Session 2.4

Establishing and Monitoring an Aseptic Workspace

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Overview

- When are aseptic operations necessary?
- Preparing the cleanroom
  - Cleaning, Monitoring, & Verification
- Preparing personnel and tools
  - Training
  - Tool sterilization
- Performing an aseptic operation
  - Monitoring for bioburden/particulate
  - Awareness of contact transfer

When are aseptic operations necessary?

- When exposing hardware with bioburden requirement only attainable by dry heat microbial reduction (DHMR) or other sterilization method
  - Requirement can not be directly verified by bioassay
  - Bioburden is verified by process
- Ideally DHMR happens as late as possible in I&T
  - At the highest possible assembly level
  - Late DHMR minimizes or eliminates need for aseptic operations
- If surfaces must be exposed, all operations must occur aseptically to prevent recontamination
  - NASA-HDBK-6022: *"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"*
  - ESA ECSS-Q-ST-70-58C: *" Action levels … during aseptic operations are: < 1 CFU/m^3 for air samples, < 400 CFU/m^2 for surfaces, < 1 CFU/glove print (5 fingers)… In some cases, aseptic operations can only be ensured if a more stringent particulate control (e.g. ISO 5) is applied"*

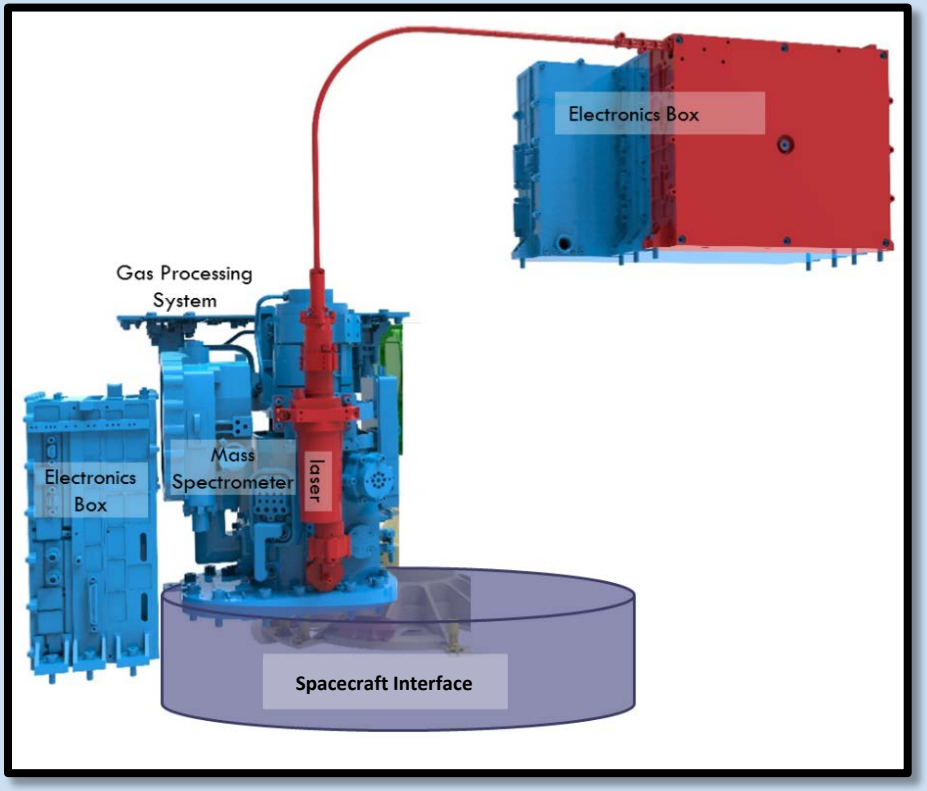
When are aseptic operations necessary?

When working with hardware that has undergone dry heat microbial reduction (DHMR) or other sterilization method!

- Bioburden requirement is verified by the sterilization process
  - Exposing the sterile surfaces risks recontamination from a non-sterile environment
- Ideally DHMR happens…
  - As late as possible in the integration and testing (I&T) process
  - At the highest possible assembly level
  - Late DHMR minimizes or eliminates need for aseptic operations
- If a sterilized surface must be exposed, all operations must meet aseptic standards
  - **NASA:** *"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)
  - **ESA:** *"Action levels … during aseptic operations are: < 1 CFU/m3 for air samples, < 400 CFU/m2 for surfaces, < 1 CFU/glove print (5 fingers)… In some cases, aseptic operations can only be ensured if a more stringent particulate control (e.g. ISO 5)"* (ECSS-Q-ST-70-58C)

MOMA-MS Aseptic Operation Example

- Mars Organic Molecule Analyzer- Mass Spectrometer (MOMA-MS)
  - On the ESA ExoMars 2020 Rover
  - Life detection mission
  - **PP Category IVb**
- Surfaces on the sample path were baked at DHMR temperatures to meet 0.03 CFU/m^2 requirement
- Re-exposure of the sample path was unavoidable due to testing configurations
- Each exposure conducted in aseptic environment or re-sterilized by localized DHMR



MOMA-MS Bioburden Requirements:

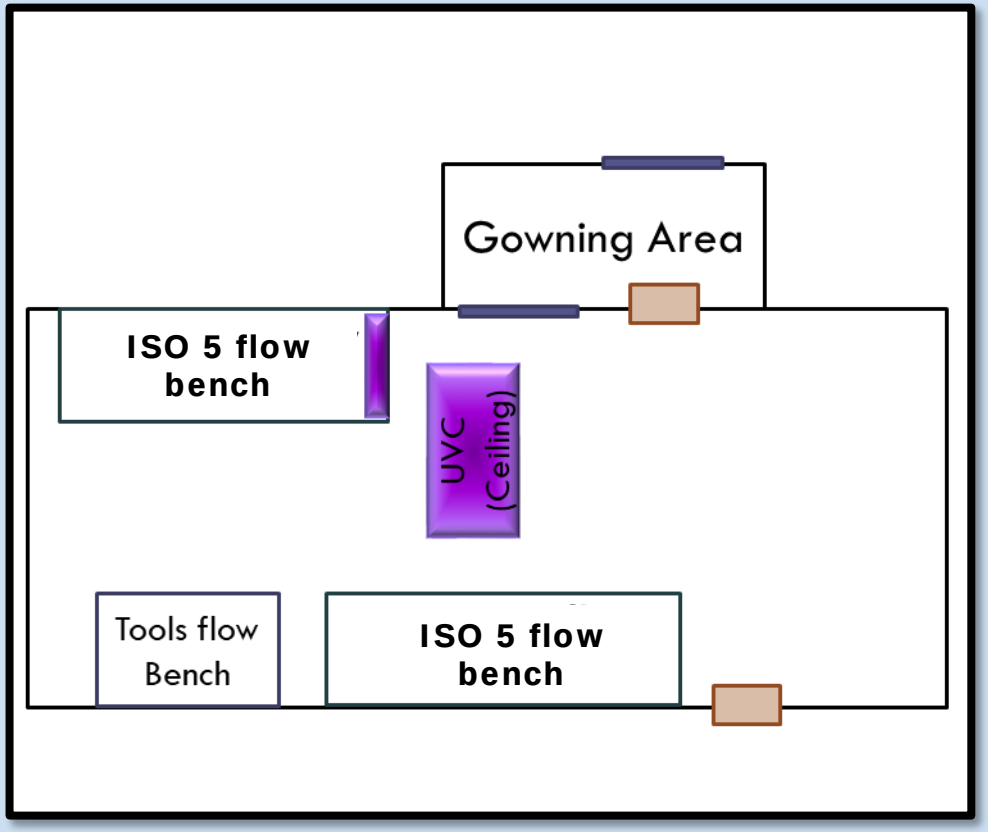
Internal sample path surfaces: 0.03 CFU/m^2

External & non-sample path surfaces: 300-1000 CFU/m^2

Overview of an aseptic operation

- Prepare an aseptic work space:
  - ISO Class 5
  - bioburden controlled and verified
- Plan and prepare the hardware:
  - Prepare sterile tools & equipment
  - Plan work to control exposure time
  - Plan work to minimize personnel and handling
- Monitor during the aseptic work
  - Continuously sample environment for particles and bioburden
  - Visually monitor hands and tools for contact transfer during operation

Cleanroom for aseptic operations



- ISO class 7 clean room
  - Maintains close to ISO 5
  - Aseptic operations take place at flow bench
- Daily cleaning:
  - Mop, alternating weekly between 70% IPA and 7% H_2O_2
  - Wipe critical surfaces with sterile 70% IPA
- Twice a week cleaning:
  - Wipe horizontal surfaces with sterile 70% IPA
  - Replace all garments
  - Run UVC lamps

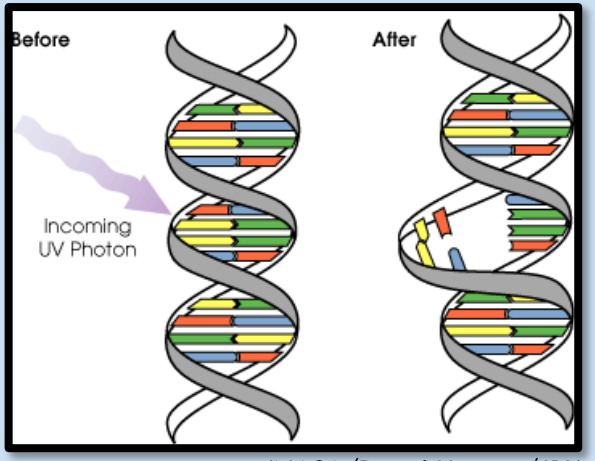
Biocidal mopping

- Cleanroom mopped daily (M-F) with either 70% IPA or 7% H_2O_2
  - Alternate between IPA and H_2O_2 weekly

- Different biocidal mechanisms to prevent selecting for resistant organisms
  - 70% IPA denatures proteins
    - (70% IPA/30% water is a more effective biocide than 100% IPA)
  - 7% H_2O_2 disinfects by oxygen radical damage to DNA and proteins

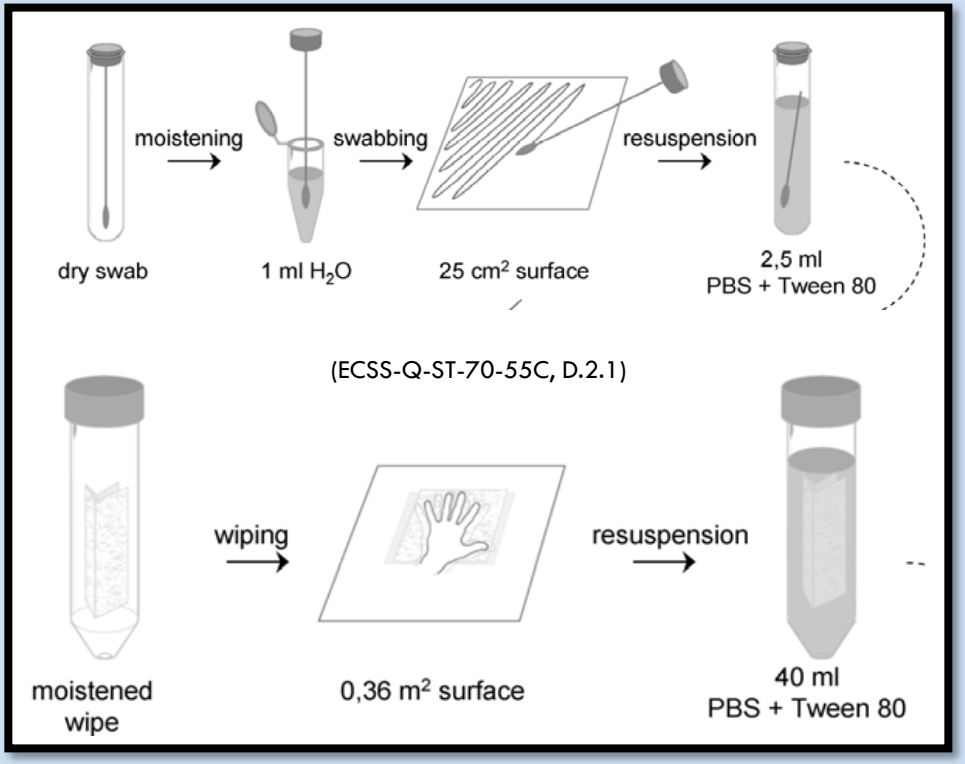
Ultraviolet light treatment of cleanroom

- Ultraviolet-C (UV-C) 100-290nm, 250-260nm is germicidal
  - Kills by crosslinking DNA, which disrupts replication
  - UV-C lamps (253nm) installed in cleanroom ceiling and a portable lamp on flow bench
  - Line of sight limited
- 22,000 µWs/cm^2 (microwatt seconds/ square centimeter) is a sufficient energy dose to kill 99% (2 log) of most common bacteria and bacterial spores on a surface
  - UV-C intensity at the floor of the measured at 30 µW/cm^2
  - 15 min exposure to reach 22,000 µWs/cm^2



(NASA/David Herring/JPL)

Facility bioburden monitoring



- Weekly room monitoring
  - General viable microbe screen (not spore specific)
  - Swab 25cm^2 or wipe up to 1m^2 surface area
  - Sample an established set of cleanroom surfaces for trending data
    - Same surfaces each time, but not necessarily exact location (ex. different locations on the same table)

ATP rapid bioburden assessment

- 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 **(same chemistry that makes fireflies glow!)**
  - Less than 5 minutes to sample
  - Used for trending and quick check

- Pre-wet swab contains *chlorhexidine digluconate and benzethonium chloride*
  - Not to be used on sensitive hardware
  - Removable by 70% IPA wiping

Airborne microbial monitoring

- **Passive monitoring:** Allowing airborne microbes to settle onto a plate surface
  - Standard practice is to use a poured plate, but gelatin filters are used in cleanroom due to off gassing concerns
  - Used to monitor immediate environment during highly sensitive activities

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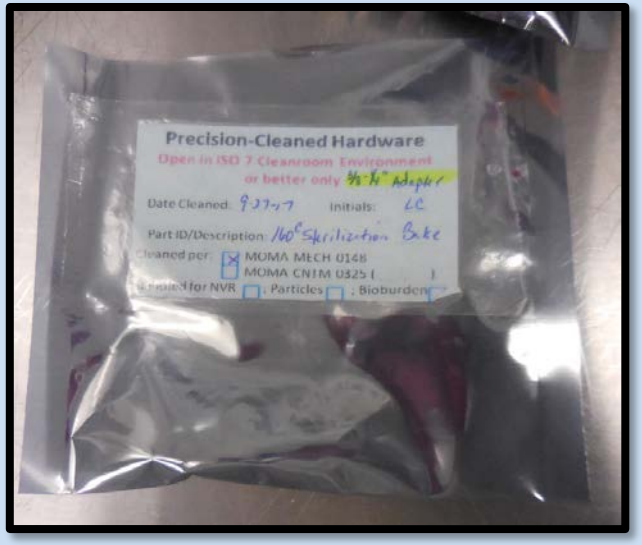
- **Active monitoring:** Pulling air through a filter which is later transferred to a plate
  - Used in cleanroom trending and to monitor immediate environment during highly sensitive activities

Biological contamination prevention - Personnel

- Personnel training
  - Everyone on the team: 1 day Planetary Protection overview
  - Everyone in the cleanroom: Standard cleanroom certification
  - **Aseptic operators only: Half-day aseptic operations training**
    - Covers: Sterile garmenting/gloves, sterile handling with a focus on contact transfer risk, tool/GSE preparation, and two-operator system for opening sterilized tools/components
- Personnel practice in aseptic environment
  - Single use sterile cleanroom coveralls, hood, and gloves
  - Two person system to manage sterile tools (pass sterile tool into workspace as needed)
  - Change gloves as needed

Tool preparation

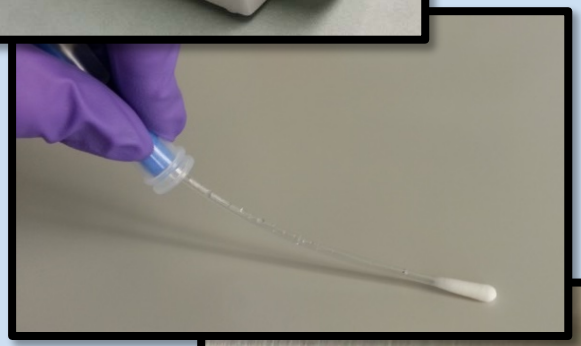


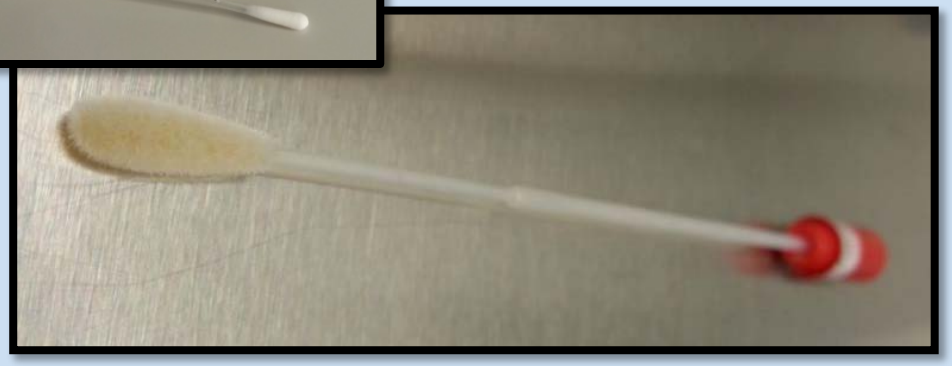
- Sterile tools
  - After precision cleaning, compatible tools are sterilized by dry heat
  - Double foil wrapped and cleanroom bagged to allow non-sterile operator to open the outer pack while the sterile operator touches only the inner wrap
  - Prepare sterile foil for sterile field and wrapping non sterile surfaces
- Non-sterile tools
  - Tools that are not compatible with sterilization will not be used in direct contact with sample path surfaces post-DHMR

Preparation for aseptic operations

- Work area:
  - 70% isopropanol and 7% hydrogen peroxide used to clean surfaces
  - When possible, sanitizing UVC lamps are also used
- Hardware:
  - Non-sterile surfaces isolated with bag or drape (where feasible)
  - Non-sterile exposed surface wiped with sterile 70% isopropanol
- All tools are cleaned and sterilized
  - Tools that cannot be sterilized are wrapped with sterile foil before being handled, and the sockets that interact directly with the hardware are sterile

Verifying aseptic work space



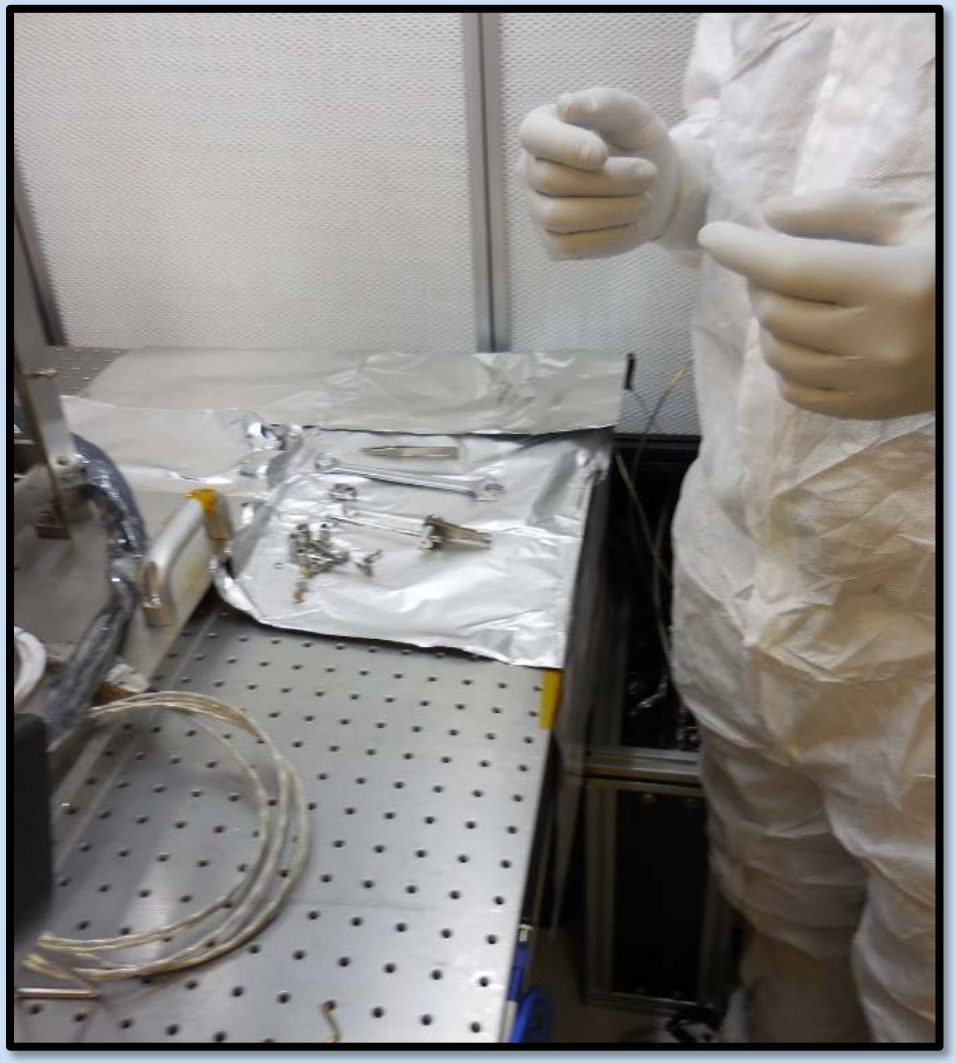


- Four forms of cleanliness verification:
  - Air samples for viable bioburden (<1 CFU/m^2)
  - ATP rapid bioassay
    - Any high ATP readings require immediate re-cleaning before swab bioassays
  - Surface samples for viable bioburden (72 h, <400 CFU/m2)
  - Particle counter monitoring (ISO Class 5)

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

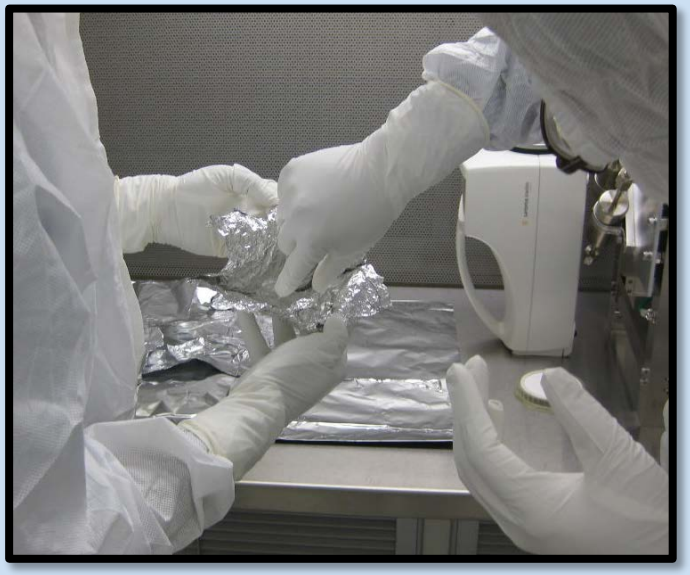
Contact transfer risk

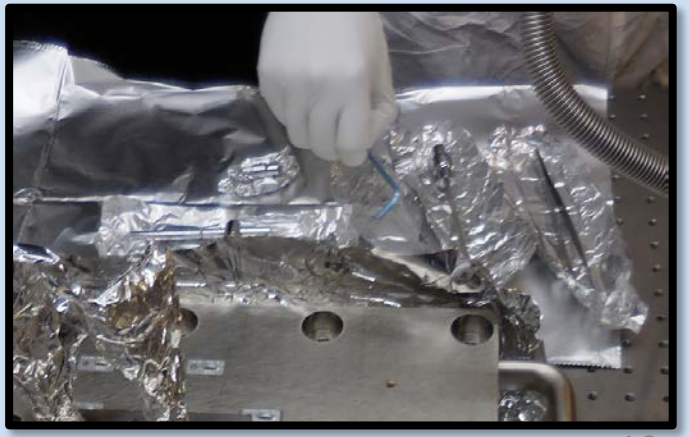
- There are two types of surfaces during an aseptic operation: sterile and clean
  - **Sterile surfaces** are those isolated since DHMR, sterile tools, and/or sterile foil
  - **Clean surfaces** include exterior surfaces of the hardware that are not maintained sterile, cleanroom tables/walls, etc.
    - Surfaces were cleaned and tested by bioassay
- Change sterile gloves as often as necessary to limit contact transfer risk between sterile and clean surfaces
- Dedicated person to monitor personnel and tool contact



Tool / part sterility

- Tool handling
  - Must only be exposed to ISO 5 or cleaner aseptic conditions
  - Must be handled wearing sterile gowning
  - Only wiped with sterile wipes
- Sterile field
  - Must only be set on sterile surface or 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

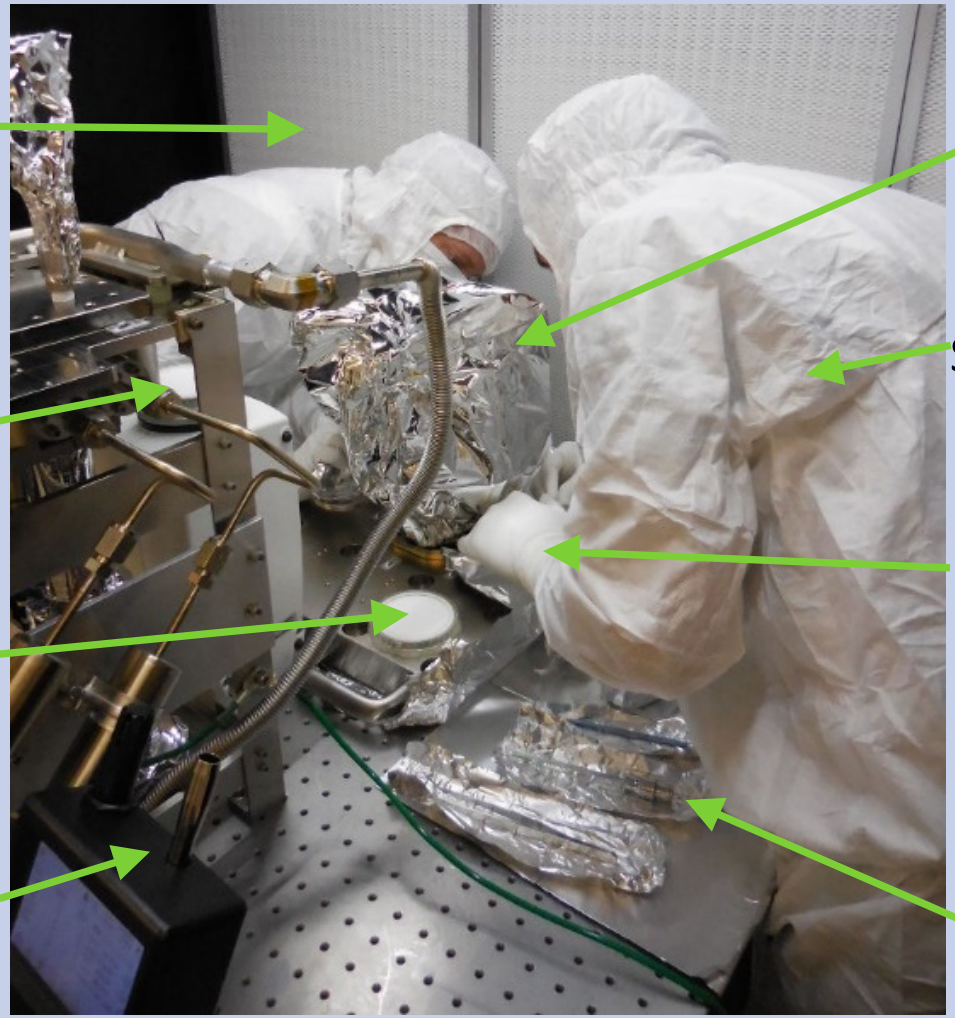




Minimize exposure time

- Even a bioburden verified ISO 5 work space may not be 100% microbe free
- Depending on the mission requirements, even 1 microorganism could be too many
  - Ex: MOMA's sample path requirement of 0.03 cfu/m^2, with <1m^2 of exposed surface during an aseptic operation)
- Longer exposure of a sensitive surface increases the risk of unacceptable contamination
- Carefully plan operations so everything is in place to open and close sensitive surfaces as quickly as possible
  - If possible, eliminate direct contact with critical surfaces
- Project-specific analysis of acceptable exposure times based on surface area exposed, bioburden trending in cleanroom, and as necessary, post exposure data from bioburden monitoring
  - Determine acceptable exposure time before operation
  - Record exposure time during operation

Sample path exposure



Review

- Prepare and verify the work space
  - ISO class 5 (class 5 cleanroom or flow bench in ISO class 7)
  - Biocidal cleaning to reduce bioburden
  - Bioburden verifications: surface and airborne bioassays
- Prepare personnel and tools
  - Cleanroom and aseptic training
  - Sterilize tools or find ways to isolate non-sterile tool surfaces
- Conduct aseptic operations carefully, quickly, and clean
  - Sterile garments and gloves (change gloves as often as needed)
  - Only sterile tools in contact with critical surfaces
  - Minimize and record exposure time