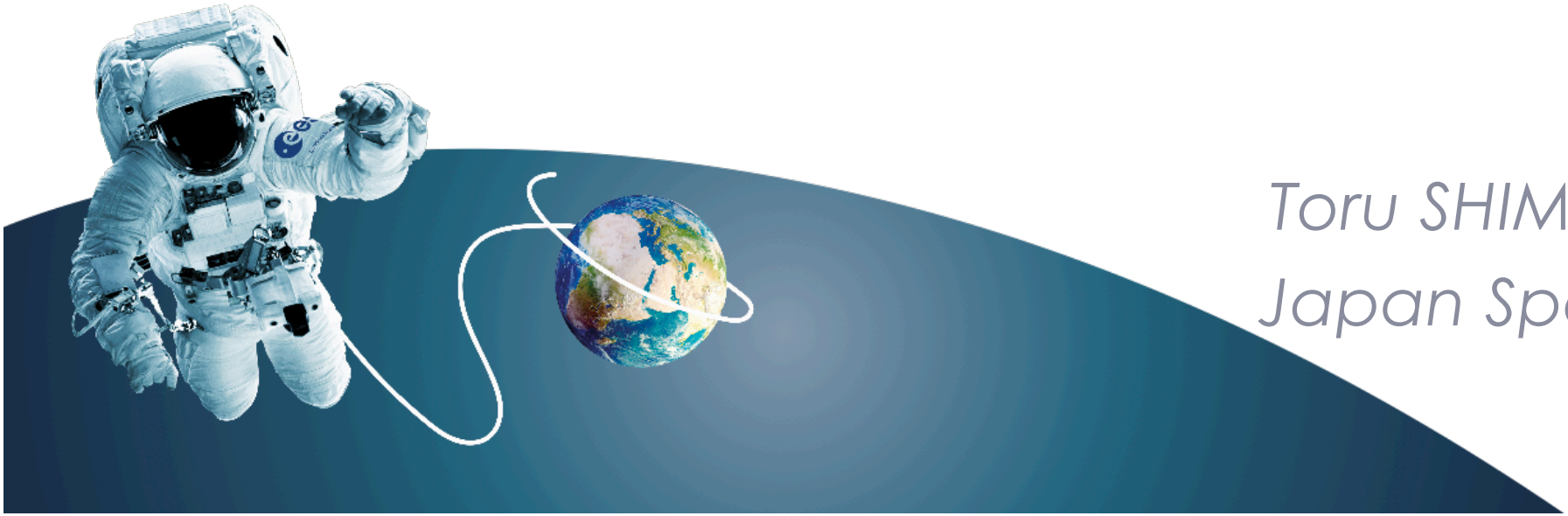
MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

Status and Future plan of JAXA microbial monitoring from ISS and beyond



Toru SHIMAZU, Ph.D.
Japan Space Forum

Introduction

The space habitat is a confined environment with a simple ecosystem that consists mainly of microorganisms and humans with some plants and rodents.

Recent studies revealed that microbes in the space habitat were mainly derived from humans and that some microorganisms brought in from the Earth have adapted to the environment. To ensure microbiological safety in the space habitat, a comprehensive analysis of environmental microbiota is needed to understand the overall microbial world in this habitat. The resulting data contribute to evidence-based microbial monitoring, and continuous microbial monitoring will provide information regarding changes in bioburden and microbial ecosystem. And future on board automatic analyze system is essential for moon / mars era.

BACTERIAL MONITORING IN THE INTERNATIONAL SPACE STATION (ISS)

- NASA, RSA and JAXA have been continuously monitoring microbes in the ISS.
- To understand the real microbial world, culture-independent approaches are required.
- Since 2009, we are performing continuous monitoring of environmental microbes in the JEM.
- Most bacteria present in JEM were constituents of human microbiota, and established through long-term operations in JEM.

JAXA Microbe Experiments

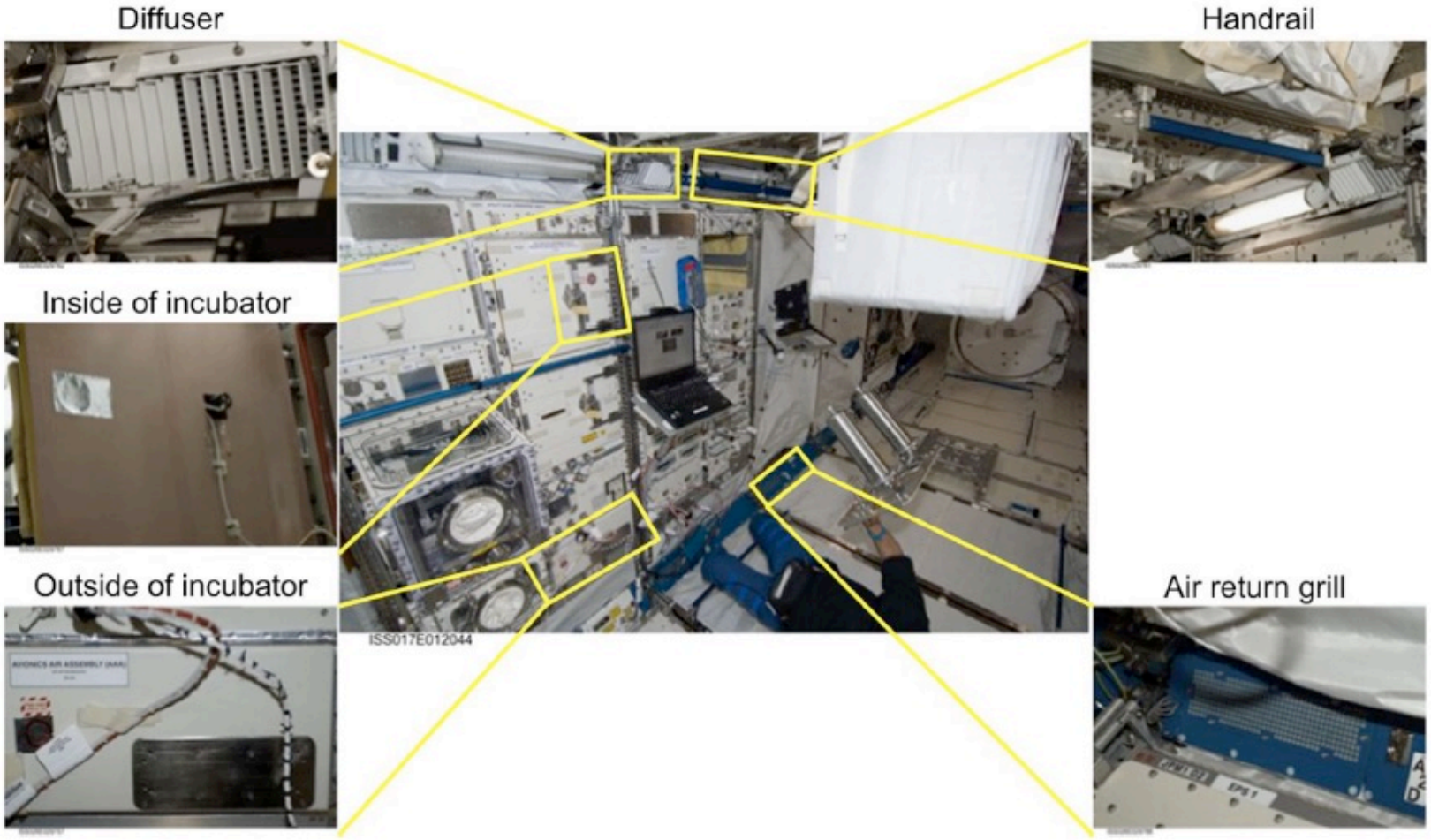
We have been performing microbial monitoring in JEM (FY2009 – 2018).

Experiments title: **“Microbe – I, II, III and IV”**

We will start new microbial monitoring in JEM (FY2020 –).

Experiments title: **“JEM-Microbe ” “Micro Monitor”**

Sampling Points in JEM



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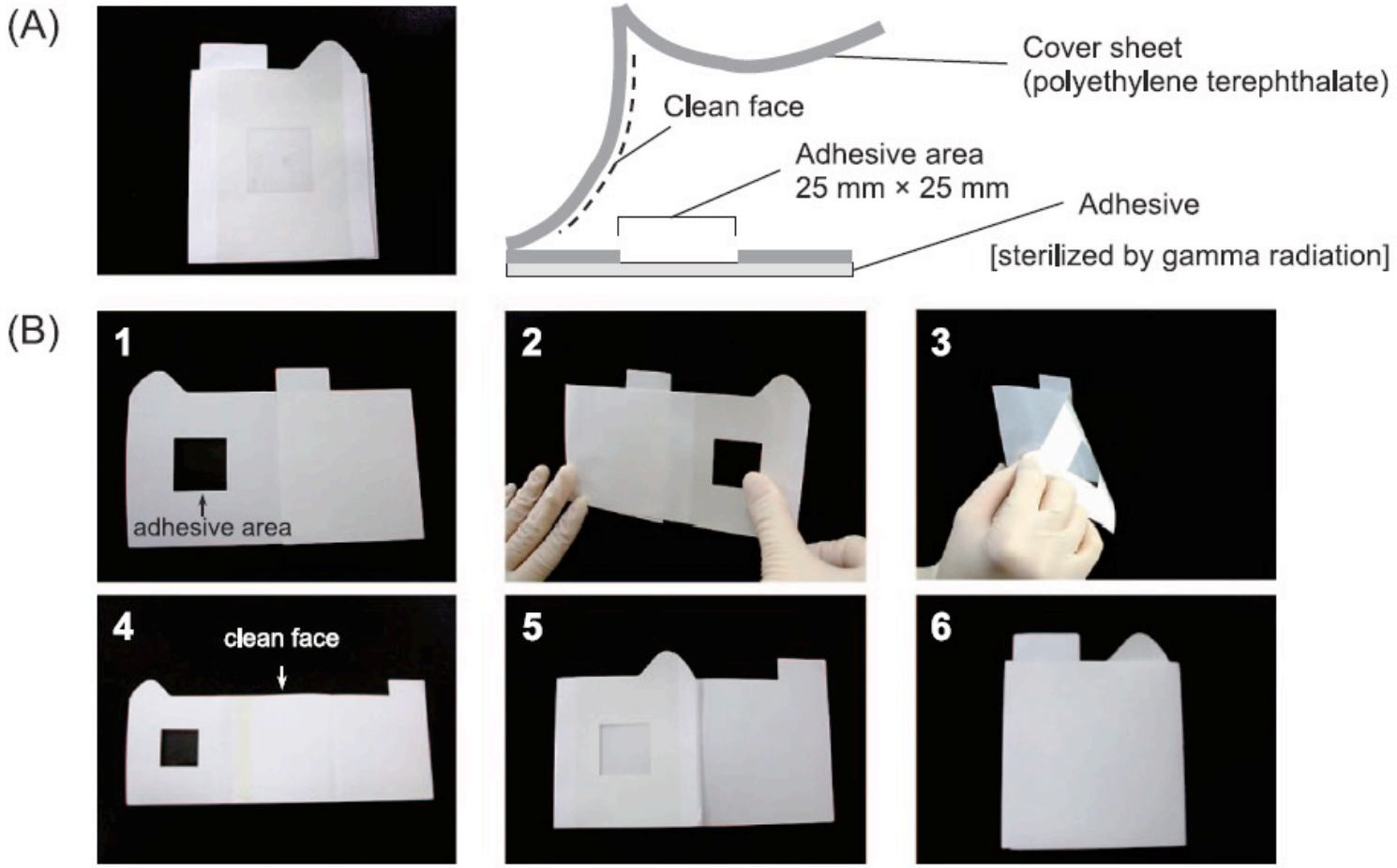
JAXA Sampling Sheet



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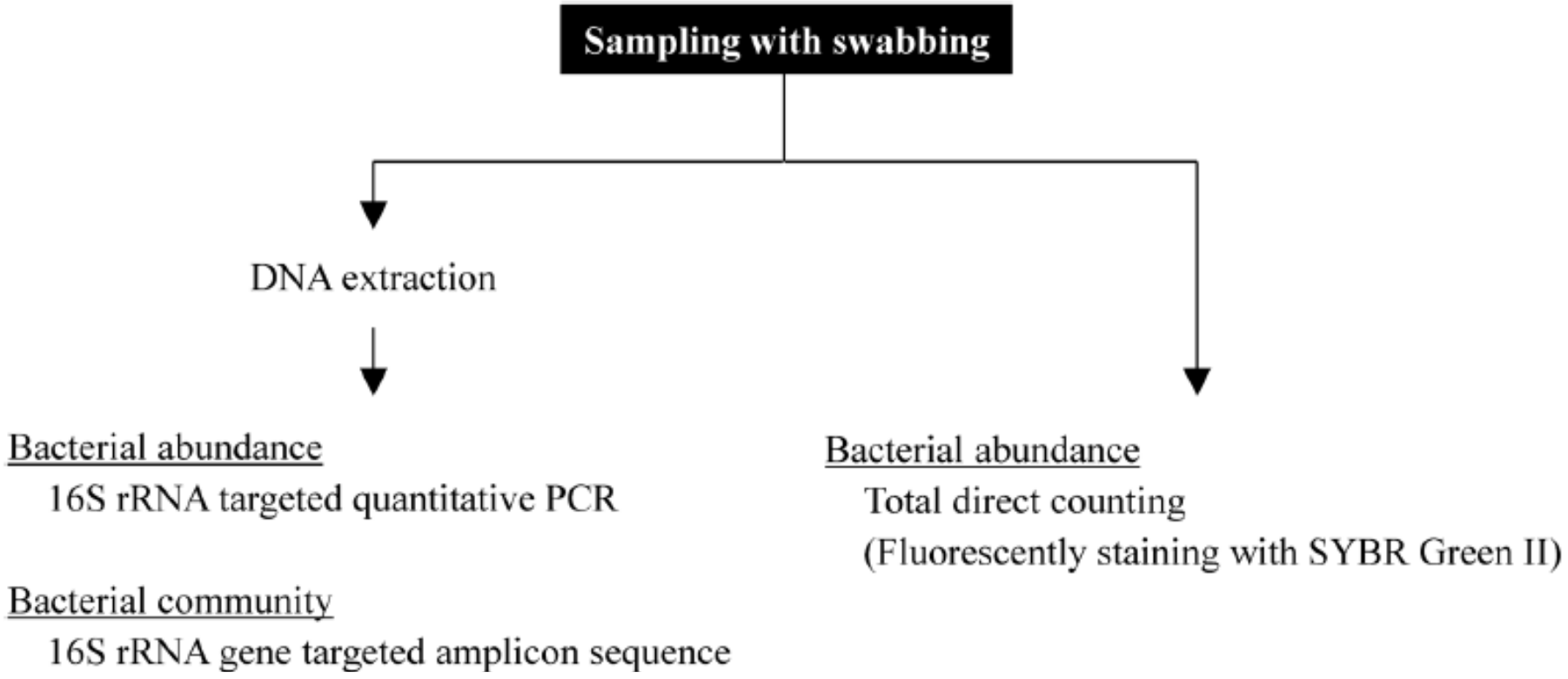
Procedure for the Microbiological Analysis



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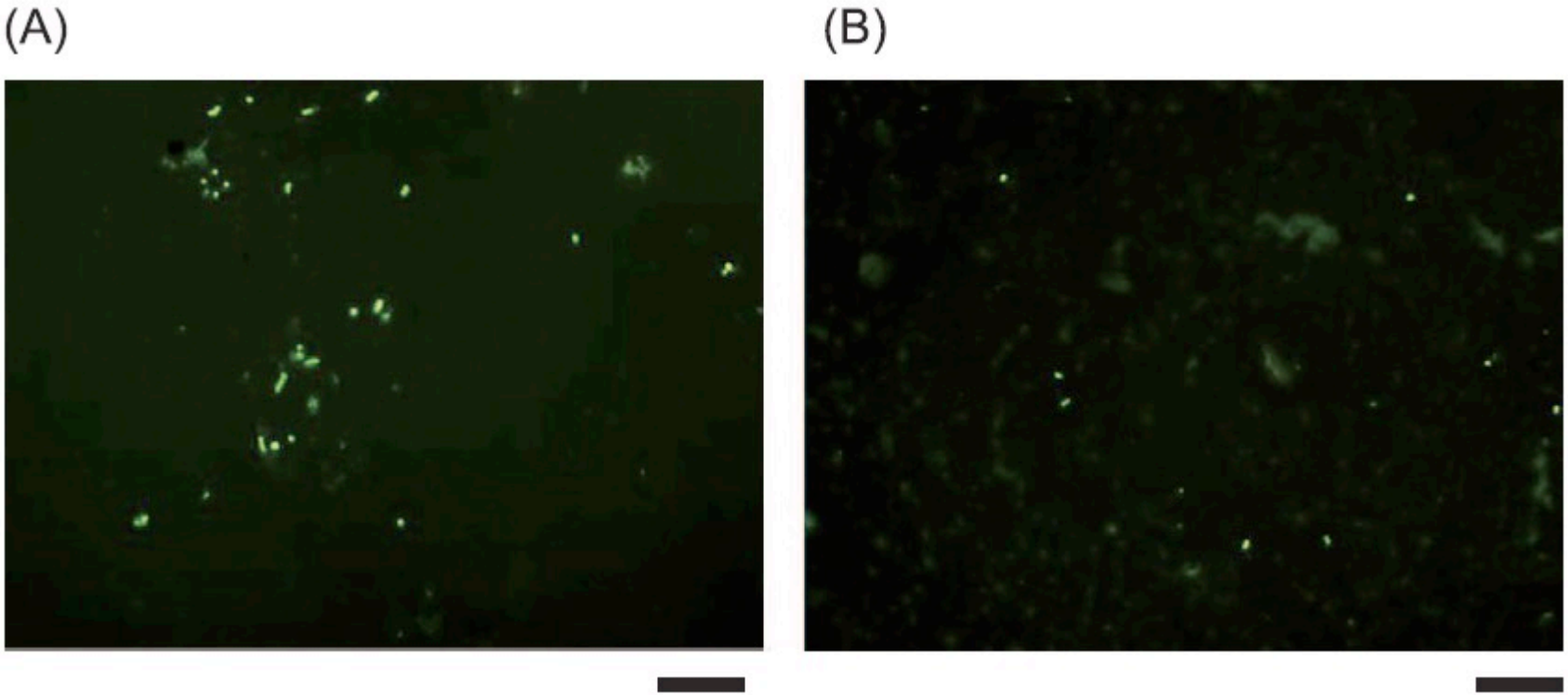
Fluorescent microscopic image of microbes



Fluorescent microscopic image of microbes collected with an adhesive sheet. Microbial cells were stained with 1×SYBR Green II (scale bars, 10 μm). (A) Mixture of A. lwoffii ATCC15390, B. subtilis 168, P. putida ATCC12633 and S. epidermidis IFO3762; (B) sample from vertical surface of the rack in our laboratory.

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Bacterial abundance

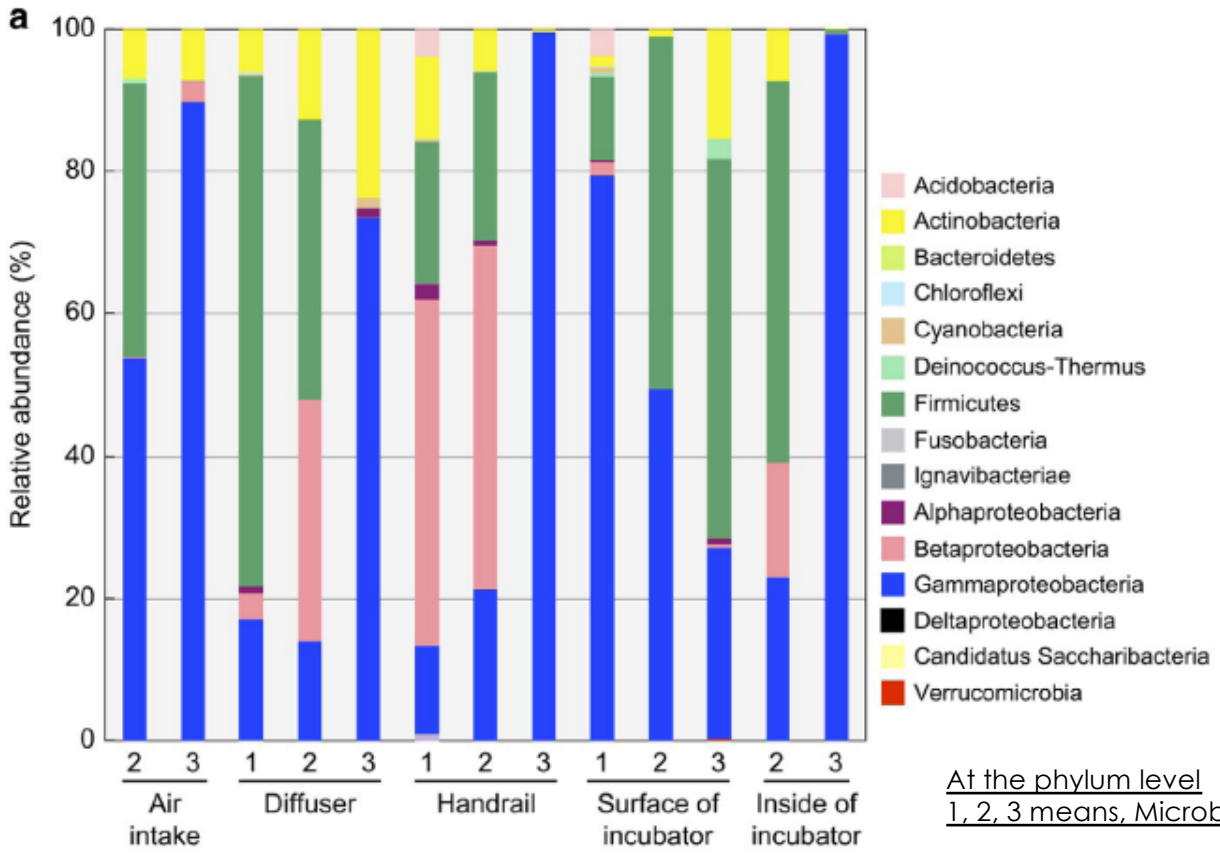
Bacterial abundance on the interior surfaces in JEM determined by fluorescent microscopy and quantitative PCR.

The results show that the bacterial number was below 104 cells/cm2.

	Microbe-I		*Microbe-II*		*Microbe-III*	
	TDC (cells/cm^2)	*qPCR (cells/cm^2)*	*TDC (cells/cm^2)*	*qPCR (cells/cm^2)*	*TDC (cells/cm^2)*	*qPCR (cells/cm^2)*
Outer surface of incubator	2×10^3	4×10^3	2×10^2	$<1\times10^2$	2×10^2	$<1\times10^2$
Air diffuser	9×10^2	2×10^3	$<2\times10^2$	3×10^2	$<2\times10^2$	$<1\times10^2$
Handrail	7×10^2	5×10^2	$<2\times10^2$	1×10^2	2×10^2	$<1\times10^2$
Air return grill	NT	NT	$<2\times10^2$	1×10^2	$<2\times10^2$	$<1\times10^2$
Internal surface of incubator	NT	NT	$<2\times10^2$	1×10^2	$<2\times10^2$	$<1\times10^2$

Abbreviations: NT, not tested; qPCR, quantitative PCR; TDC, total direct counting with fluorescent microscopy.

Bacterial Community Structure on the Interior Surfaces in JEM for 3 years



At the phylum level
1, 2, 3 means, Microbe-I, -II, -III

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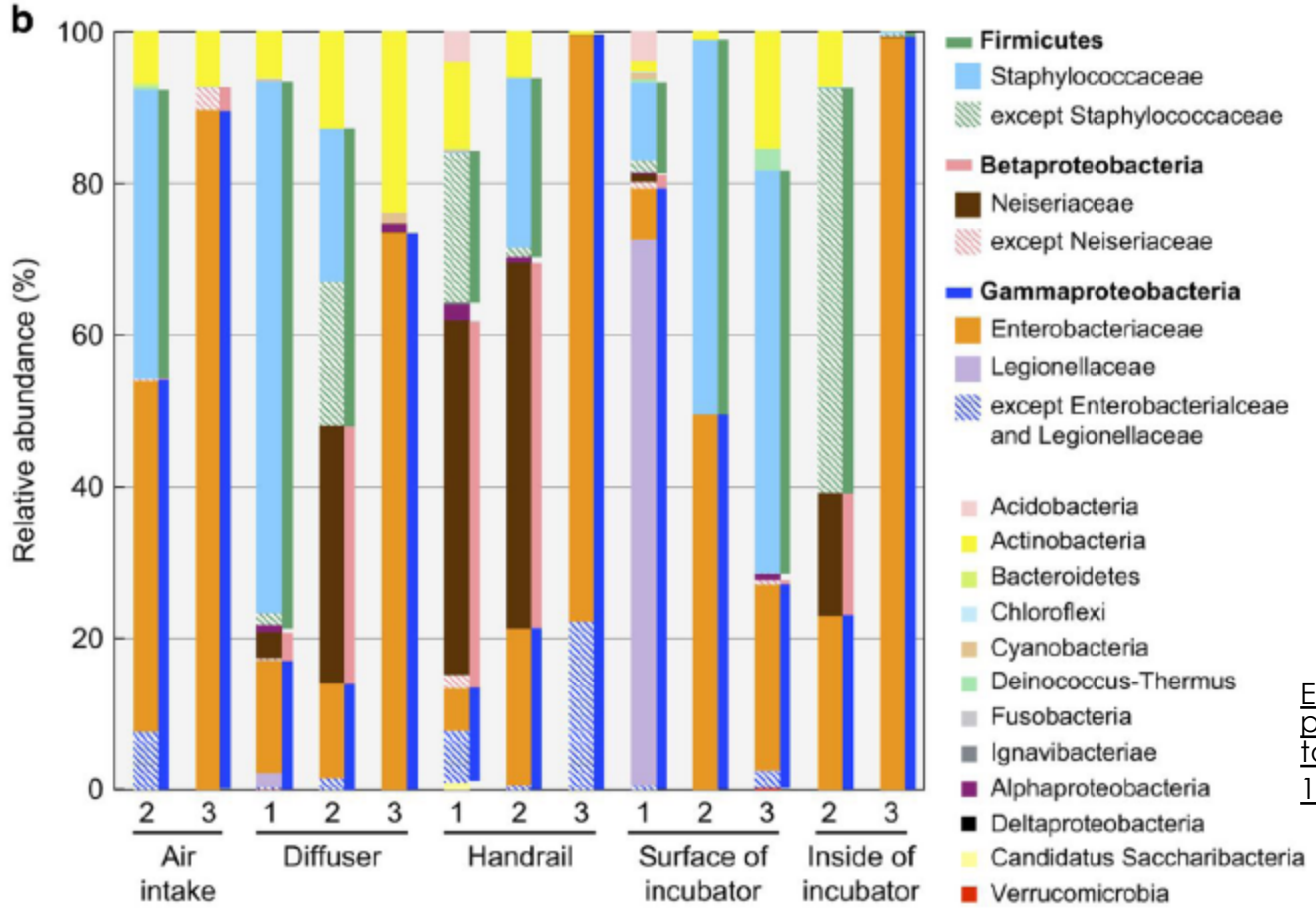
Bacterial Community Structure on the Interior Surfaces in JEM for 3 years



Expanding beta- and gamma-proteobacteria and Firmicutes to the family level

1, 2, 3 means, Microbe-I, -II, -III

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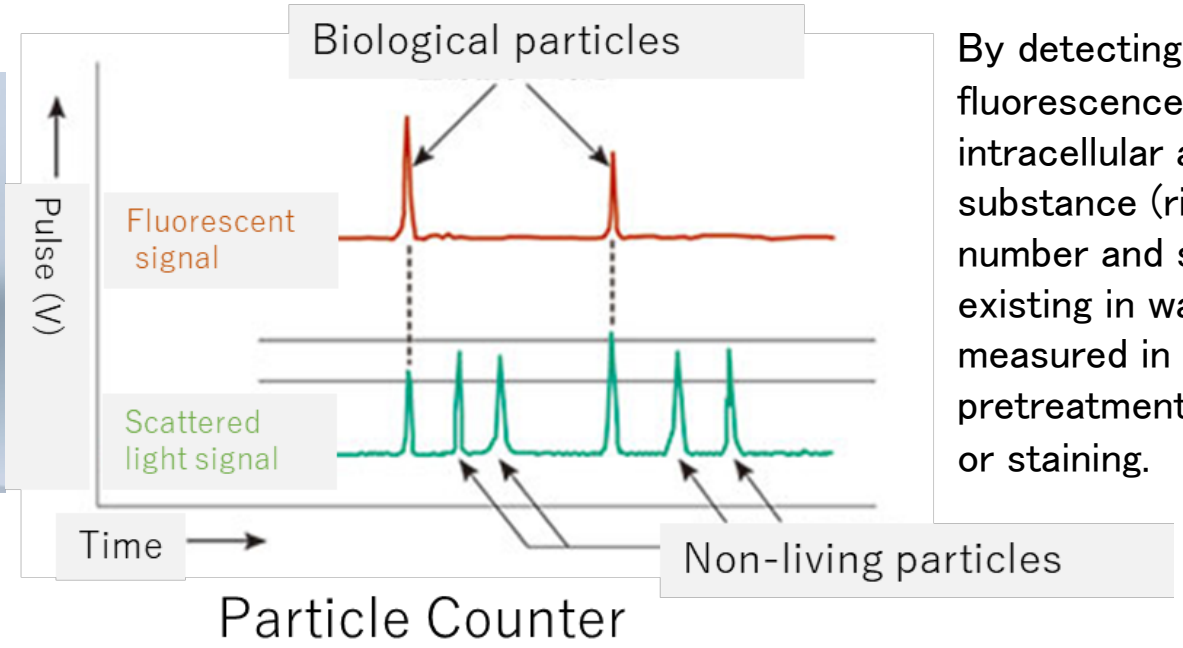
Future analysis on board ISS and beyond

- We are planning to utilize an automated fluorescence particle counter to detect microbes on board ISS and beyond

Particle Counter
RION Co.,Ltd.



Particle Counter

By detecting the fluorescence emitted by the intracellular autofluorescent substance (riboflavin), the number and size of bacteria existing in water can be measured in real time without pretreatment such as culture or staining.

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THANK YOU.

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