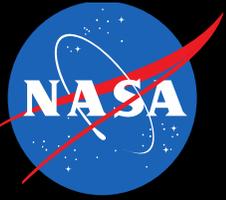


# ISS EXTERNAL MICROORGANISMS: COLLECTING PLANETARY PROTECTION SAMPLES DURING EXTRAVEHICULAR ACTIVITY

National Aeronautics and  
Space Administration



Aaron B. Regberg, Mary Sue Bell, Richard E. Davis,  
Martin L. Tschirschwitz, Sarah Wallace

PPP.3 0005-24

07/14/2024





- **COSPAR/NASA Planetary Protection Knowledge Gaps for Crewed Missions**
- Prototype Hardware
- Ground Test Results
- Flight Hardware
- ISS Sampling Plan

# COSPAR AND NASA HAVE DEFINED PLANETARY PROTECTION KNOWLEDGE GAPS THAT NEED TO BE ADDRESSED BEFORE CREWED MISSIONS ARRIVE AT MARS



## BACKGROUND

Some organisms can survive exposure to space!



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

images-  
assets.nasa.gov/image/iss018e03922  
7/iss018e039227~orig.jpg

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

# KNOWLEDGE GAP 2H. WHAT MICROBIAL CONTAMINANTS WOULD VENT FROM AN EXTRAVEHICULAR ACTIVITY (EVA) SUIT OR OTHER VEHICLES?



- 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?



Apollo 12 Mission image - Astronaut Bean deploys ALSEP Central Station

# KNOWLEDGE GAP 2B. WHAT LEVEL OF NON-VIABLE MICROBIAL CONTAMINATION ESCAPE IS ACCEPTABLE?



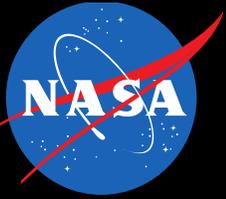
## Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions

G Kminek, BC Clark, CA Conley, MA Jones, M Patel, MS Race, MA Rucker, O Santolik, B Siegel & JA Spry (Eds.)

- 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?

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



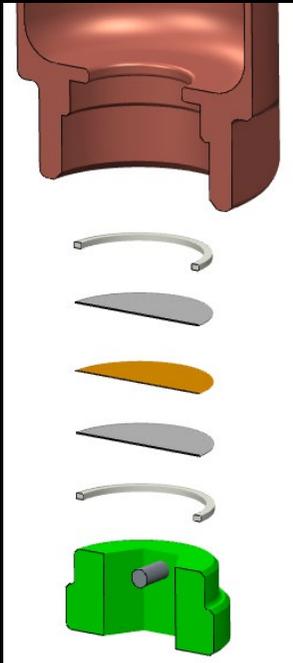


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# PROTOTYPE PAYLOAD 8-SAMPLE EVA SWAB KIT



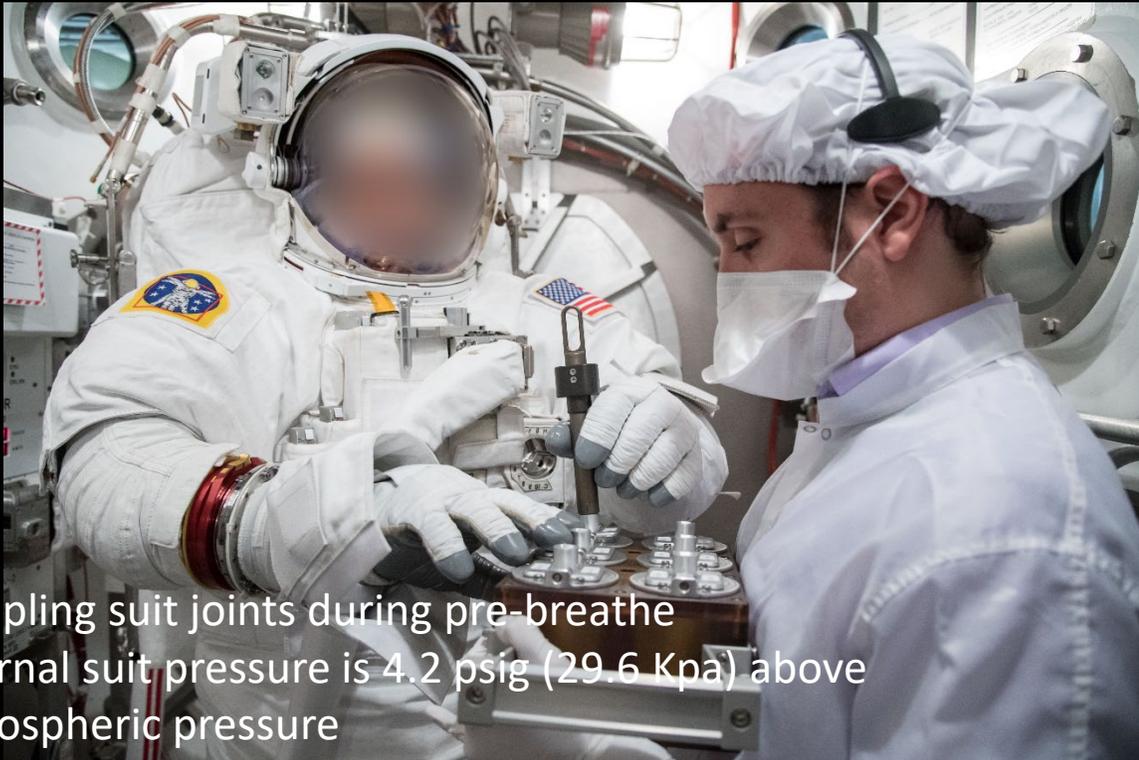
- 6 on top, 2 on bottom (not shown)
- 0.22 micron pore Microbial filter in bottom of each container to accommodate pressure changes



Repurposed Shuttle tile repair handle

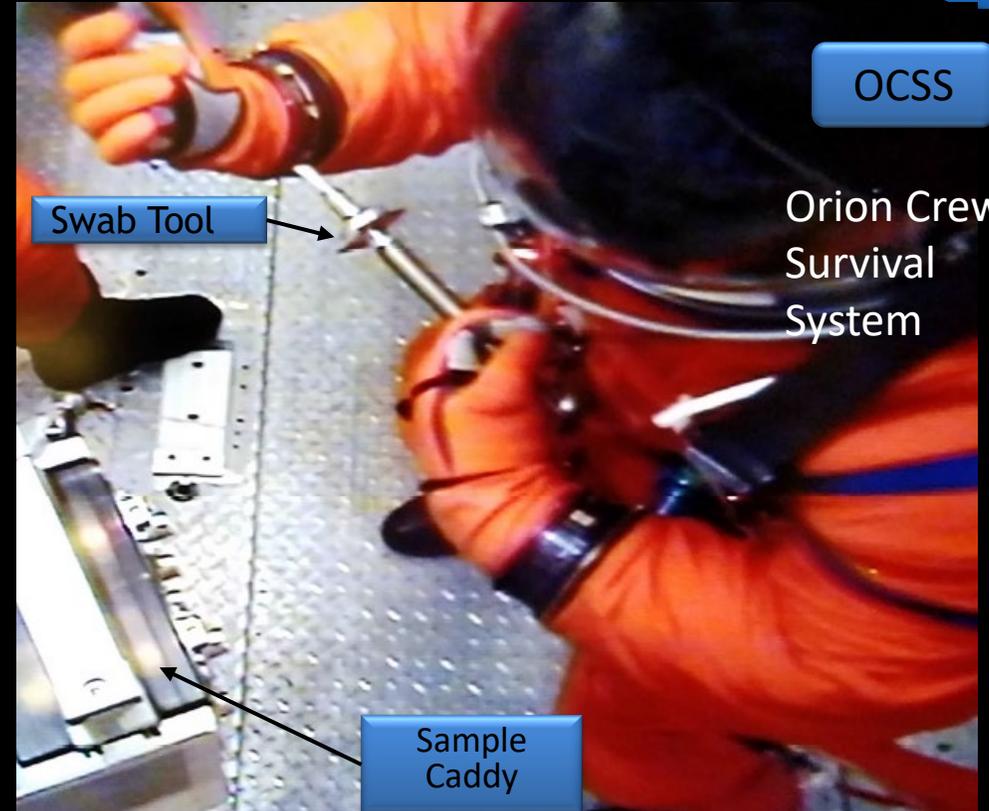
Rucker, Michelle A., Drew Hood, Mary Walker, Kasthuri J. Venkateswaran, and Andrew C. Schuerger. 2018. "EVA Swab Tool to Support Planetary Protection and Astrobiology Evaluations." In 2018 IEEE Aerospace Conference, 1–9. IEEE. <https://doi.org/10.1109/AERO.2018.8396381>.

# GROUND TESTING WITH FLIGHT LIKE ISS AND ORION SUITS



Sampling suit joints during pre-breathe  
Internal suit pressure is 4.2 psig (29.6 Kpa) above atmospheric pressure

**NOTE: we would NOT expose EMU wrist joints on ISS**

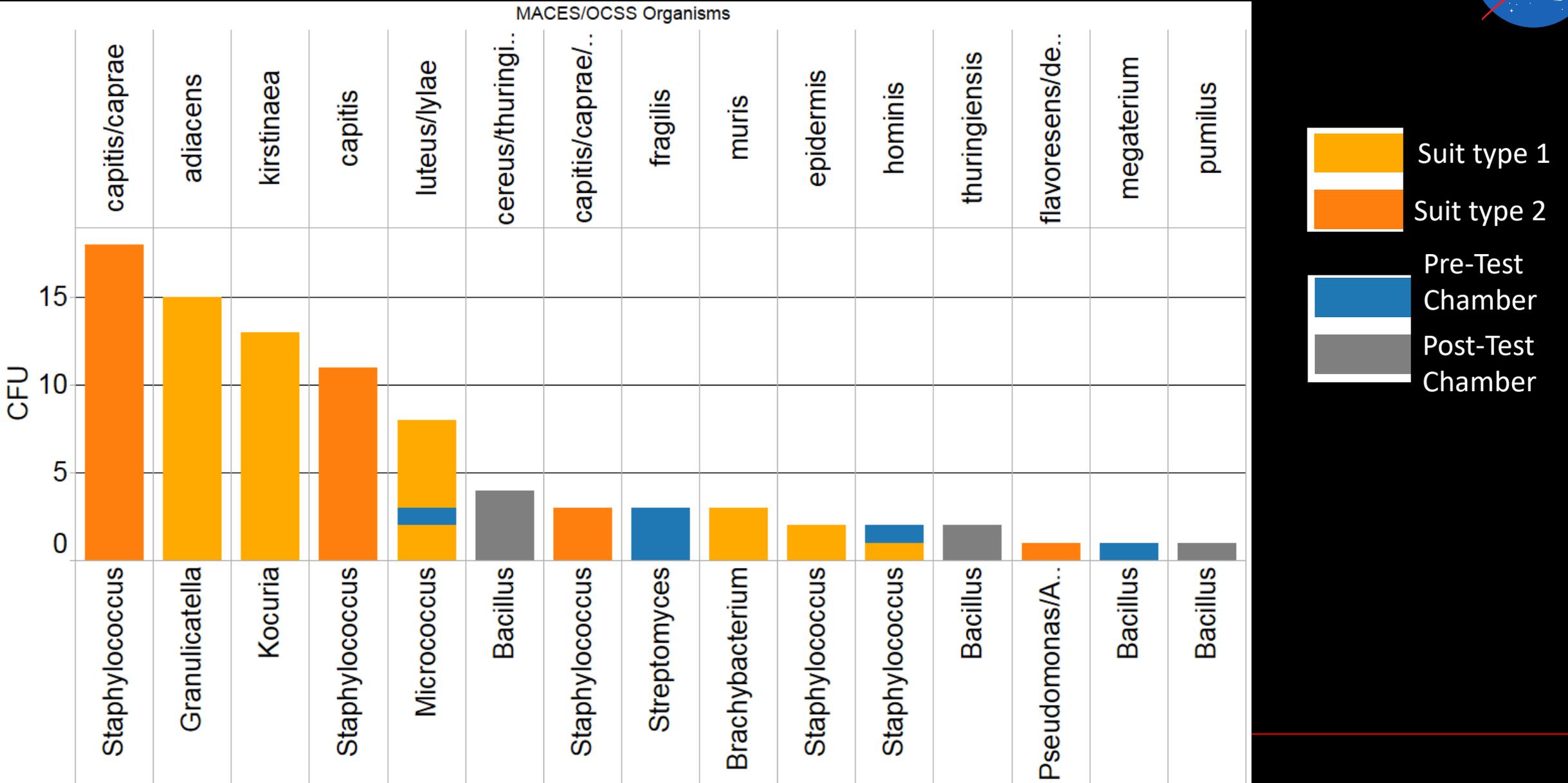
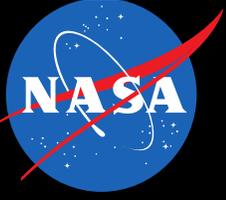


Sampling suit at vacuum in the 11 ft. chamber at Johnson Space Center  
Internal suit pressure is 4.2 psia 29.6 Kpa)

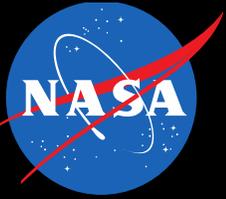


- COSPAR/NASA Planetary Protection Knowledge Gaps for Crewed Missions
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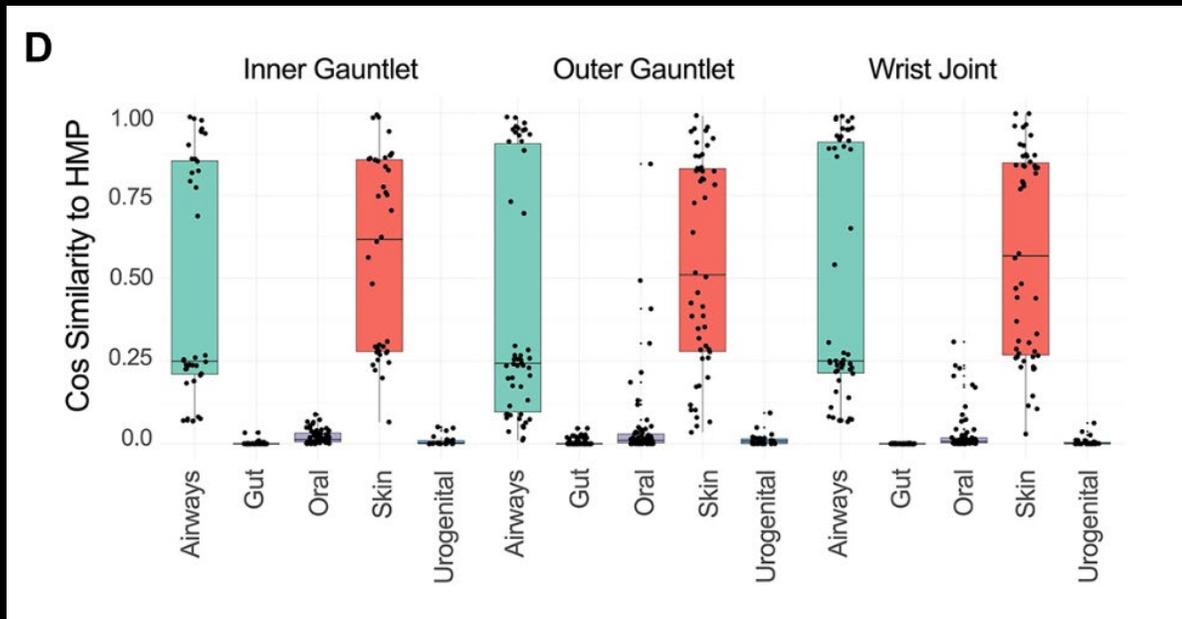
# 4-6 HOURS OF VACUUM DOES NOT KILL BACTERIA ON SPACE SUIT SURFACES



# MICROBES ON SPACESUITS ARE SIMILAR TO MICROBES FROM HUMAN SKIN AND AIRWAYS



- We used Shotgun metagenomic sequencing to further characterize the microbial community in these samples
- Spacesuits are cleaner than human skin but external surfaces are not sterile.



Danko, D., Malli Mohan, G. B., Sierra, M. A., Rucker, M., Singh, N. K., Regberg, A. B., et al. (2021). Characterization of Spacesuit Associated Microbial Communities and Their Implications for NASA Missions. *Frontiers in Microbiology*, 12, 1900. <https://doi.org/10.3389/fmicb.2021.608478>

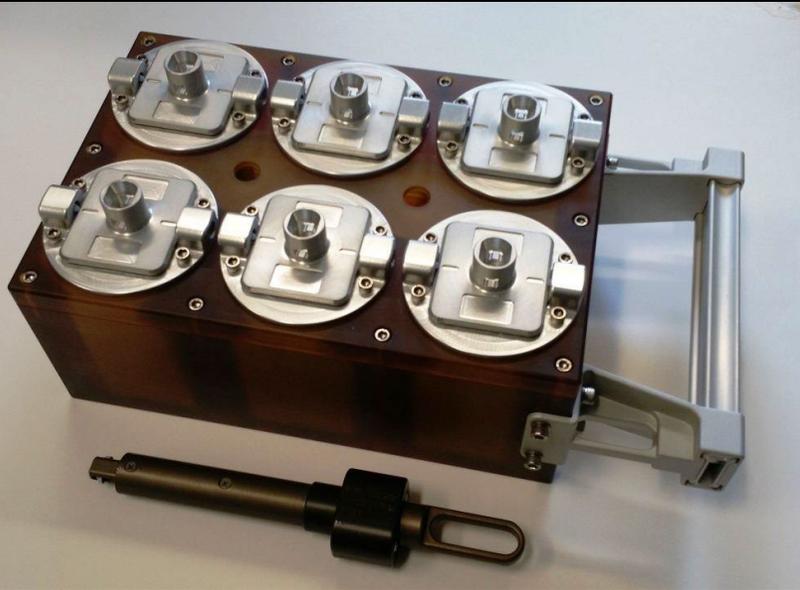


David Danko



- COSPAR/NASA Planetary Protection Knowledge Gaps for Crewed Missions
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- **Flight Hardware**
- ISS Sampling Plan

# HARDWARE DESIGN WAS UPDATED BASED ON GROUND TESTING RESULTS AND NASA EVA SAFETY REQUIREMENTS

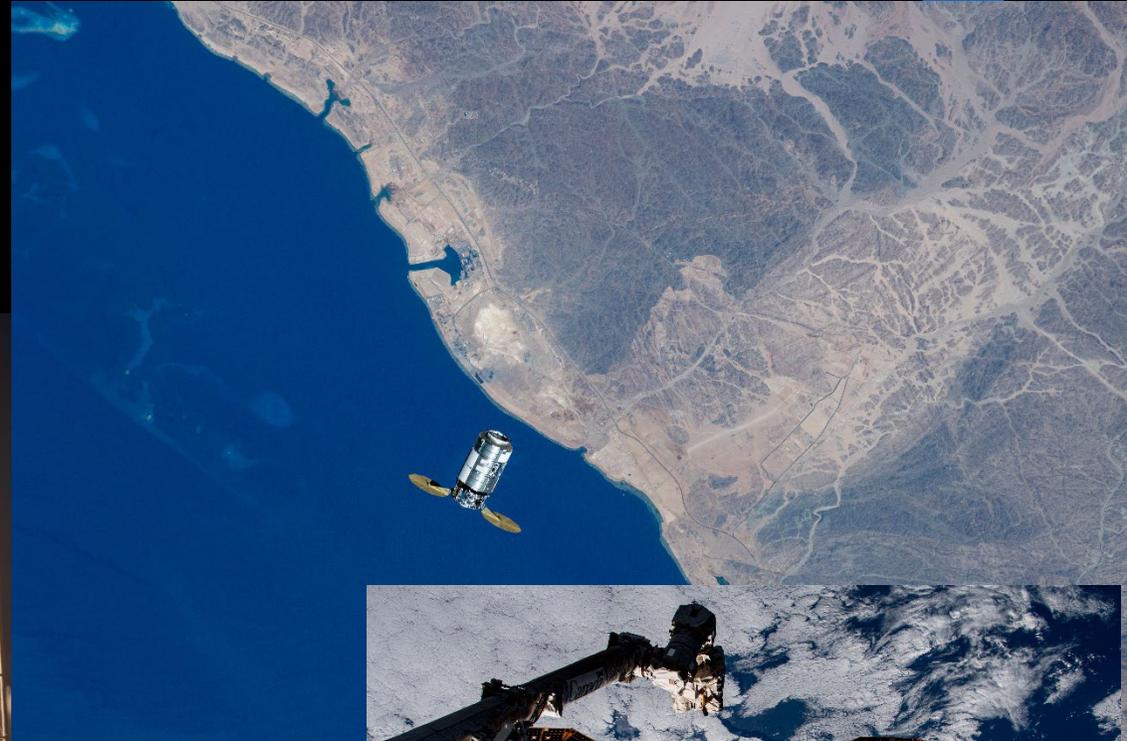


5 years

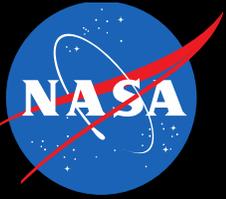


Delivered for launch in August 2023

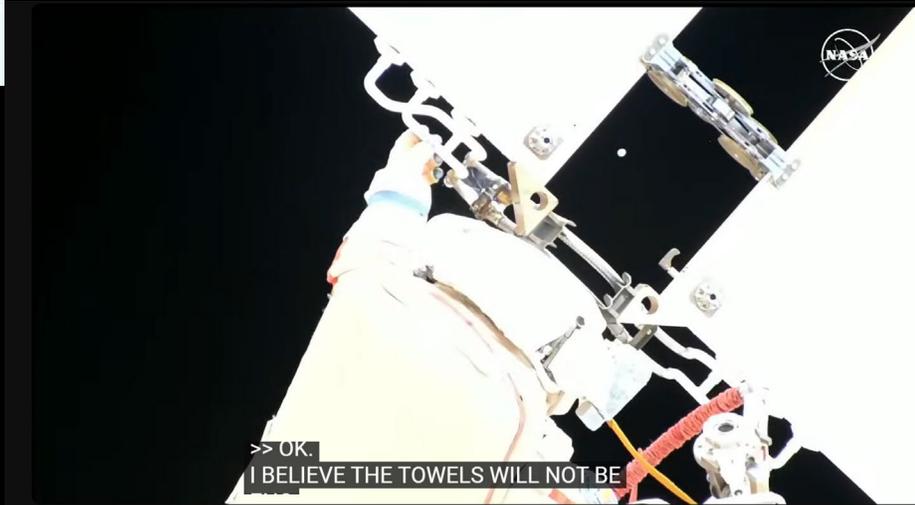
# LAUNCHED ON NG-19 IN AUGUST OF 2023



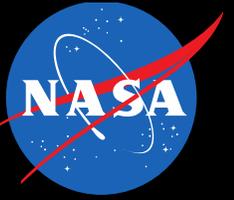
# EVA DELAYED FROM OCTOBER 2023 TO 6/13/2024



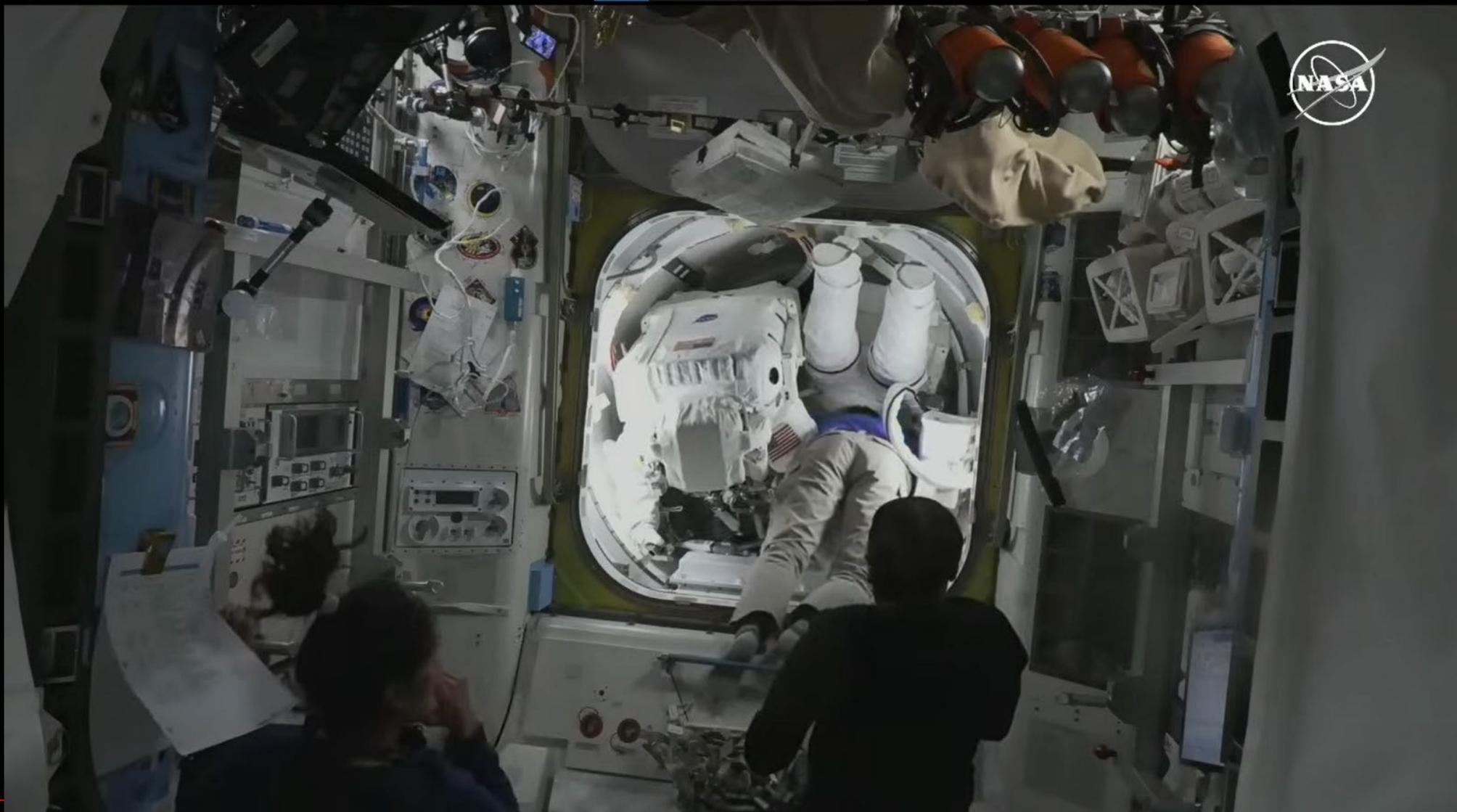
- Radiator coolant leak delayed US EVA's
- Coolant is non-toxic silicone oil



# 6/13 EVA SCRUBBED DUE TO SUIT DISCOMFORT RESCHEDULED FOR 6/24



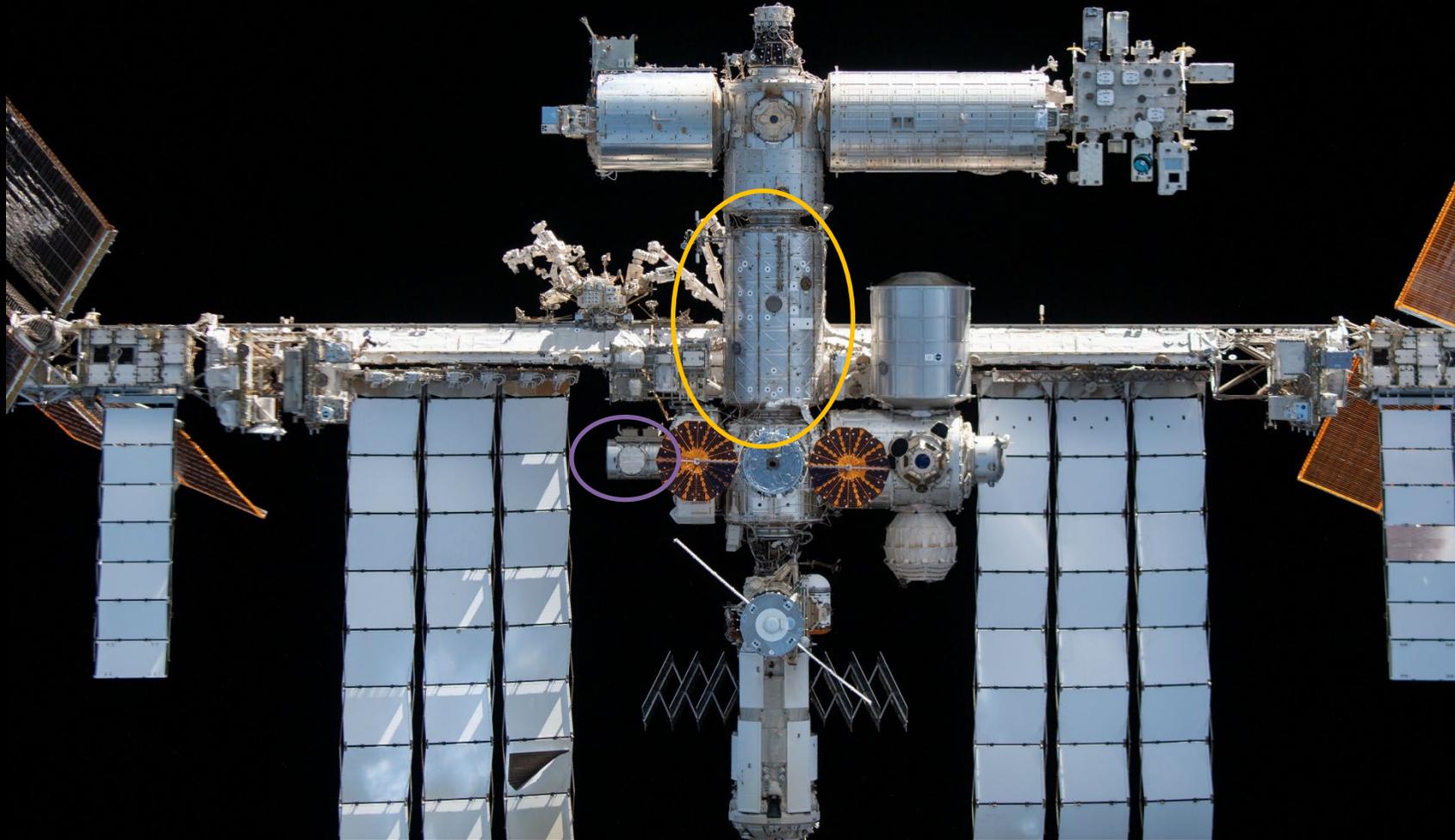
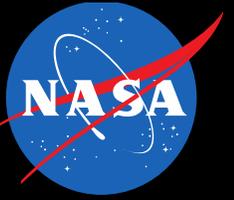
# 6/24 EVA SCRUBBED DUE TO AN UMBILICAL WATER LEAK RESCHEDULED FOR 7/29



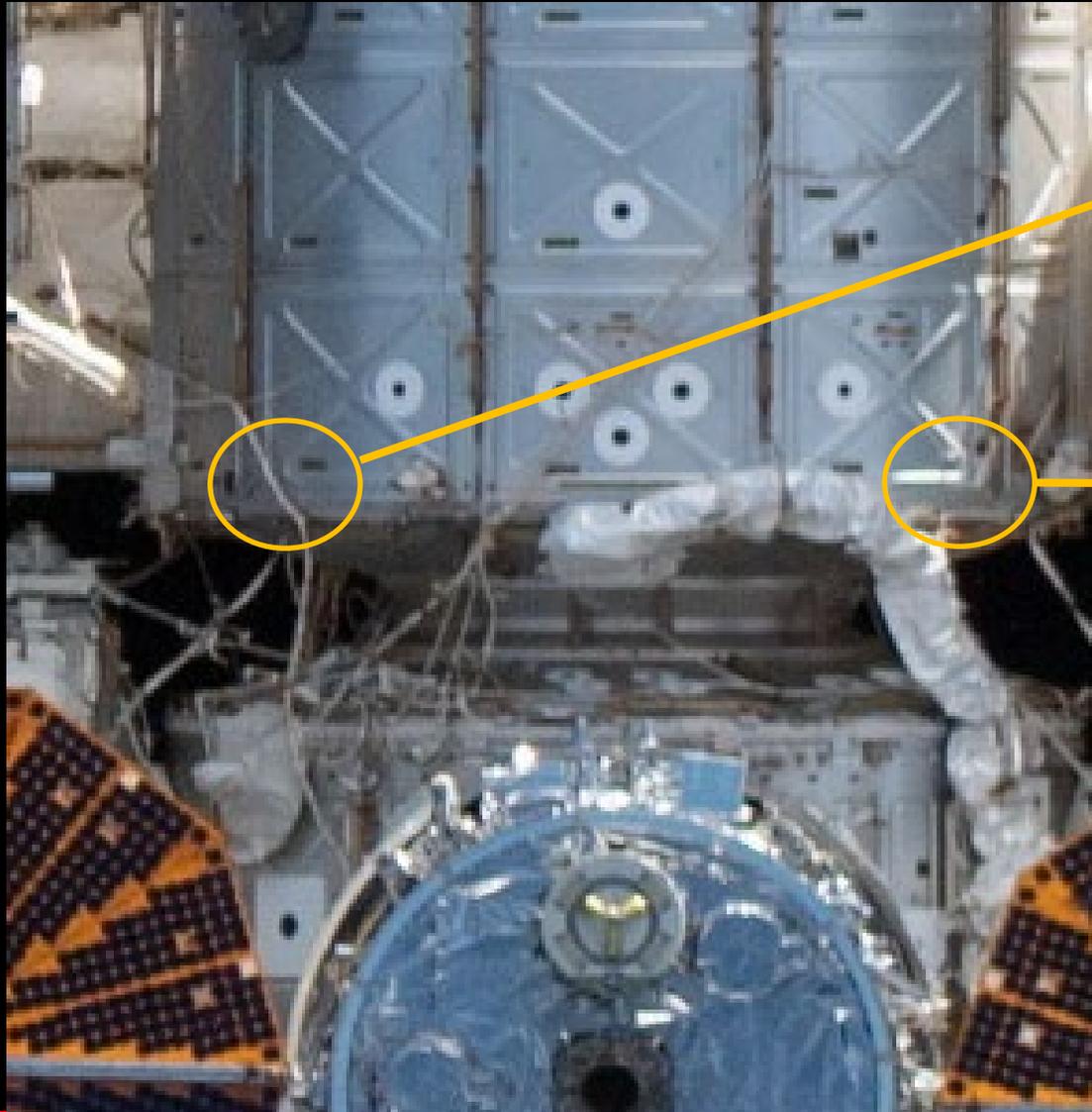


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# SAMPLE LOCATIONS NEAR THE AIRLOCK AND ON THE LAB MODULE



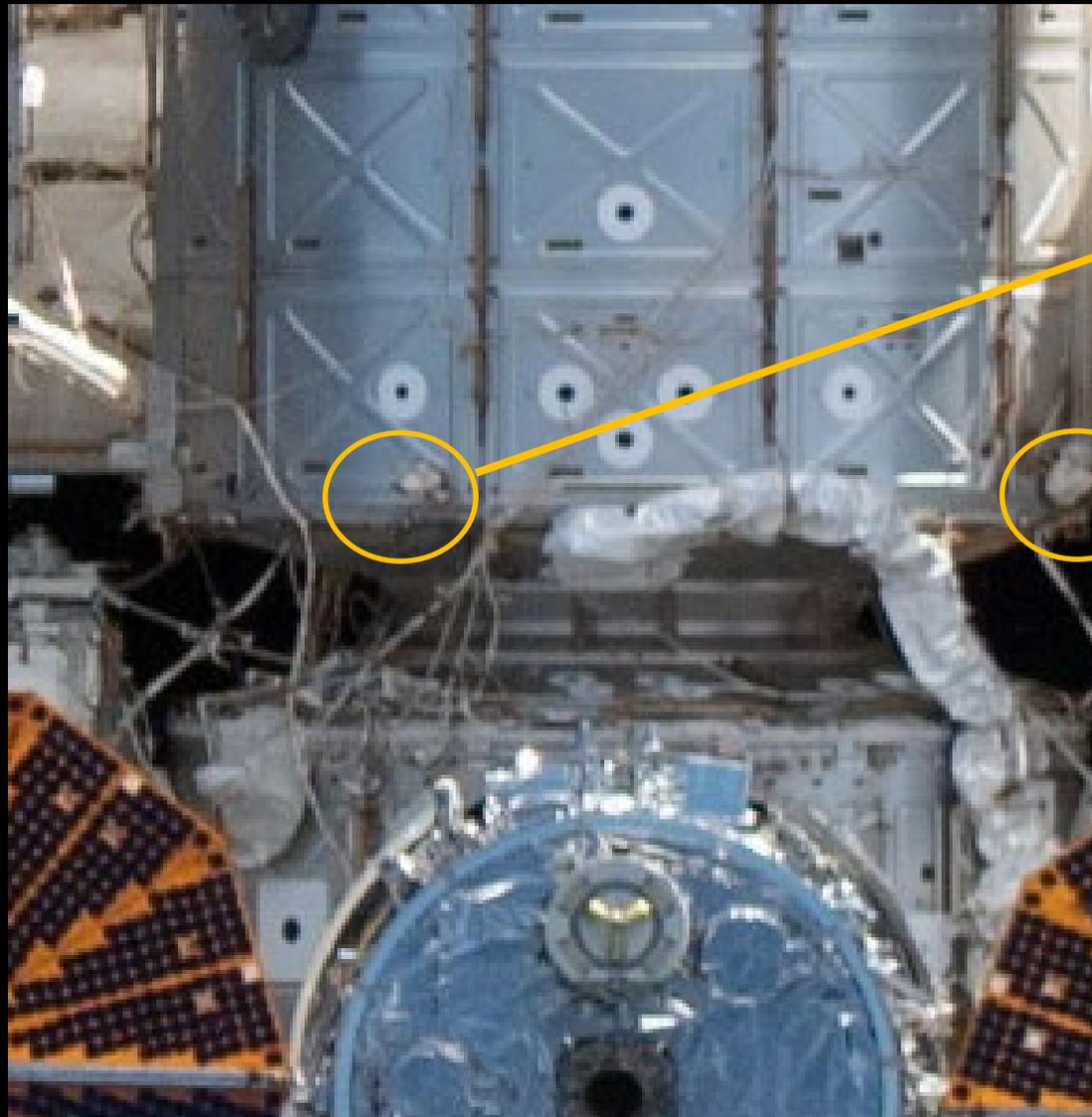
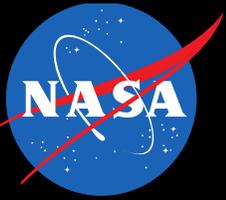
# SAMPLE LOCATIONS ON THE LAB MODULE



Carbon Dioxide Vent

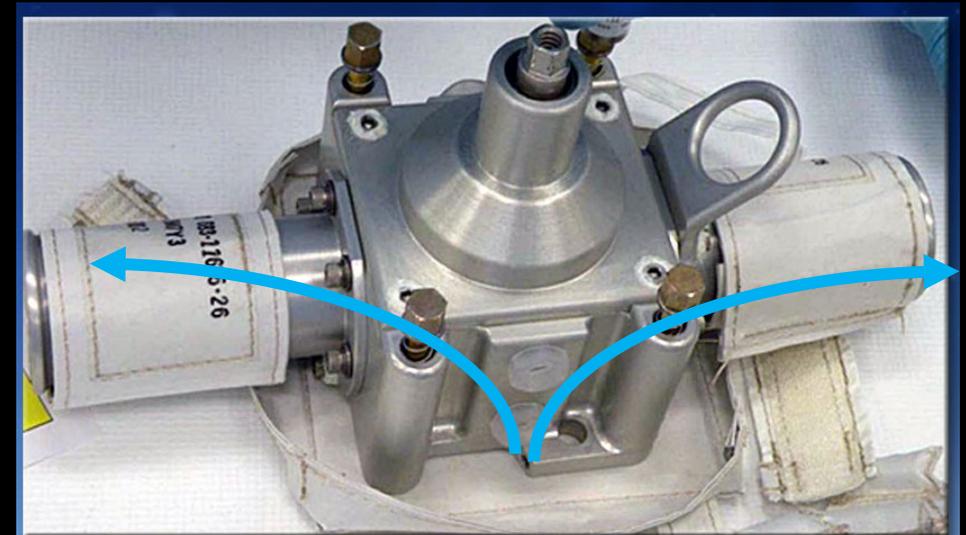
Vacuum Exhaust Vent

# SAMPLE LOCATIONS ON THE LAB MODULE

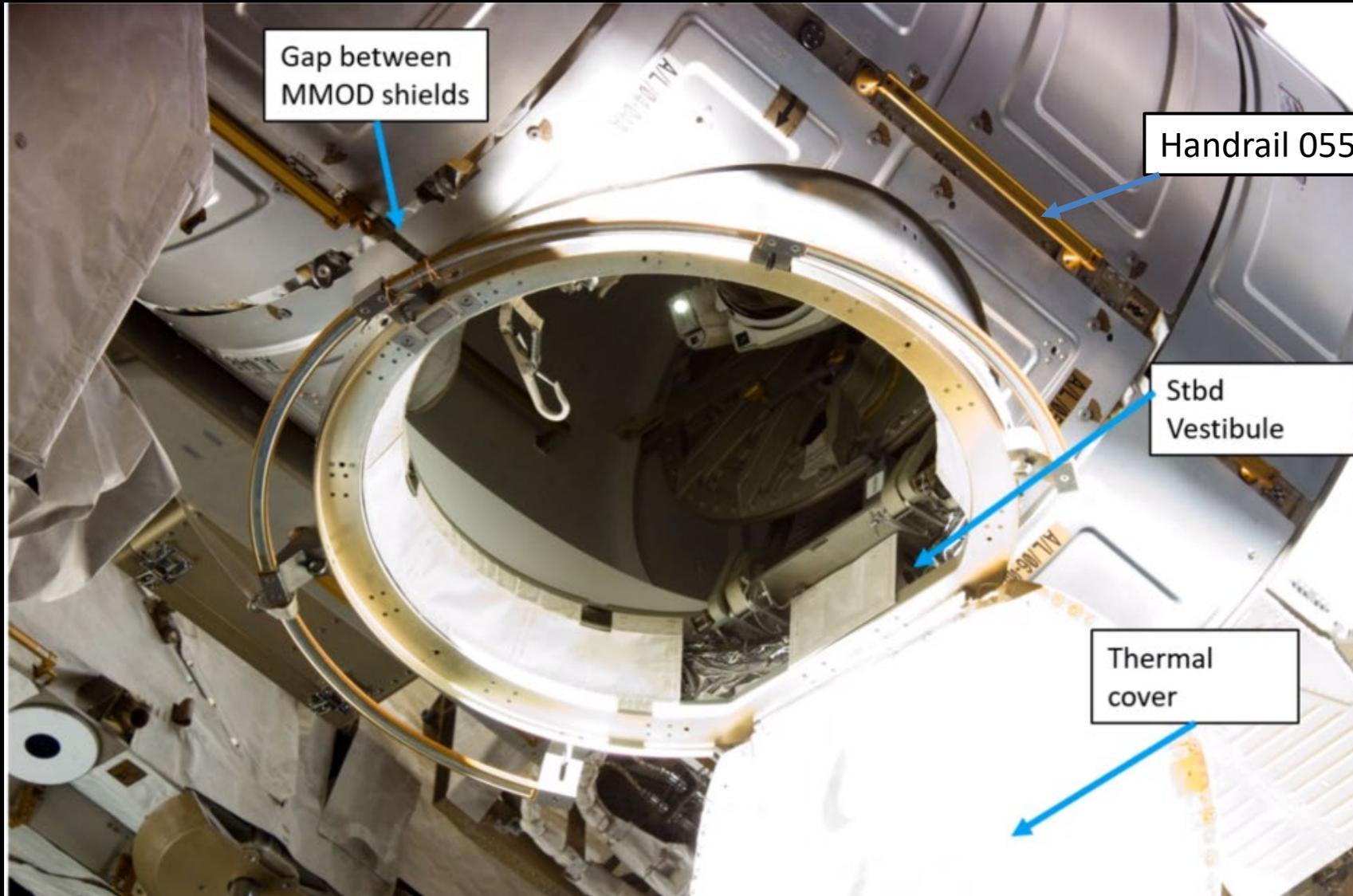
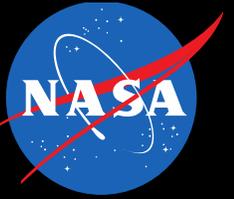


Carbon Dioxide Vent

Vacuum Exhaust Vent



# SAMPLE LOCATIONS AROUND THE AIRLOCK



# RUSSIA HAS ALSO SAMPLED THEIR 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<sup>1</sup> · Elena V. Shubralova<sup>2</sup> · Svetlana V. Fialkina<sup>1,3</sup> · Aleksandr A. Guridov<sup>1</sup> · Natalia D. Novikova<sup>1</sup> · Oleg S. Tsygankov<sup>4</sup> · Pavel S. Lianko<sup>2</sup> · Oleg I. Orlov<sup>1</sup> · Sergey P. Morzunov<sup>5,6</sup> · Albert A. Rizvanov<sup>5,6</sup> · Irina V. Nikolaeva<sup>7</sup>

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

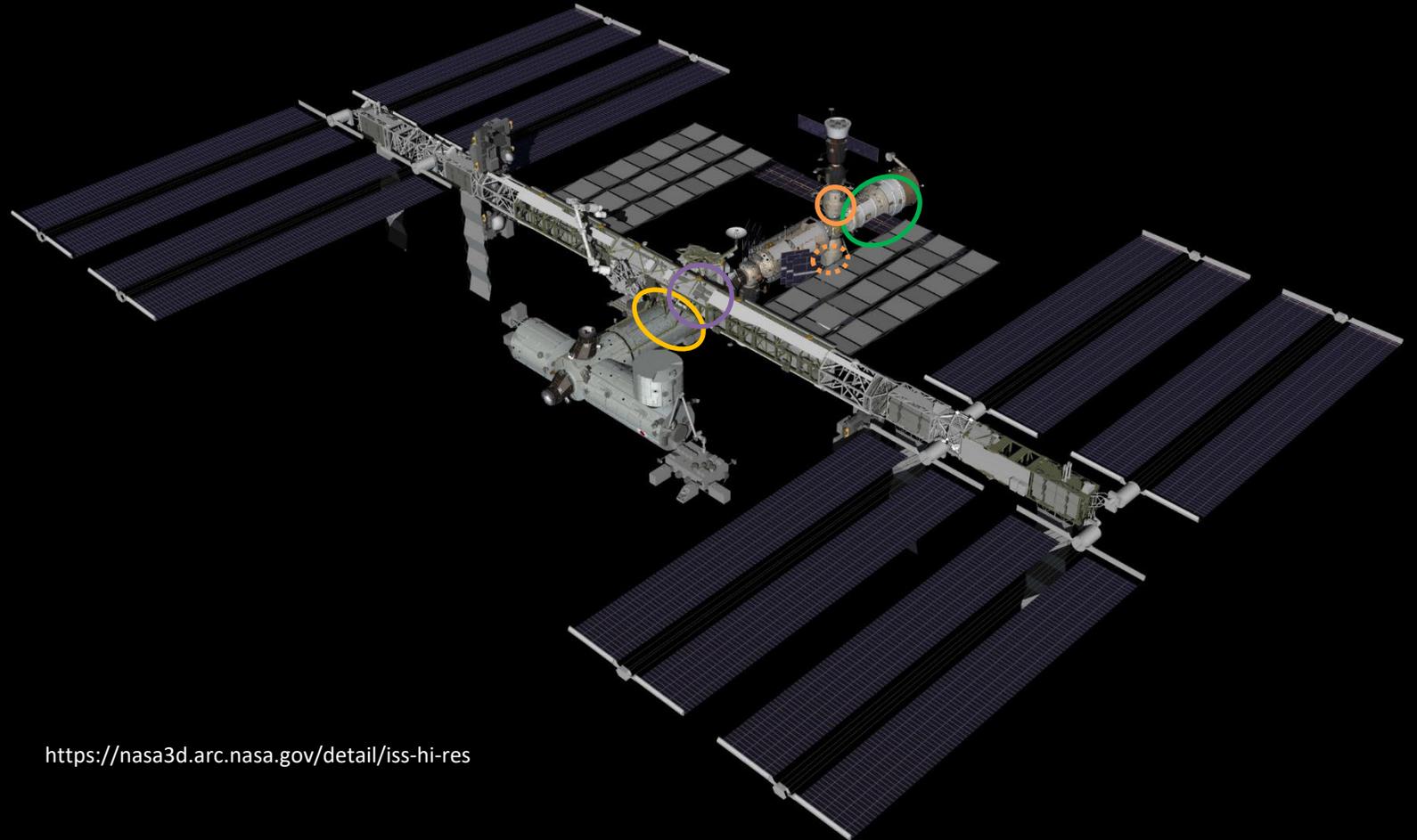
EVA/date	Location sampled	Swab appearance
EVA-25 15 November 2010	Valves of trace contamination removal unit	Black spots
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Black spots
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Gray spots
EVA-38 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

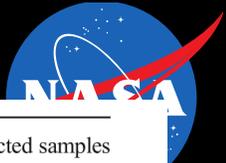
SM, SERVICE MODULE

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>

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**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

**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	<i>Bacillus licheniformis</i>
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Black spots	<i>Bacillus sphaericus</i> ; <i>B. subtilis</i>
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module "SEARCH"	Gray spots	<i>B. subtilis</i>
EVA-38 19 June 2014	SM, window 2	Black spots	<i>Bacillus pumilus</i>
EVA-41 10 August 2015	SM, between tubes of radiator (STR)	Black spots	<i>Bacillus pumilus</i> ; <i>Aureobasidium</i> sp.
EVA-42 26 February 2016	The exit porthole of the module "PIRS" surface	Gray spots	<i>Bacillus sphaericus</i>
EVA-42 26 February 2016	The exit porthole of the module "PIRS," window, and hatch frame	Black and gray spots, bright grains	<i>Bacillus licheniformis</i> ; <i>Bacillus pumilus</i> ; assemblage of <i>Agrococcus jenensis</i> , <i>Skermanella aerolata</i> , <i>Deinococcus aerolatus</i> , and <i>Staphylococcus hominis</i>

EVA, extravehicular activities

VL2, exit porthole 2

SM, SERVICE MODULE

STR, thermal control system

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

# CONCLUSIONS



- NASA has developed and tested a tool kit for collecting microbiological samples during EVA.
- Results from this experiment should help close planetary protection knowledge gaps for crewed missions and allow us to develop life support systems that minimize forward contamination.
- The payload is on station, and we hope to collect samples in July of 2024.
- Working with flight operations on a science payload has been good practice for conducting EVA science on Artemis missions.
- The tool kit could also be used to collect organic. contamination knowledge samples on other crewed or robotic missions.

