but is unavoidable
  - Aseptic ISO class 5 work space will be established by cleaning with multiple biocidal solutions confirmed by bioburden monitoring
- All personnel in the clean room during sample path exposure activities must be trained in sterile gowning practices and aseptic handling
- During sample path exposure, sterile gowning, sterile gloves, sterile tools, and aseptic handling will be implemented
- Compromise in aseptic handling can result in high cost to schedule and risk to hardware.


Credit: NASA

Acknowledgements

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