ISS External Microorganisms: A Payload to Assess Biological Cleanliness Outside the International Space Station

Aaron B. Regberg

Mary Sue Bell , Jamie Miccuilla Patrick S.G. Chain, Richard E. Davis, Andrew J. Hatch, Paul Li Martin Tschirschwitz, and Sarah Wallace

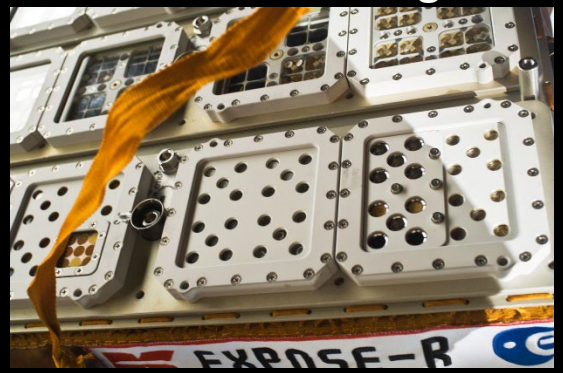
03/26/2026

National Aeronautics and Space Administration

Leaking Vehicles and Suits Could Impede our Search for Signs of Life on Other Planets

BACKGROUND

Some organisms can survive exposure to space!



images-assets.nasa.gov/image/iss018e039227/iss018e039227~orig.jpg

Cyano-bacteria, lichen and fungi survived up to 500+ days outside ISS

Tardigrades survived extended ISS exposure… *and then reproduced*

We also know that all crewed, pressurized volumes will leak or vent

ISSUE

But we *don't* know what's actually leaking/venting from our current systems, how long those organisms could survive, or how far they may travel under destination conditions

Does proximity to a warm spacecraft matter?

How close can crew get without compromising science?

How far could our hitchhikers spread

The answers will drive element design (i.e. closed vs. open ECLS), where we place elements, and who/how we collect science samples

https://www.nasa.gov/sites/default/files/atoms/files/moon-investments-prepare-us-for-mars.pdf

Knowledge Gap 2H. What microbial contaminants would vent from an extravehicular activity (EVA) suit or other vehicles?

Apollo 12 Mission image - Astronaut Bean deploys ALSEP Central Station

- Do we need to filter all of our vented products?
- How close can an astronaut get to a sample without contaminating it?
  - Should we use robots to collect and contain sensitive samples?

Kminek G. et al. 2018. *Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions*, Houston Texas: Lunar Planetary Institute. 28 p.

Knowledge Gap 2B. What level of non-viable microbial contamination escape is acceptable?


https://www.nasa.gov/image-article/ammonia-pictured-venting-outside-of-international-space-station/

- Do we need to filter all of our vented products?
- How close can an astronaut get to a sample without contaminating it?
  - Should we use robots to collect and contain sensitive samples?
- What if our spacecraft create an artificial habitable zone?

Kminek G. et al. 2018. *Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions*, Houston Texas: Lunar Planetary Institute. 28 p.

Cosmonauts Collect culturable bacteria and Fungi from the Exterior of ISS

BioNanoScience
https://doi.org/10.1007/s12668-019-00712-1

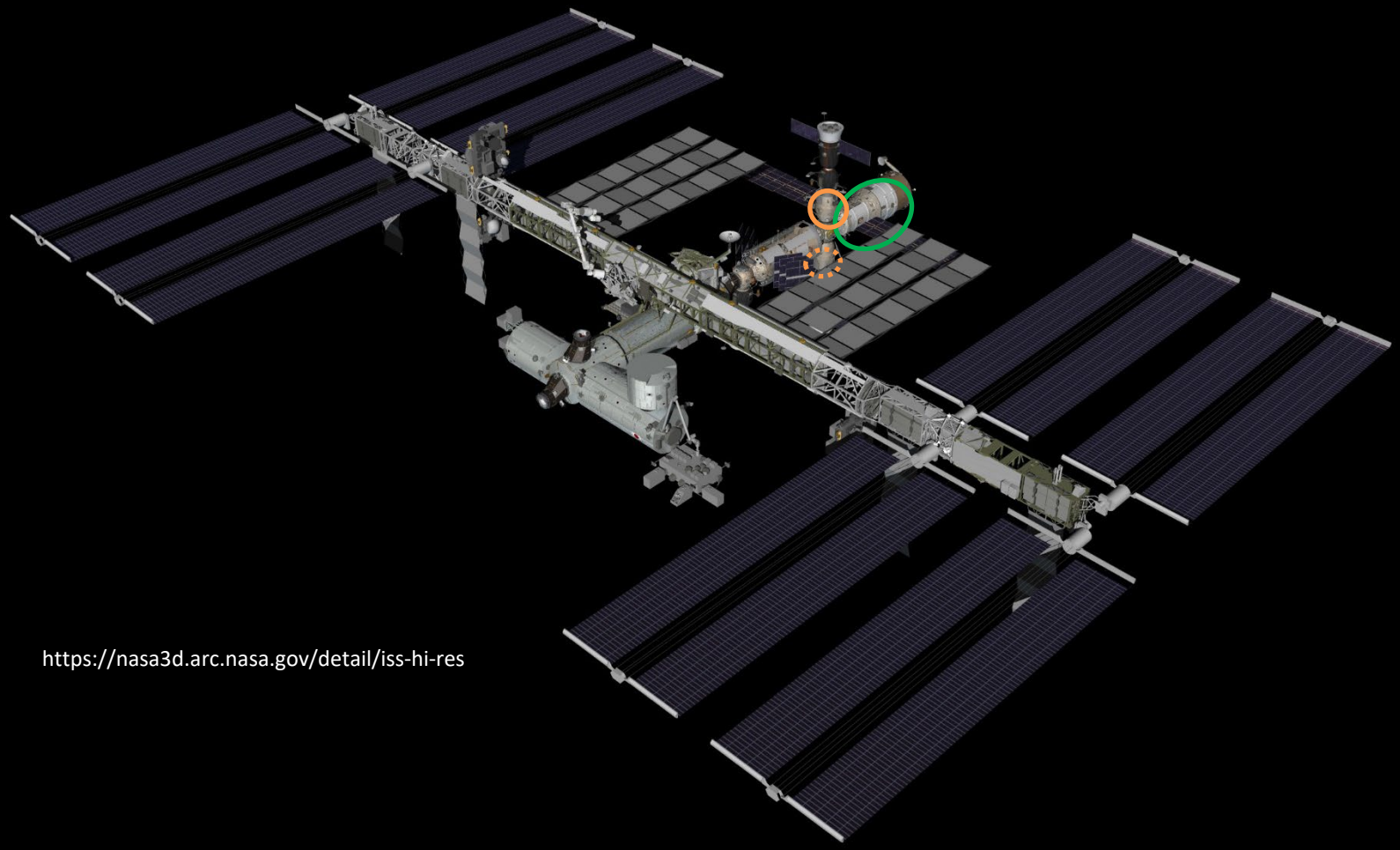
Microbiological Investigation of the Space Dust Collected from the External Surfaces of the International Space Station

Elena A. Deshevaya[1] · Elena V. Shubralova[2] · Svetlana V. Fialkina[1,3] · Aleksandr A. Guridov[1] · Natalia D. Novikova[1] · Oleg S. Tsygankov[4] · Pavel S. Lianko[2] · Oleg I. Orlov[1] · Sergey P. Morzunov[5] · Albert A. Rizvanov[5,6] · Irina V. Nikolaeva[7]

Table 4 Microorganisms isolated on the ISS external surfaces in SE TEST

EVA/date	Location sampled	Swab appearance
EVA-25 15 November 2010	Valves of trace contamination removal unit	Black spots
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Black spots
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Gray spots
EVA-38 19 June 2014	SM, window 2	Black spots
EVA-41 10 August 2015	SM, between tubes of radiator (STR)	Black spots
EVA-42 26 February 2016	The exit porthole of the module "PIRS" surface	Gray spots
EVA-42 26 February 2016	The exit porthole of the module "PIRS," window, and hatch frame	Black and gray spots, bright grains

EVA, extravehicular activities
VL2, exit porthole 2
SM, SERVICE MODULE
STR, thermal control system



https://nasa3d.arc.nasa.gov/detail/iss-hi-res

RESULTS FROM THE RUSSIAN SEGMENT

BioNanoScience
https://doi.org/10.1007/s12668-019-00712-1

Microbiological Investigation of the Space Dust Collected from the External Surfaces of the International Space Station

Elena A. Deshevaya[1] · Elena V. Shubralova[2] · Svetlana V. Fialkina[1,3] · Aleksandr A. Guridov[1] · Natalia D. Novikova[1] · Oleg S. Tsygankov[4] · Pavel S. Lianko[2] · Oleg I. Orlov[1] · Sergey P. Morzunov[5] · Albert A. Rizvanov[5,6] · Irina V. Nikolaeva[7]

Table 4 Microorganisms isolated on the ISS external surfaces in SE TEST

EVA/date	Location sampled	Swab appearance	Microorganisms
EVA-25 15 November 2010	Valves of trace contamination removal unit	Black spots	*Bacillus licheniformis*
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Black spots	*Bacillus sphaericus*; *B. subtilis*
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Gray spots	*B. subtilis*
EVA-38 19 June 2014	SM, window 2	Black spots	*Bacillus pumilus*
EVA-41 10 August 2015	SM, between tubes of radiator (STR)	Black spots	*Bacillus pumilus*; *Aureobasidium* sp.
EVA-42 26 February 2016	The exit porthole of the module "PIRS" surface	Gray spots	*Bacillus sphaericus*
EVA-42 26 February 2016	The exit porthole of the module "PIRS," window, and hatch frame	Black and gray spots, bright grains	*Bacillus licheniformis*; *Bacillus pumilus*; assemblage of *Agrococcus jenensis*, *Skermanella aerolata*, *Deinococcus aerolatus*, and *Staphylococcus hominis*

EVA, extravehicular activities
VL2, exit porthole 2
SM, SERVICE MODULE
STR, thermal control system

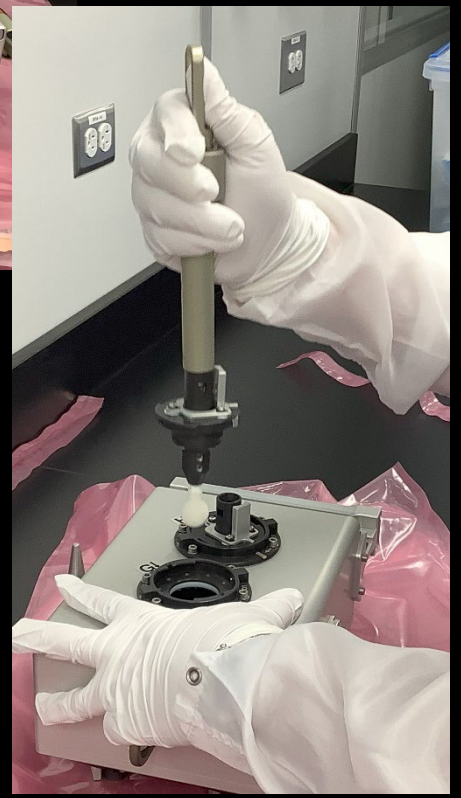
Table 2 The dust sampling chronology

EVA	Date	Cosmonauts who collected samples
EVA-25 (two samples)	15.11.2010	F. Yurchikhin
EVA-35 (two samples)	22.08.2013	A. Misurkin
EVA-38 (two samples)	19.06.2014	O. Artemiev
EVA-39 (two samples)	18.08.2014	O. Artemiev
EVA-40 (two samples)	20.10.2014	A. Samokutiaev
EVA-41 (five samples)	10.08.2015	M. Kornienko
EVA-42 (three samples)	26.02.2016	O. Volkov, Yu. Malenchenko

- All identified organisms also found inside ISS
- Only able to culture organisms from stained samples
- Not able to cultivate microorganisms from beneath thermal blankets

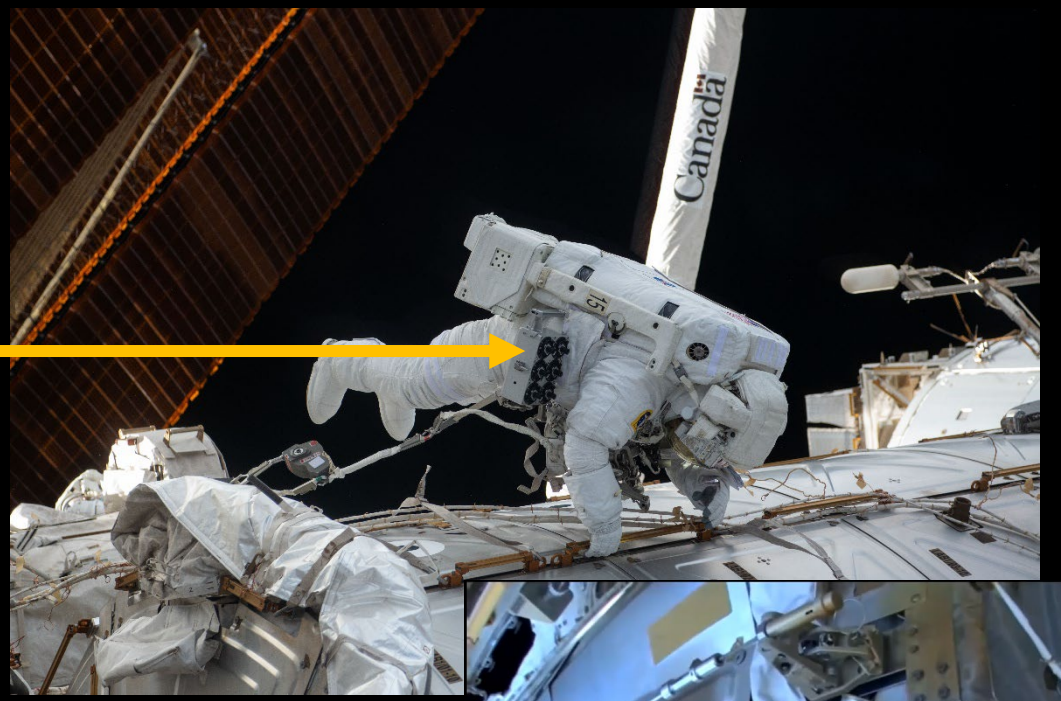
Space Walk Sampling Caddy

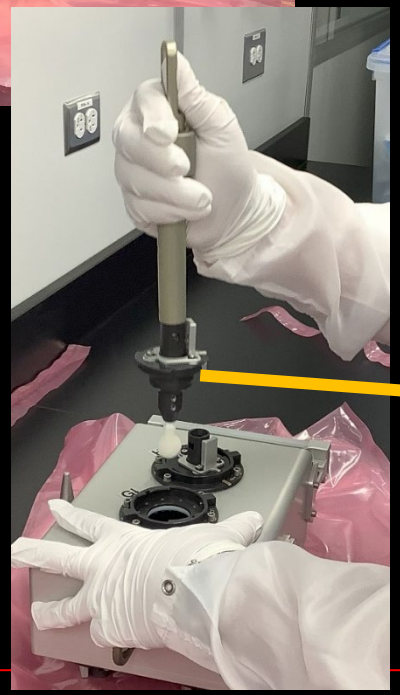
5 years

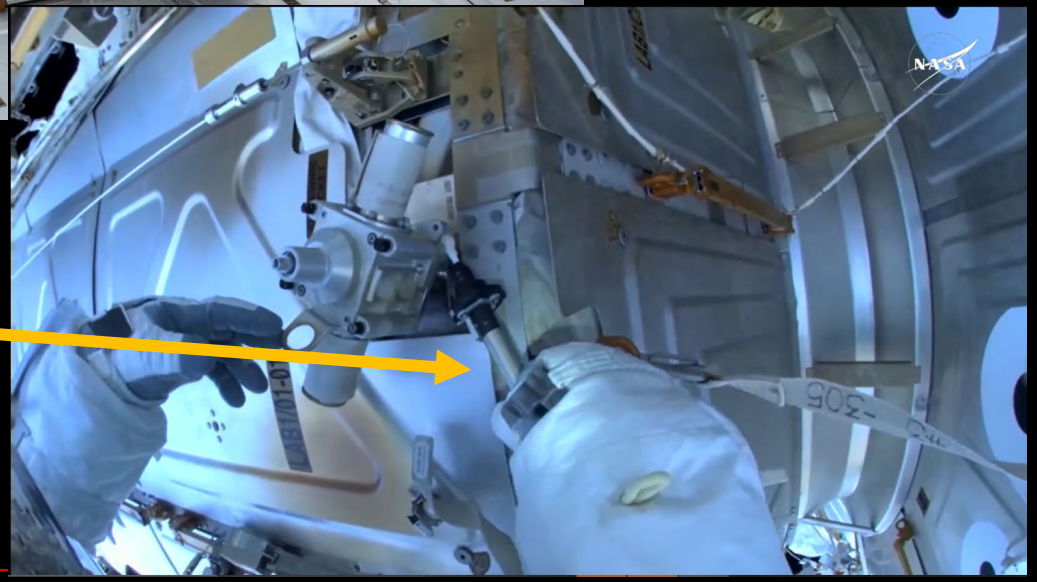


Delivered for launch in August 2023
EVA conducted Jan. 2025

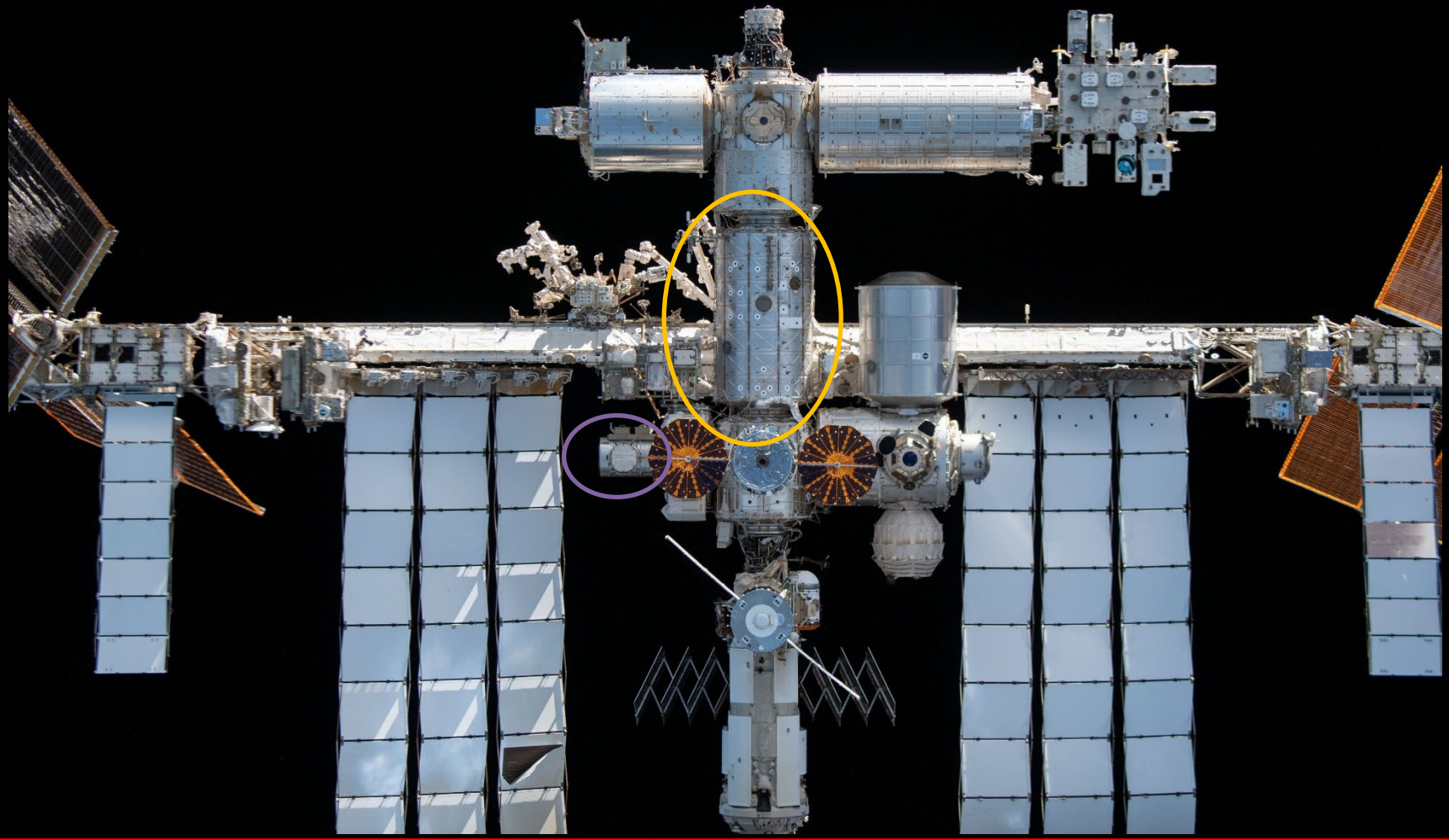
Space Walk Sampling Caddy has 8 Swabs





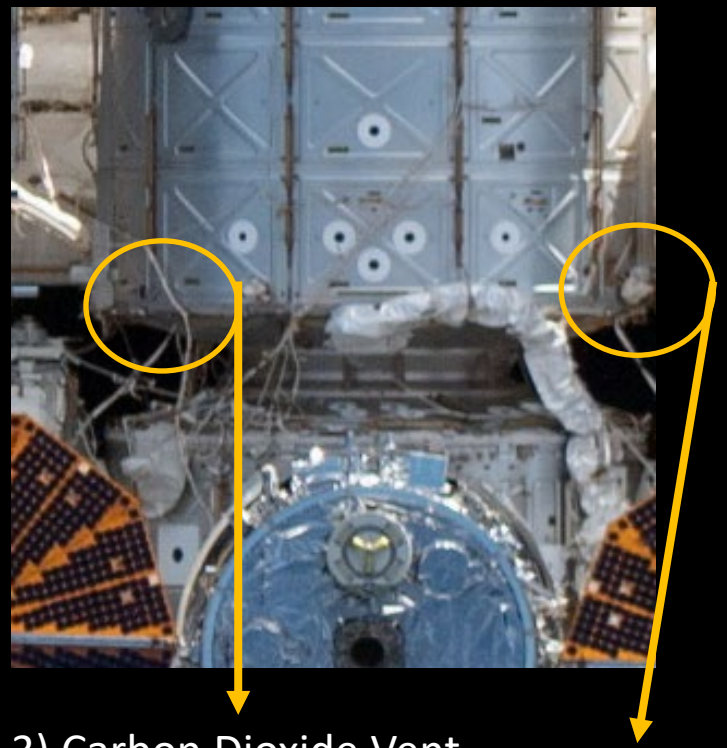


Sample Locations Near the Airlock and on the Lab Module



https://www.nasa.gov/image-article/space-station-pictured-from-spacex-crew-dragon-3/

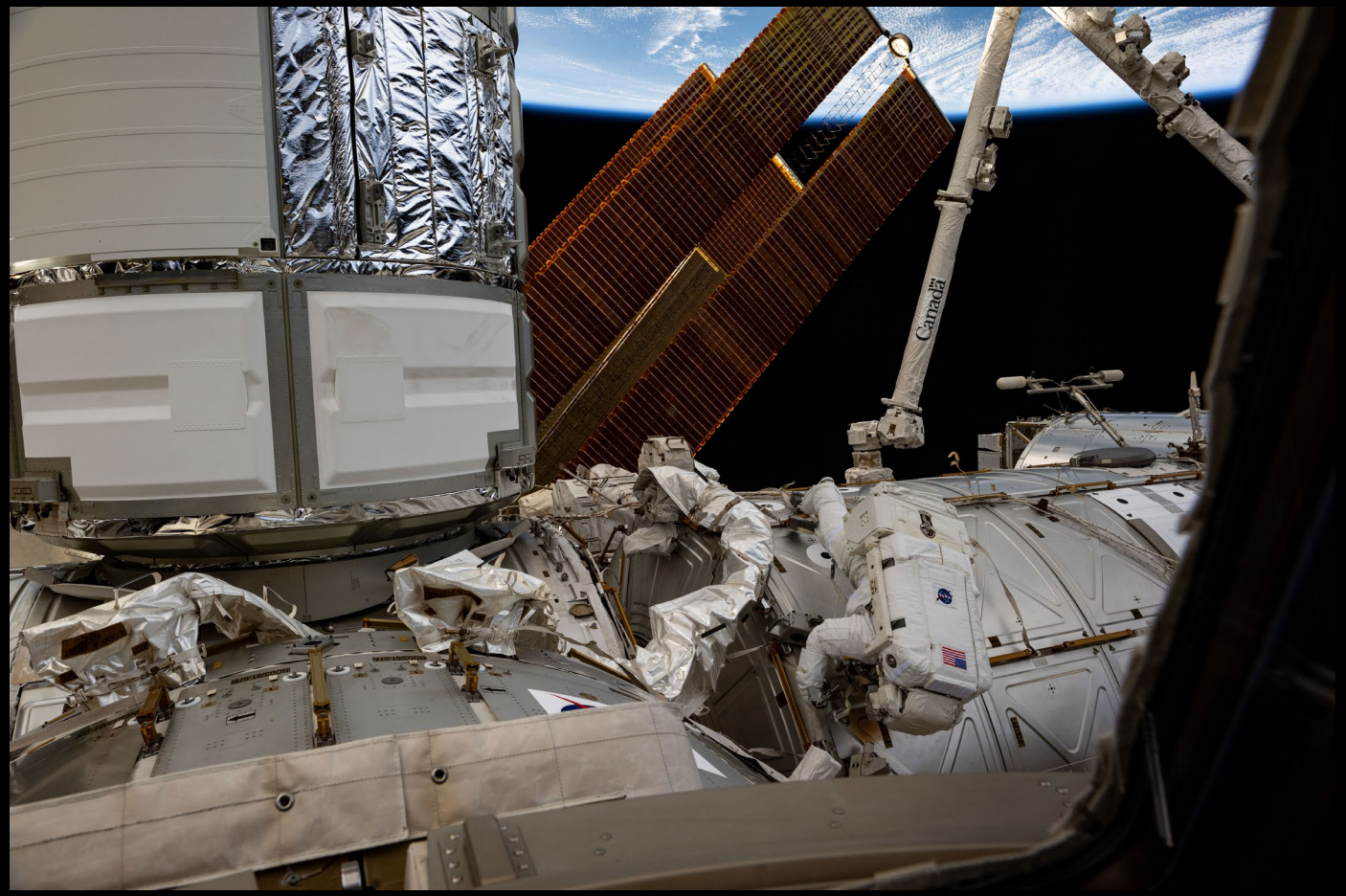
Sample Locations on the Lab Module



3) Carbon Dioxide Vent

1) “Atmosphere Sample”: Open a swab at this location but do not touch it to any surface

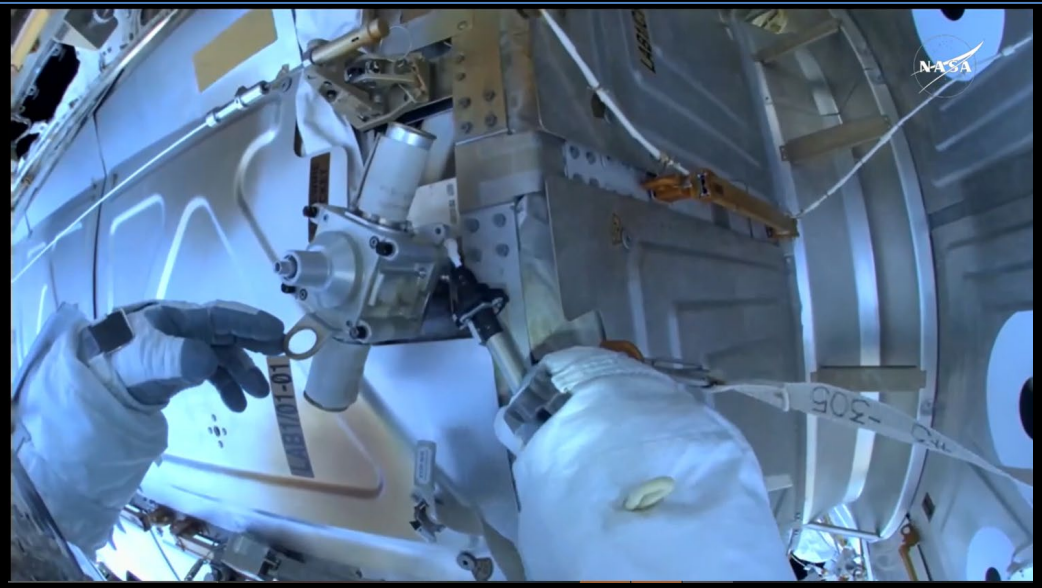
2) Vacuum Exhaust Vent



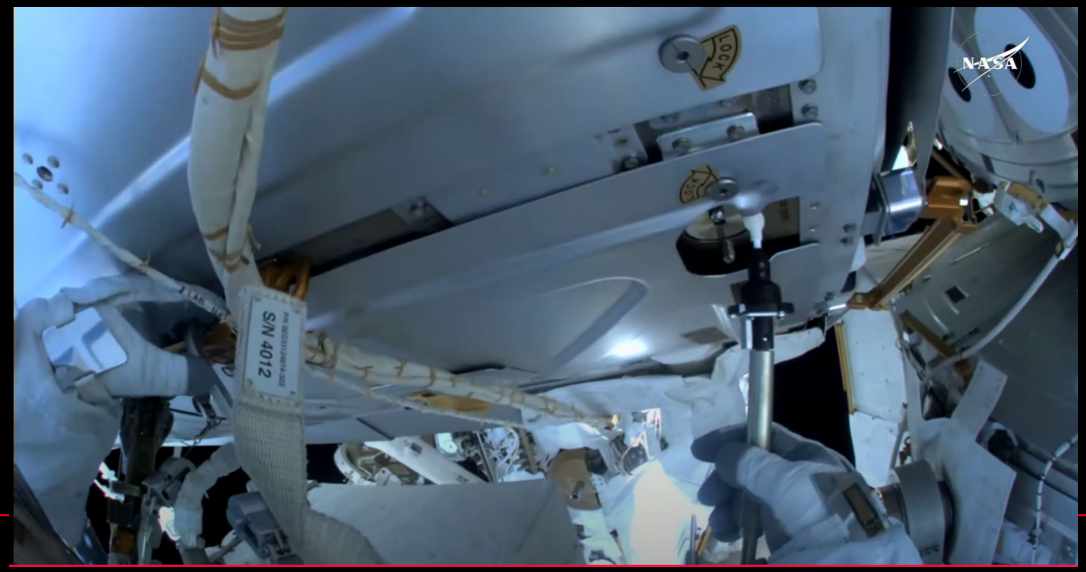
https://www.nasa.gov/image-article/space-station-pictured-from-spacex-crew-dragon-3/

Sample Locations on the Lab

1) “Atmosphere Sample”: Open a swab at this location but do not touch it to any surface

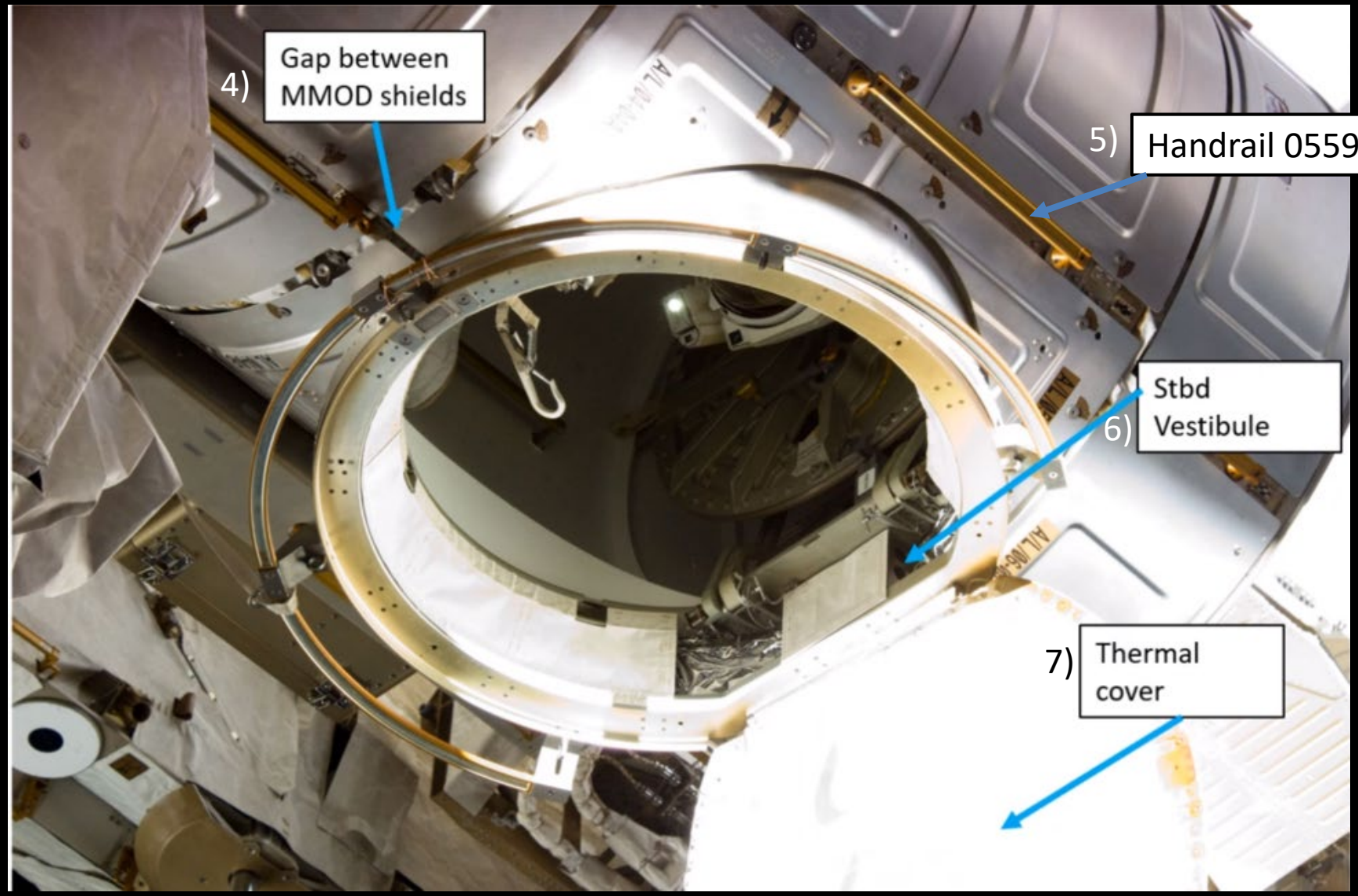


2) Vacuum Exhaust Vent



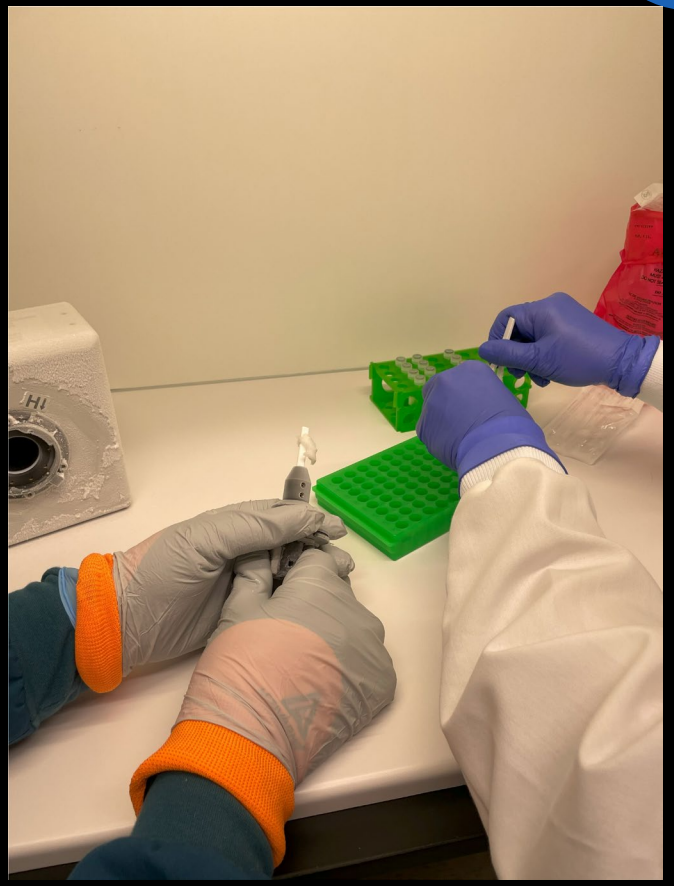
3) Carbon Dioxide Vent

Sample Locations Around the Airlock



8) Negative Control: Swab never opened in space

DNA Extraction on the Ground

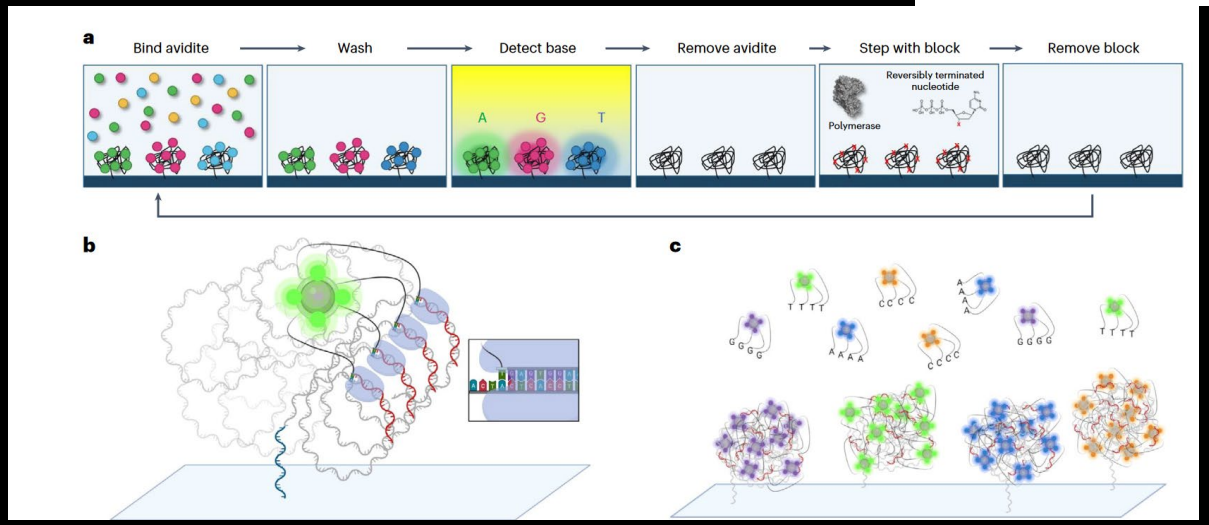


Bead beating to release cells from the swab head

Qiagen UCP DNA Extraction Kit

Spike in controls (zymo) added to several blanks

Metagenomic Sequencing with Los Alamos National Lab (Patrick Chain)



Element Biosciences Aviti Sequencing Platform
uses rolling circle amplification to increase signal to noise ratio

Arslan S. et al. 2024. Sequencing by avidity enables high accuracy with low reagent consumption. *Nature Biotechnology* 42:132–138.

- Element Biosciences Aviti Sequencer
- Data shared and processed using EDGE Bioinformatics
- Sequence Based / Taxonomic Analysis
  - BLASTnt, GOTTCHA2, Kraken2, PanGIA
- Metagenome Assembly
  - CONCOCT, MaxBin2, MetaBAT2

Detected Similar Organisms as Those Cultured from Russian Samples

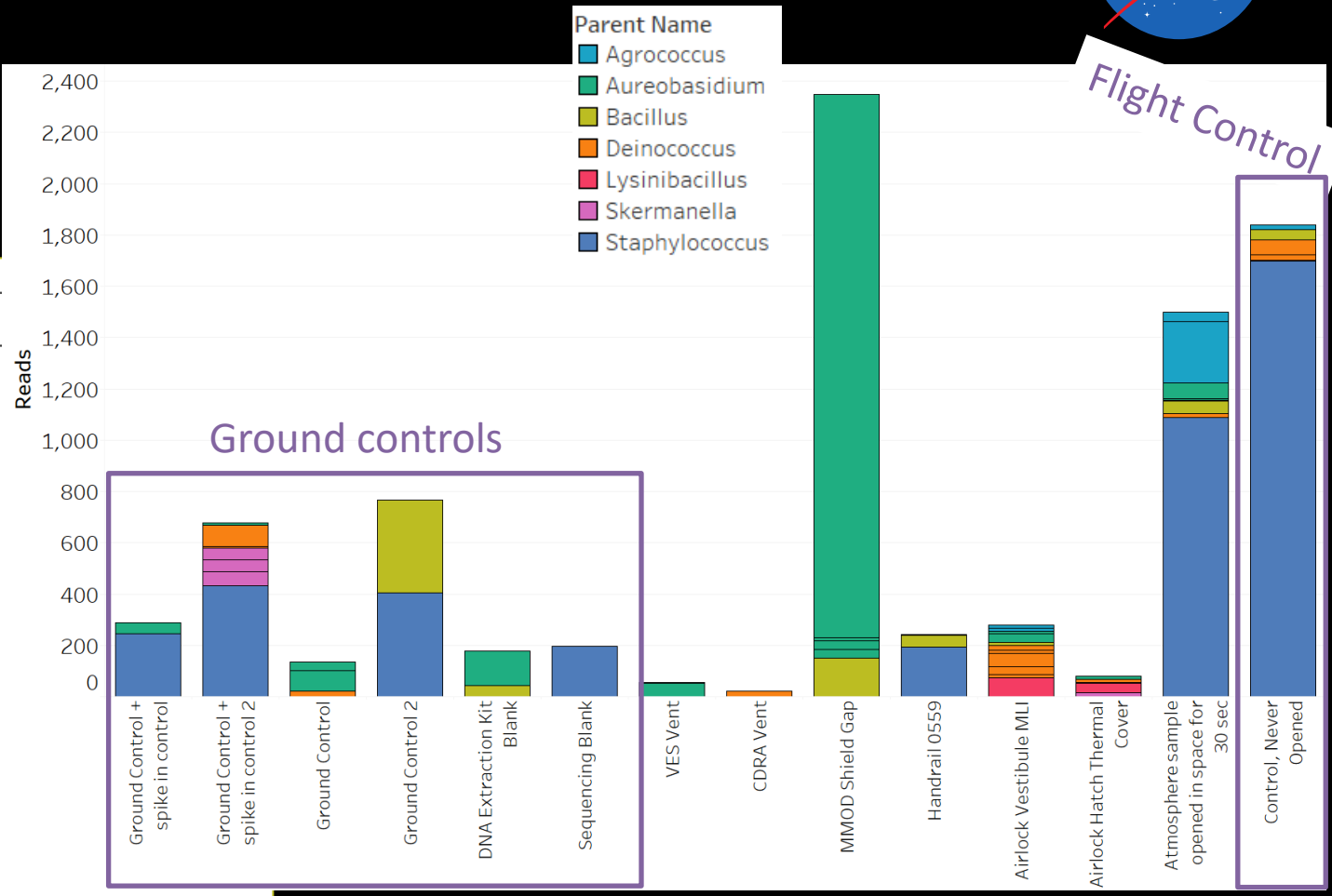
BioNanoScience
https://doi.org/10.1007/s12668-019-00712-1

Microbiological Investigation of the Space Dust Collected from the External Surfaces of the International Space Station

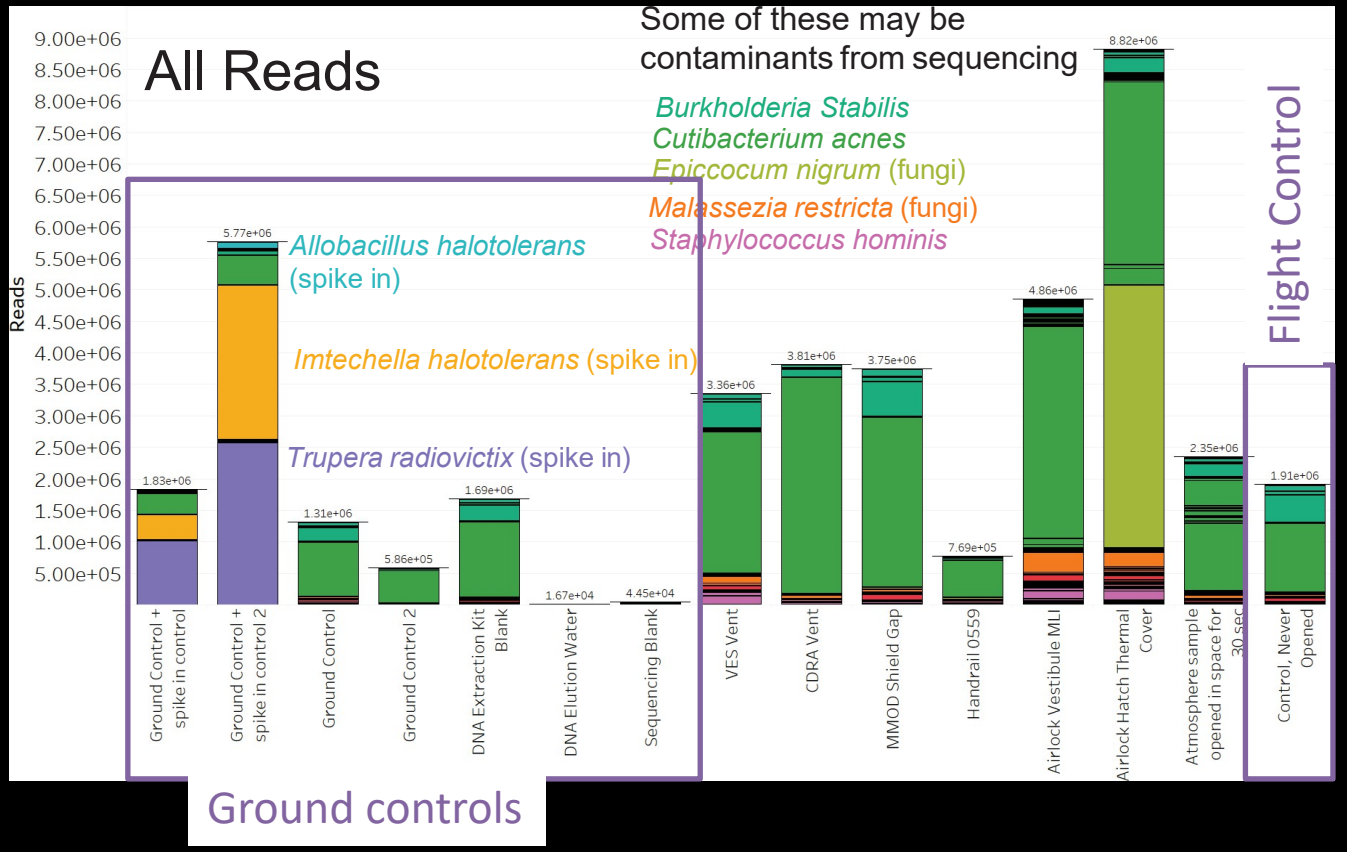
Elena A. Deshevaya[1] · Elena V. Shubralova[2] · Svetlana V. Fialkina[1,3] · Aleksandr A. Guridov[1] · Natalia D. Novikova[1] · Oleg S. Tsygankov[4] · Pavel S. Lianko[2] · Oleg I. Orlov[1] · Sergey P. Morzunov[5] · Albert A. Rizvanov[5,6] · Irina V. Nikolaeva[7]

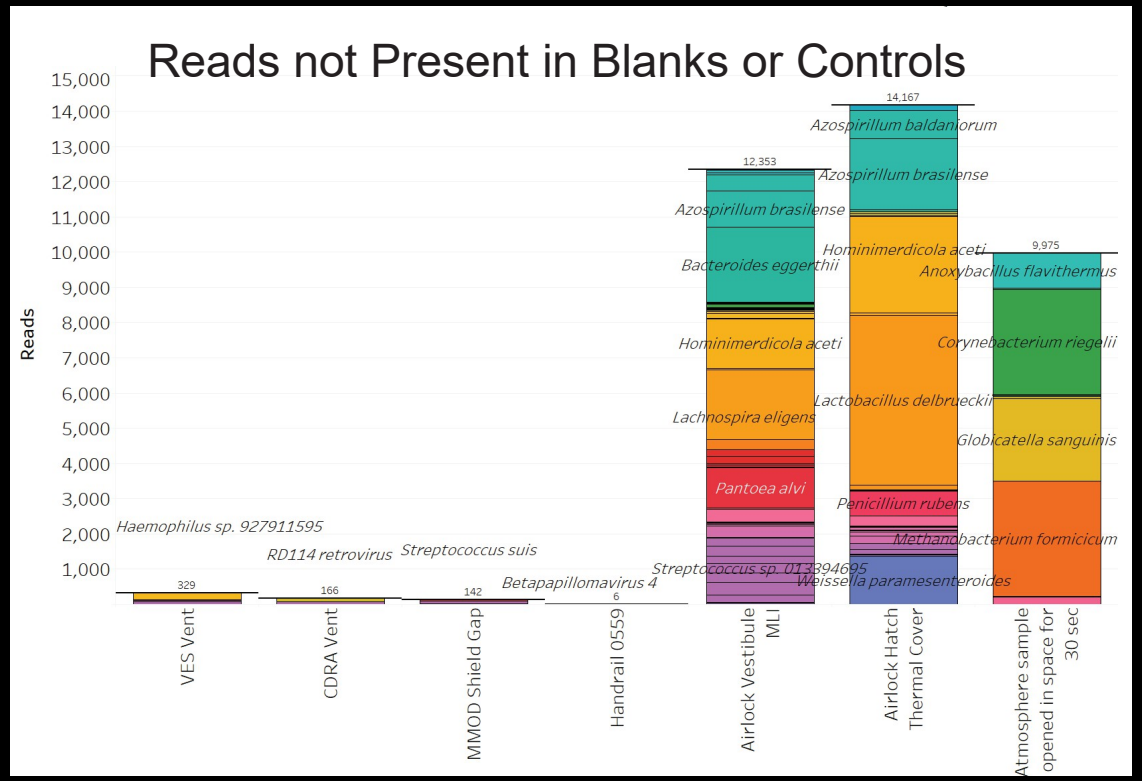
EVA/date	Location sampled	Swab appearance	Microorganisms
EVA-25 15 November 2010	Valves of trace contamination removal unit	Black spots	*Bacillus licheniformis*
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Black spots	*Bacillus sphaericus*; *B. subtilis*
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Gray spots	*B. subtilis*
EVA-38 19 June 2014	SM, window 2	Black spots	*Bacillus pumilus*
EVA-41 10 August 2015	SM, between tubes of radiator (STR)	Black spots	*Bacillus pumilus*; *Aureobasidium* sp.
EVA-42 26 February 2016	The exit porthole of the module "PIRS" surface	Gray spots	*Bacillus sphaericus*
EVA-42 26 February 2016	The exit porthole of the module "PIRS," window, and hatch frame	Black and gray spots, bright grains	*Bacillus licheniformis*; *Bacillus pumilus*; assemblage of *Agrococcus jenensis*, *Skermanella aerolata*, *Deinococcus aerolatus*, and *Staphylococcus hominis*

EVA, extravehicular activities
VL2, exit porthole 2
SM, SERVICE MODULE
STR, thermal control system



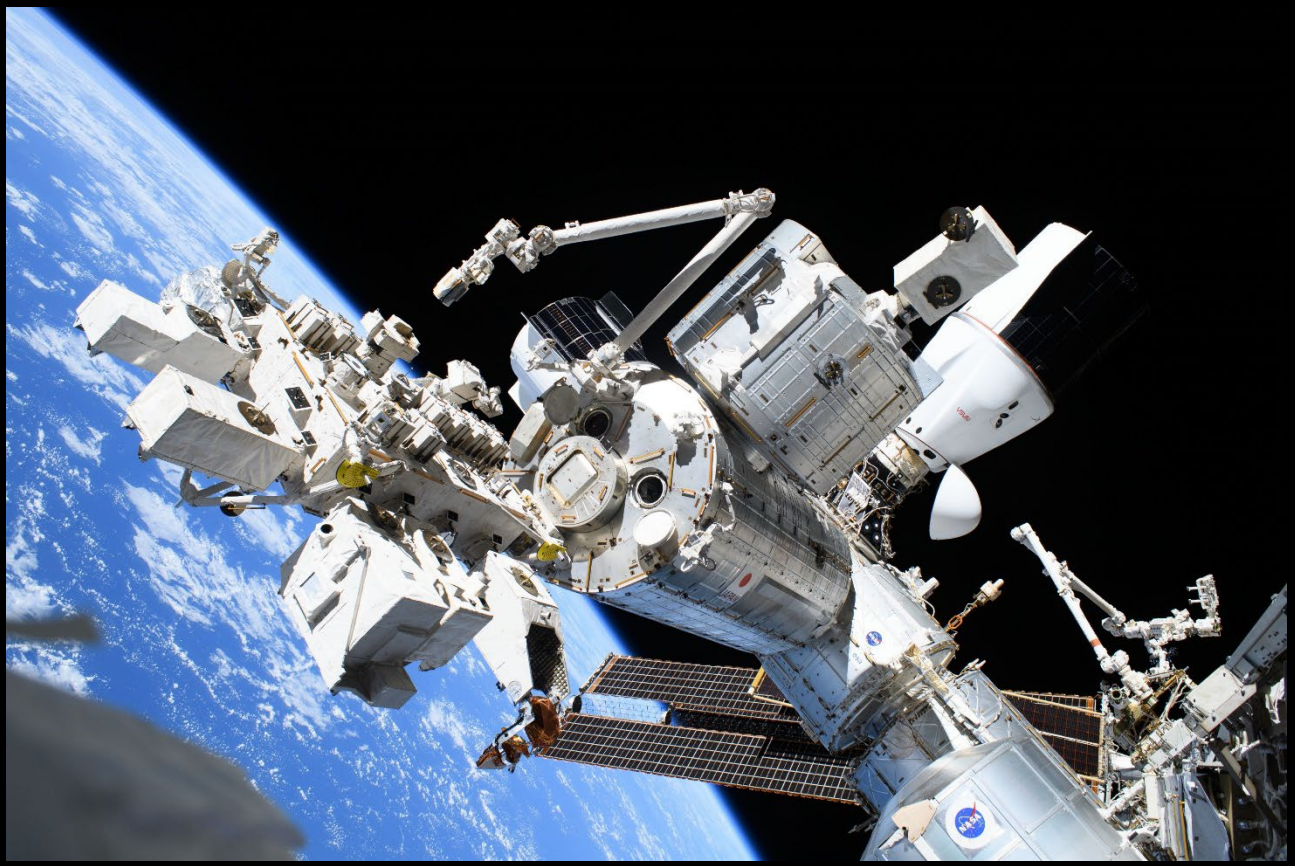
Does the Atmosphere around the suit have its own microbiome?





Observe similar clustering using binning methods

We are Planning a Second EVA



NASA: https://flic.kr/p/2r2h3WA

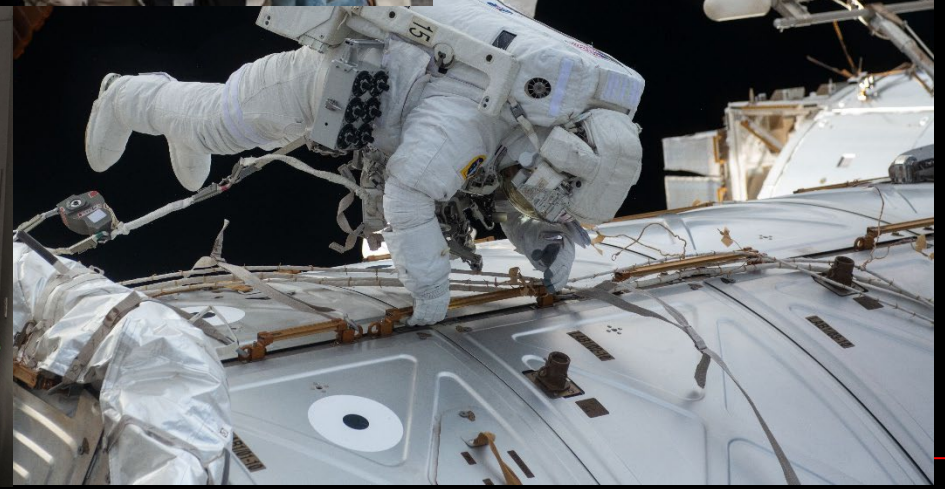
- Originally Scheduled for 01/08/2026
  - On the timeline for 3/18/2026, but not accomplished
- Planned Sampling Locations
  - Replicate “atmosphere” sample and extend exposure time (30 sec. vs. 2 min.)
  - Replicate airlock samples
  - Sample Spacesuit Glove
- Sample Wishlist
  - Science Airlock on JEM

Conclusions and Future Work

- Results represent a first step towards a forward contamination risk assessment for crewed missions
- Vents on ISS do not appear to be leaking much if any detectable DNA
- Crewed airlock the suit itself do appear to be sources of DNA from inside station
- Metagenomic sequencing has improved in recent years and appears to be a viable option for even ultra-low biomass samples.
- Data processing pipelines need work if we want to use this for routine monitoring
- How do we use shotgun metagenomics for monitoring?
  - Standardized protocols, methods and bioinformatic pipelines
  - DNA free reagents and consumables

https://www.flickr.com/photos/nasa2explore/54312641779/in/album-72177720312887519

Conclusions and Future Work



- Vents on ISS do not appear to be leaking much if any detectable DNA
- Crewed airlock and the suit itself do appear to be sources of DNA from inside station
- Metagenomic sequencing has improved in recent years and appears to be a viable option for even ultra-low biomass samples.

  Work needed to be able to extract and analyze these samples in space.

  Data processing pipelines need work if we want to use this for routine monitoring.

  How do we use shotgun metagenomics for monitoring?
  - Standardized protocols, methods and bioinformatic pipelines
  - DNA free reagents and consumables

https://www.flickr.com/photos/nasa2explore/54312641779/in/album-72177720312887519