

# MARS SAMPLE RETURN: PREPARING FOR BIOLOGICALLY SENSITIVE SAMPLES

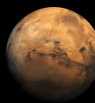
National Aeronautics and  
Space Administration



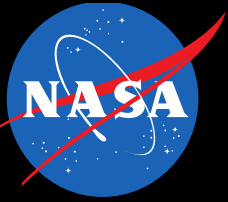
Aaron B. Regberg PhD

NASA Johnson Space Center

01/08/2025



# WHAT DO WE NEED TO DO DIFFERENTLY FOR MARS SAMPLE RETURN?



Chevaya Falls Sample

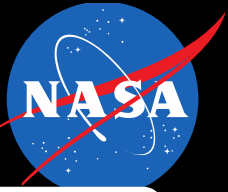
- **Science Objectives**

- **Protect the Precious Cargo:** Preserve the integrity of the samples by minimizing exposure to high temperatures and magnetic fields, maintaining mechanical integrity, and avoiding potential contamination.
- **Geology:** Reconstruct the formation and alteration history of the returned samples to transform our understanding of the geological processes and environments of Mars.
- **Astrobiology:** Determine the potential astrobiological significance of the Martian geological record represented by the samples, including whether the samples contain evidence of past life on Mars.
- **Planetary Processes:** Provide new insights into planetary-scale formation and evolution in the inner solar system.
- **Prepare for Humans on Mars:** Identify and characterize potential risks and opportunities for future human missions.



# THE ASTROMATERIALS ACQUISITION AND CURATION OFFICE

*Our Past 50+ years – planning for and curating multiple collections*



## Lunar (1969)

Apollo program lunar rocks and soils; Luna samples



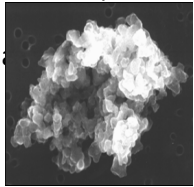
## Meteorite (1977)

Antarctic Search for Meteorites (ANSMET) program



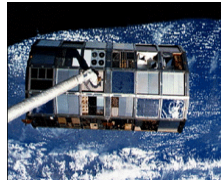
## Cosmic Dust (1981)

Cosmic dust grains from Earth's stratosphere



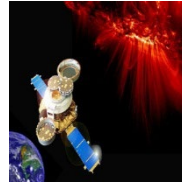
## Micro-particle (1985)

Space exposed hardware from spacecraft



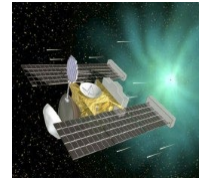
## Genesis (2004)

Genesis solar wind samples at Earth-Sun L1 point



## Stardust (2006)

Cometary and interstellar samples from Comet Wild 2



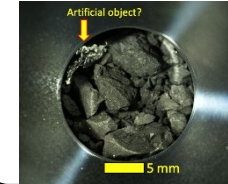
## Hayabusa (2012)

Samples collected from JAXA asteroid mission to Itokawa



## Hayabusa II (2020)

Subset of samples collected from JAXA asteroid mission to 162173 Ryugu



## OSIRIS-REx (2023)

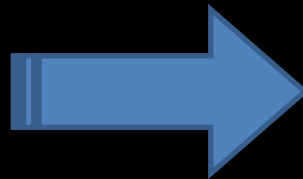
Asteroid sample return from 101955 Benu



## Our Near Future . . .

### Artemis (2027)

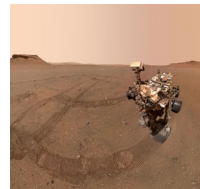
Lunar South Pole. Cold Sample return?



## Our More Distant Future . . .

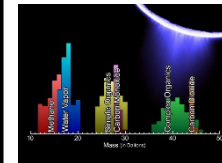
### Mars

(~2030s)

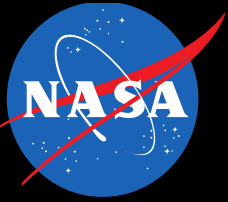


(est. 1964)

### Comet Enceladus Europa Ceres



# FOR HISTORICAL COLLECTIONS INORGANIC PARTICLES AND UNWANTED OXIDATION WERE THE PRIMARY CONTAMINATION CONCERNS



Meteorite (ISO 7 equivalent)



Lunar (ISO 6 Equivalent)

- Use cleanrooms to minimize particulate contamination
- Store samples in an inert environment (ultra pure nitrogen)
- Limit the types of material we use to interact with the samples
  - Stainless steel
  - Aluminum
  - Teflon

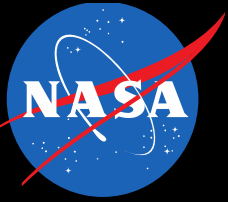


Stardust (ISO 5 Equivalent)



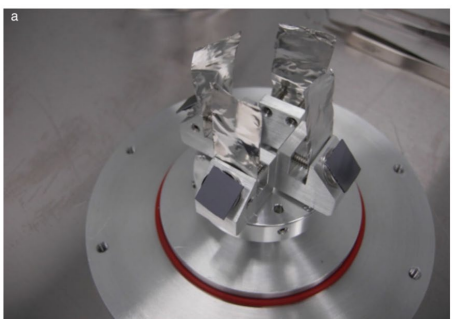
Genesis (ISO 4 Equivalent)

# COLLECTIONS ALSO HAVE ORGANIC CONTAMINATION REQUIREMENTS



- Apollo 11
  - Total Organic Contamination <math><1.0 \mu\text{g C/g}</math>
- Apollo 12 – 17
  - Total Organic Contamination <math><0.1 \mu\text{g C/g}</math>
- Osiris REx
  - A/2 500 ng/ cm<sup>2</sup> NVR (Non-Volatile Residue)
  - <math><180 \text{ ng/cm}^2</math> of amino acids

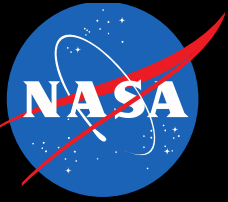
B. R. Simoneit and D. A. Flory (1970) Apollo 11, 12 and 13 Organic Contamination Monitoring History, UC Berkeley



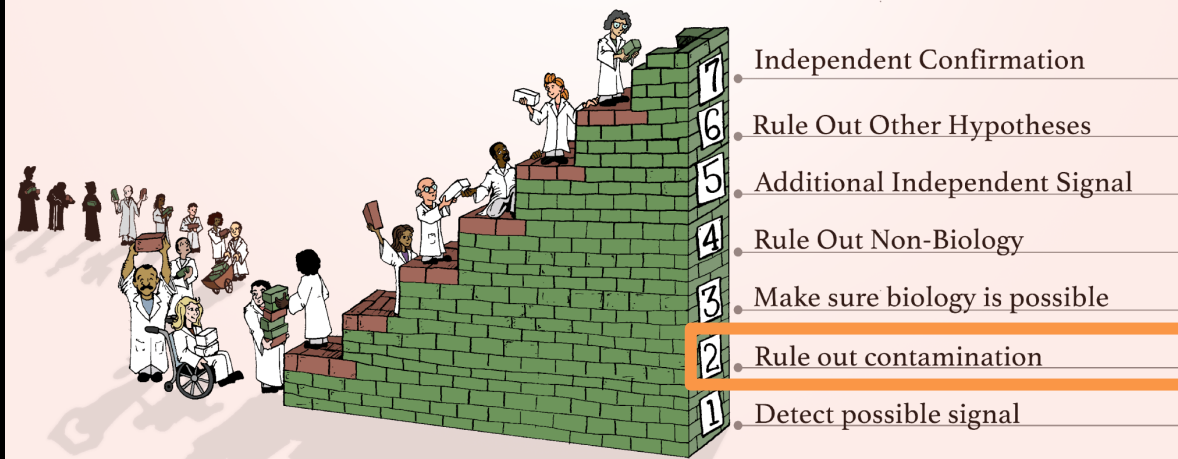
Dworkin, J. P., Adelman, L. A., Ajluni, T., Andronikov, A. V., Aponte, J. C., Bartels, A. E., et al. (2018). OSIRIS-REx Contamination Control Strategy and Implementation. *Space Sci Rev*, 214(9). <https://doi.org/10.1007/s11214-017-0439-4>



# OUR CURATION LABS WILL NEED TO MEET THIS STANDARD

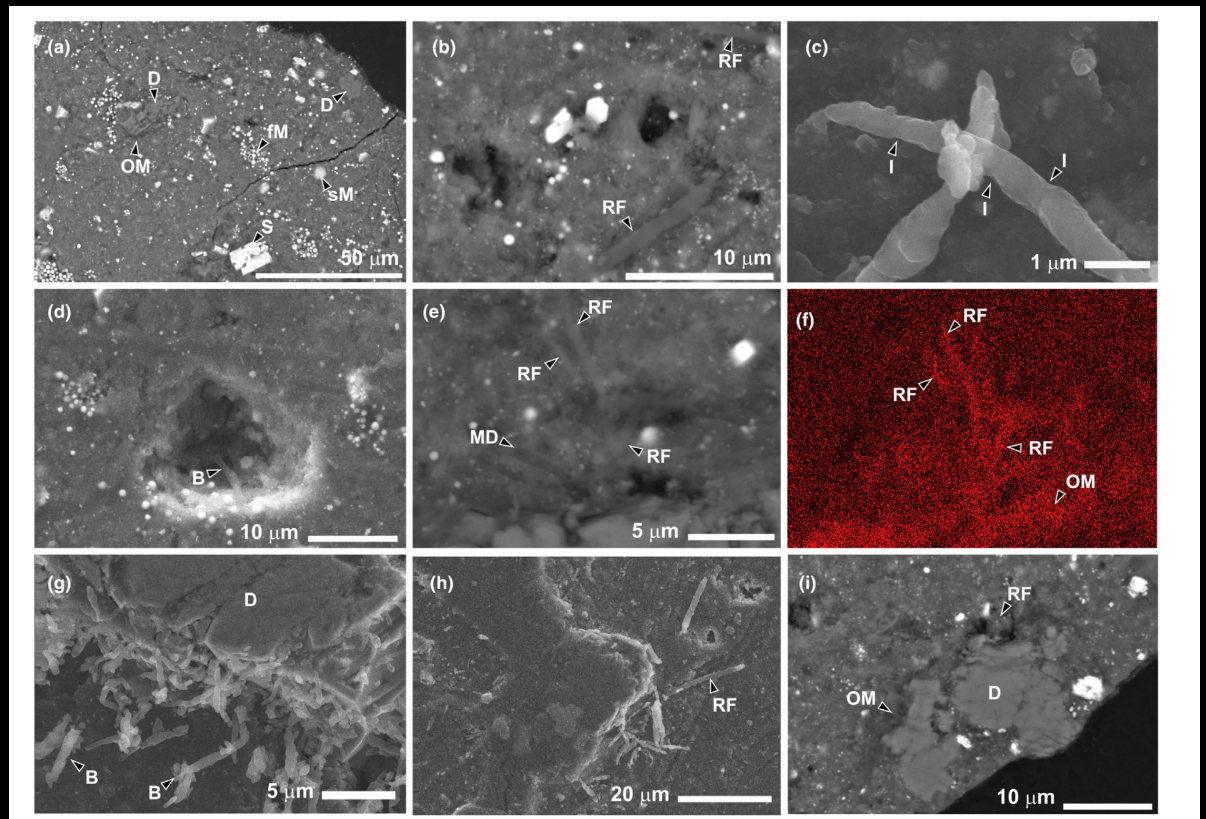


## CoLD SCALE Confidence of Life Detection



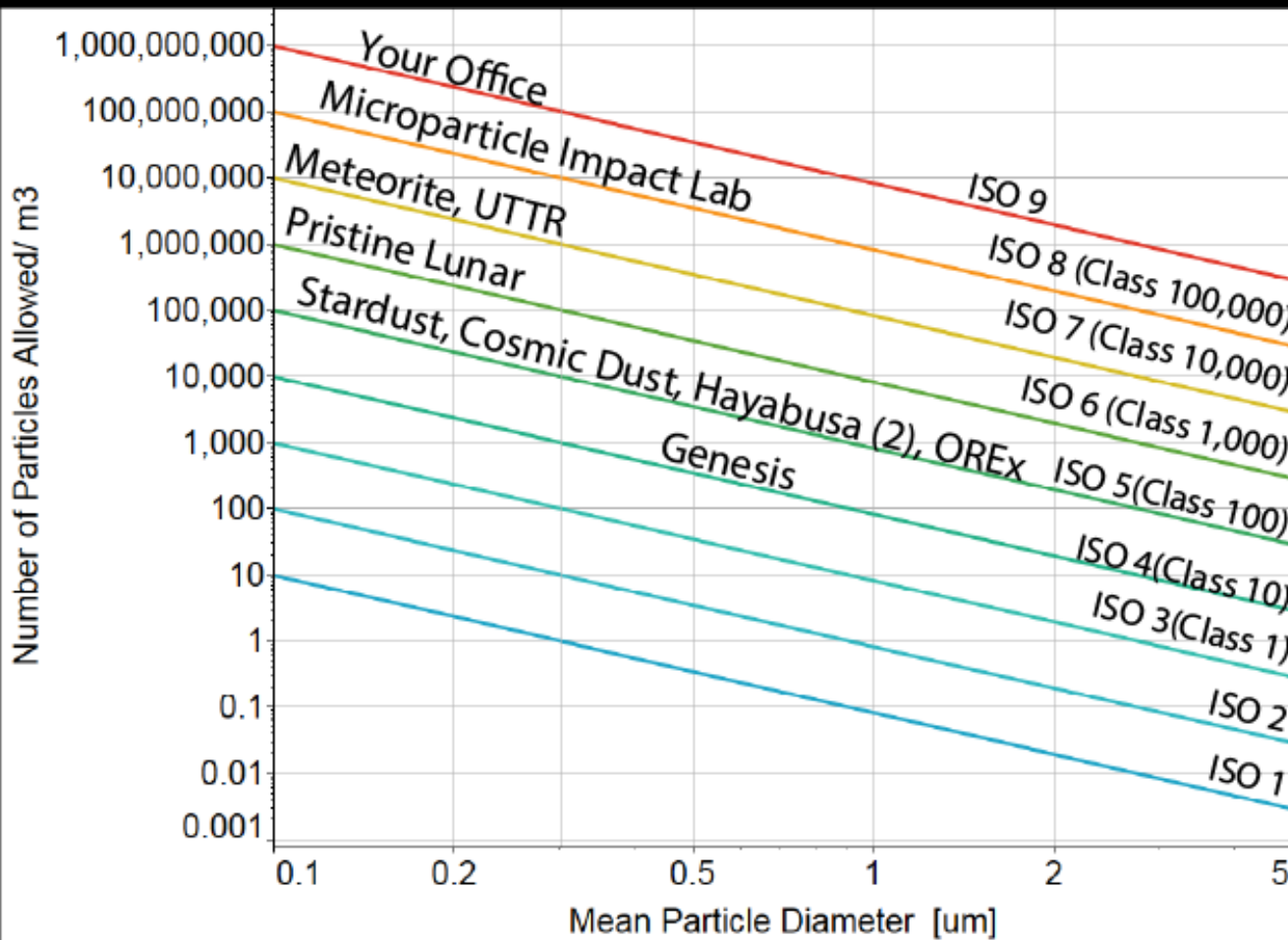
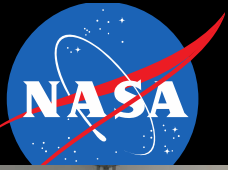
NASA / Aaron Gronstal

## Potential biological contamination of Ryugu occurred outside JAXA cleanrooms

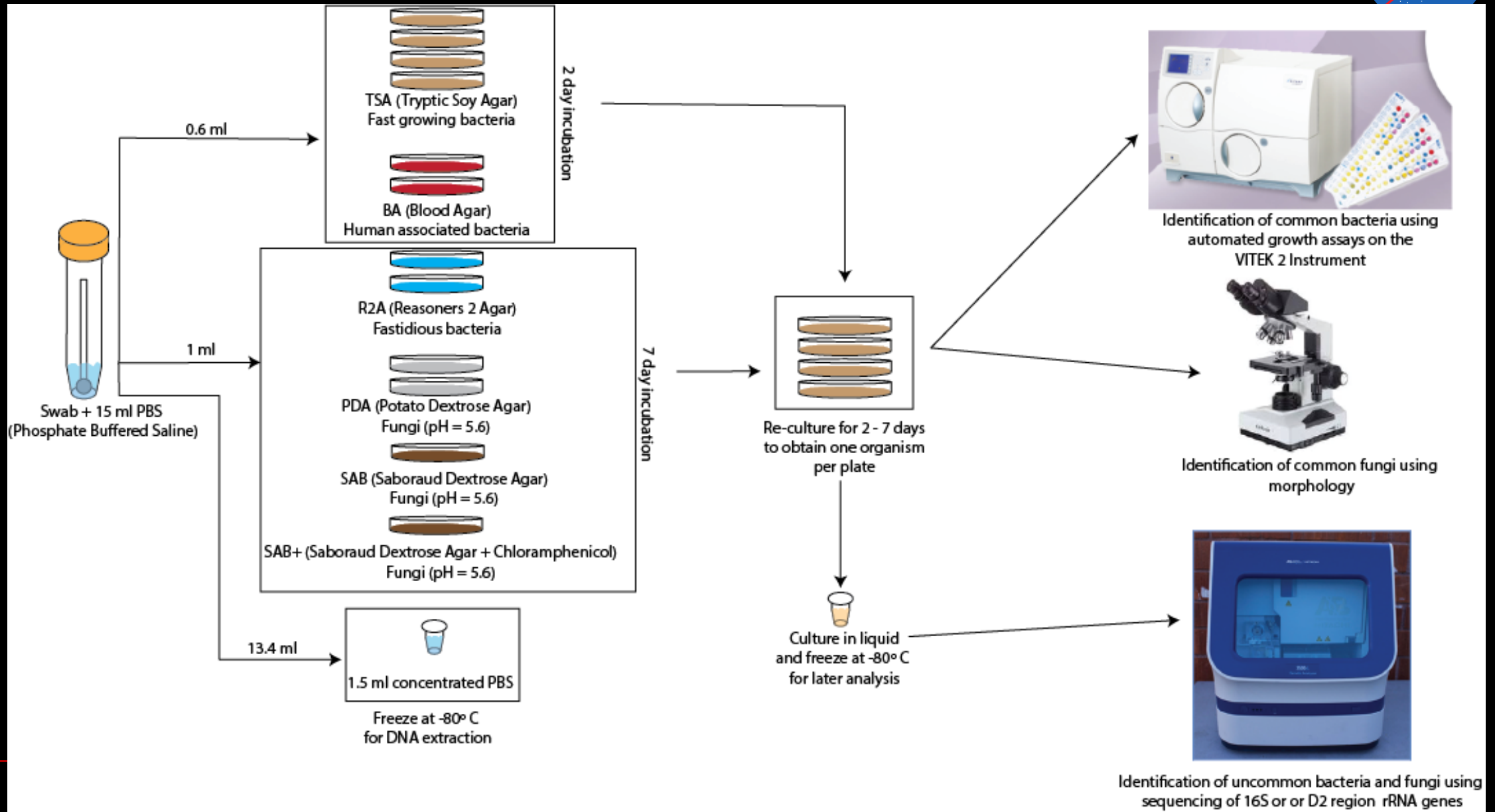


Genge, M. J., Almeida, N., Van Ginneken, M., Pinault, L., Preston, L. J., Wozniakiewicz, P. J., et al. (2024). Rapid colonization of a space-returned Ryugu sample by terrestrial microorganisms. *Meteoritics & Planetary Science* n/a. doi: [10.1111/maps.14288](https://doi.org/10.1111/maps.14288)

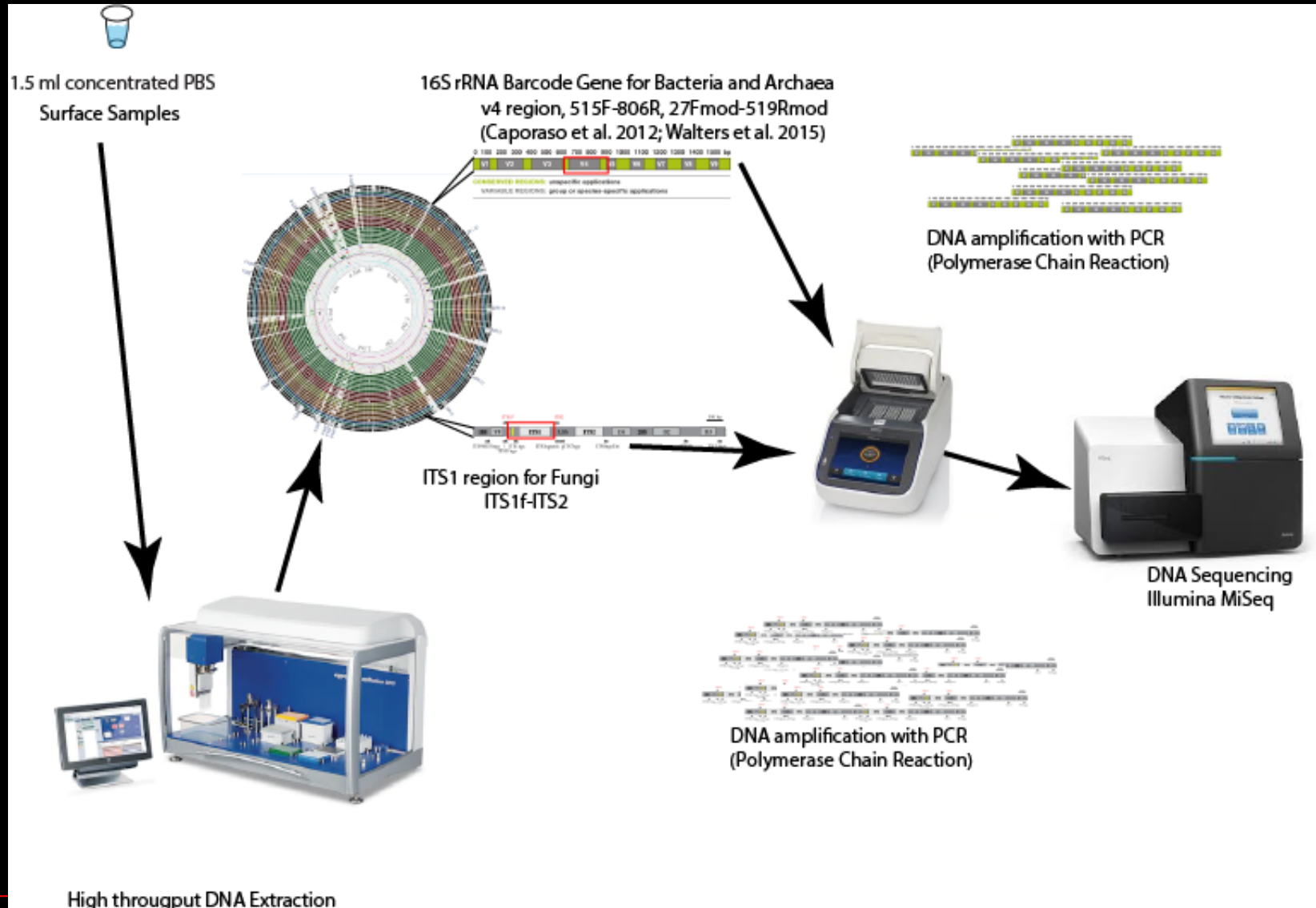
# NASA IS PREPARING BY MONITORING MICROBIAL CONTAMINATION IN EXISTING COLLECTIONS



# USE CULTURE-BASED METHODS TO CHARACTERIZE CLEAN LAB MICROBIOLOGY



# USE CULTURE-INDEPENDENT METHODS TO CHARACTERIZE CLEAN LAB MICROBIOLOGY



# RECOVERY RATE IS OUR PREFERRED MONITORING METRIC

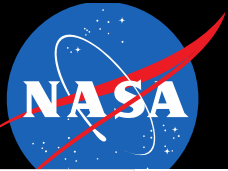


$$\text{Recovery Rate} = \frac{\text{\# of samples with } >0 \text{ CFU}}{\text{\# total number of samples collected during a sampling event}}$$

**Table 3. Suggested Initial Contamination Recovery Rates in Aseptic Environments<sup>a</sup>**

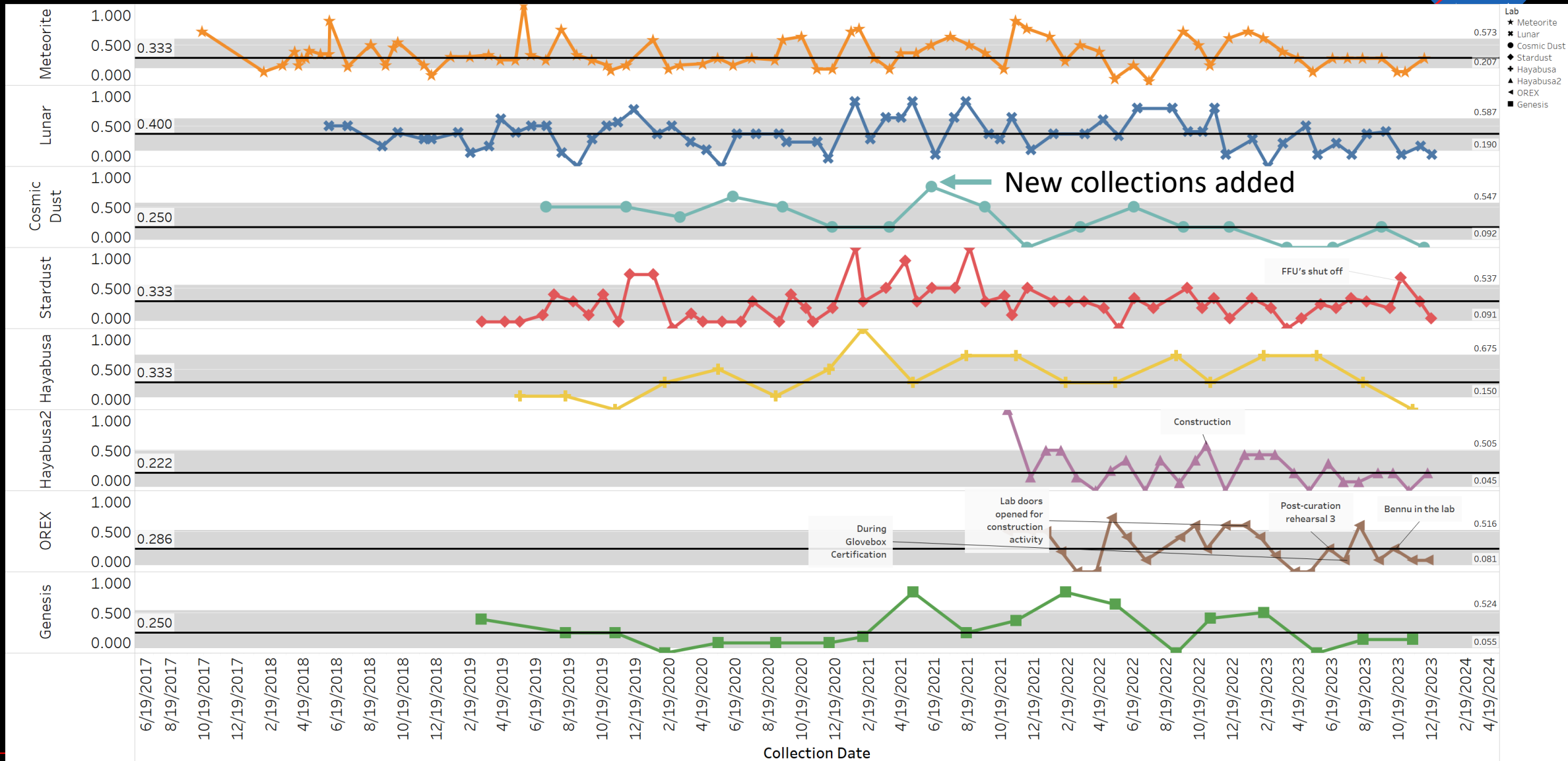
<b>Room Classification</b>	<b>Active Air Sample (%)</b>	<b>Settle Plate (9 cm) 4 h Exposure (%)</b>	<b>Contact Plate or Swab (%)</b>	<b>Glove or Garment (%)</b>
Isolator/Closed RABS (ISO 5 or better)	<0.1	<0.1	<0.1	<0.1
ISO 5	<1	<1	<1	<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

# FACILITIES ISSUES AND USAGE AFFECT LAB MICROBIOLOGY

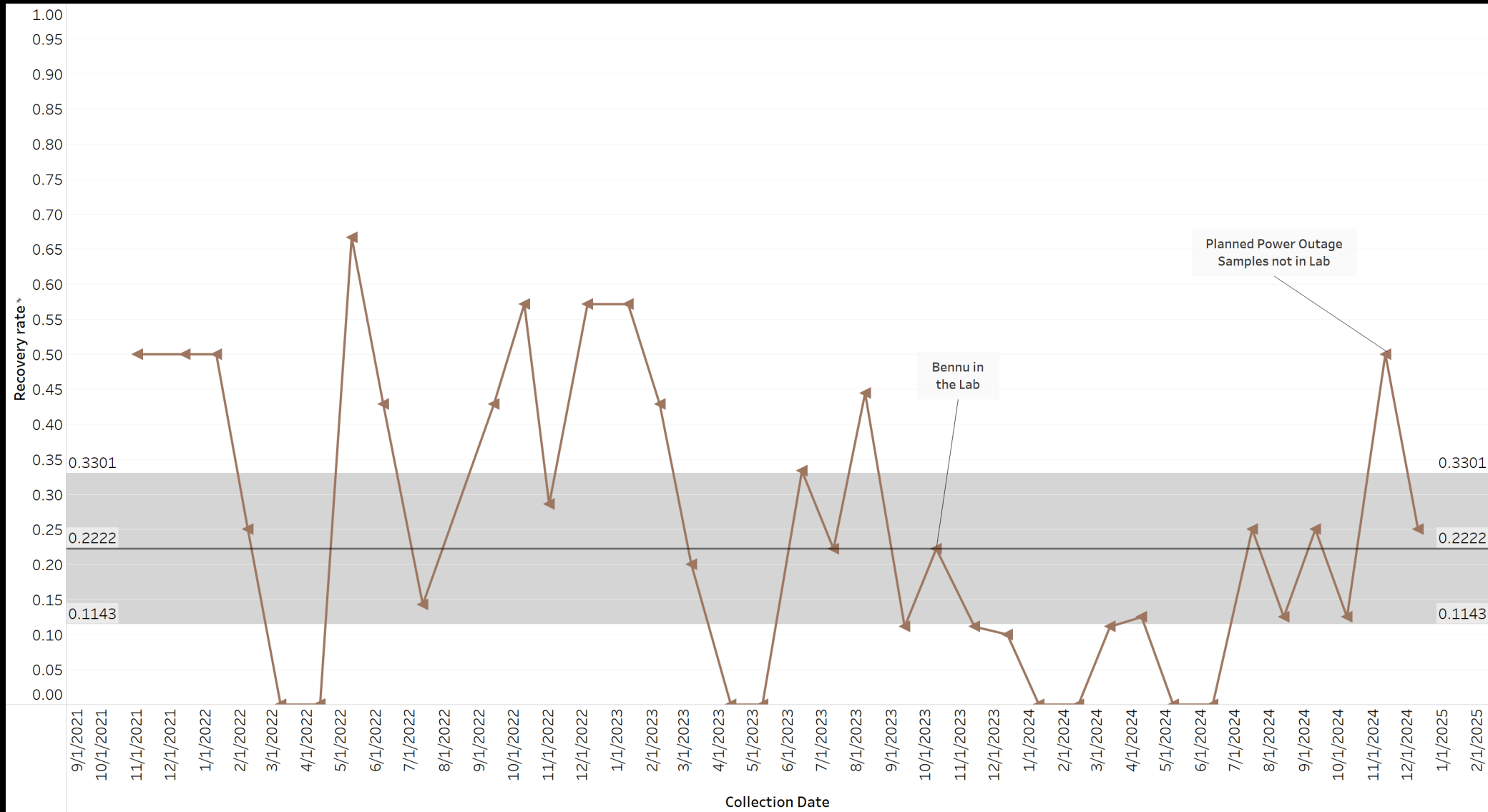
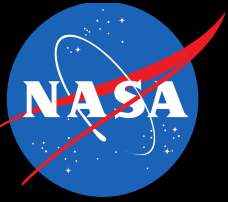


ISO 7

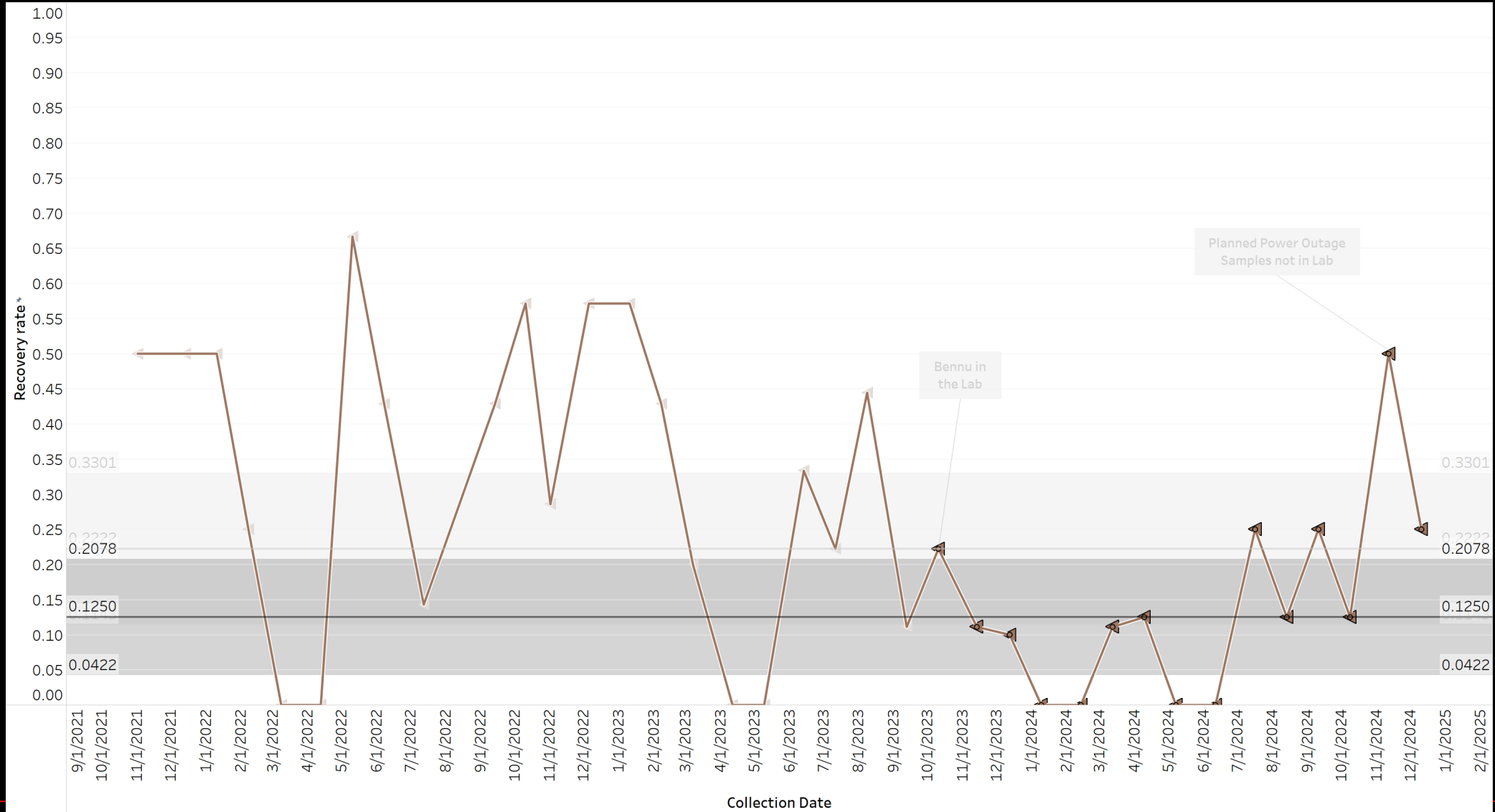
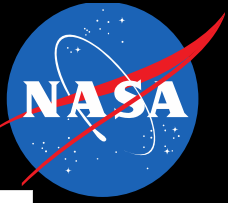
ISO 4



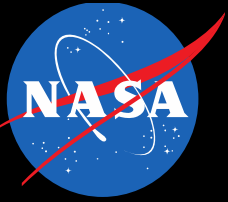
# EXTRA CLEANING PROCEDURES KEEP RECOVERY RATE LOWER



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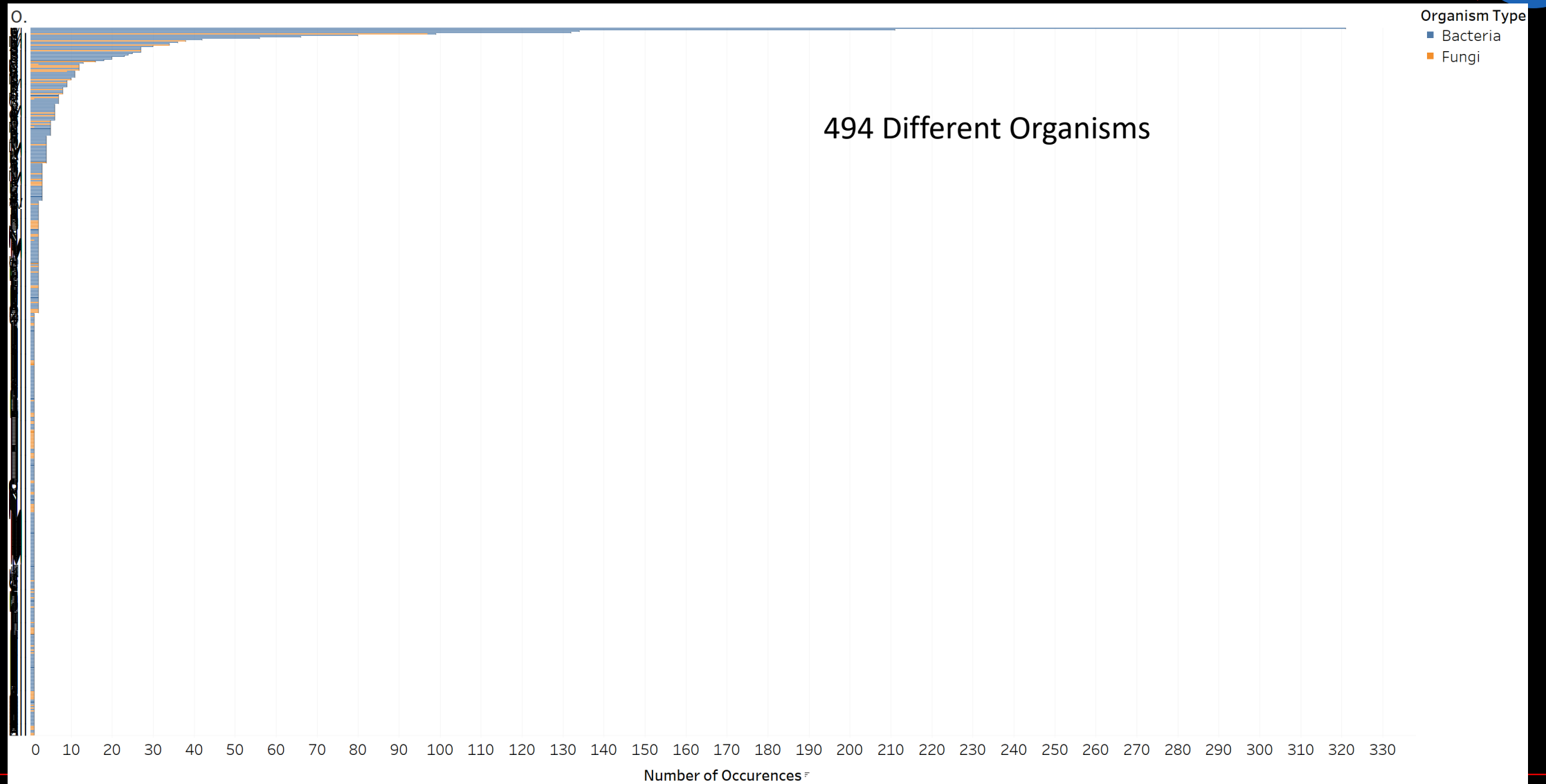


# ADDITIONAL CLEANING MEASURES IMPLEMENTED TO MINIMIZE BIOLOGICAL AND ORGANIC CONTAMINATION FOR OSIRIS REX

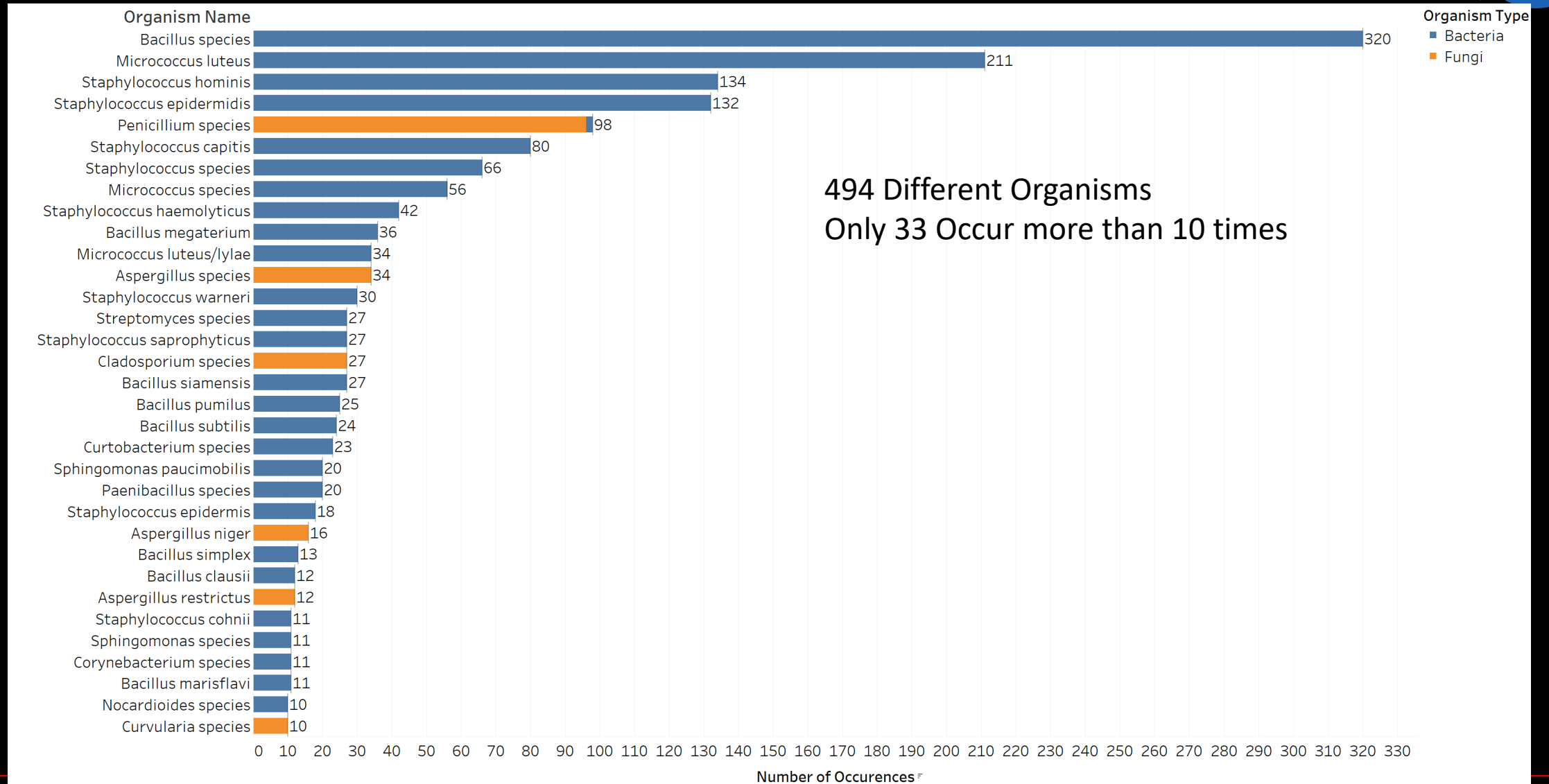


- **Bioburden Reduction**
  - Autoclave tools and sample containers
  - Disinfect surfaces and gloveboxes with 7.5 wt%  $H_2O_2$ 
    - 30 min. contact time for aluminum
    - 6 hr contact time for Teflon, glass and stainless steel
    - Also provides some oxidation of organic compounds
- **Organic Reduction**
  - Bake containers and tools at  $500^{\circ}C$  for up to 6 hours
  - Also sterilizes

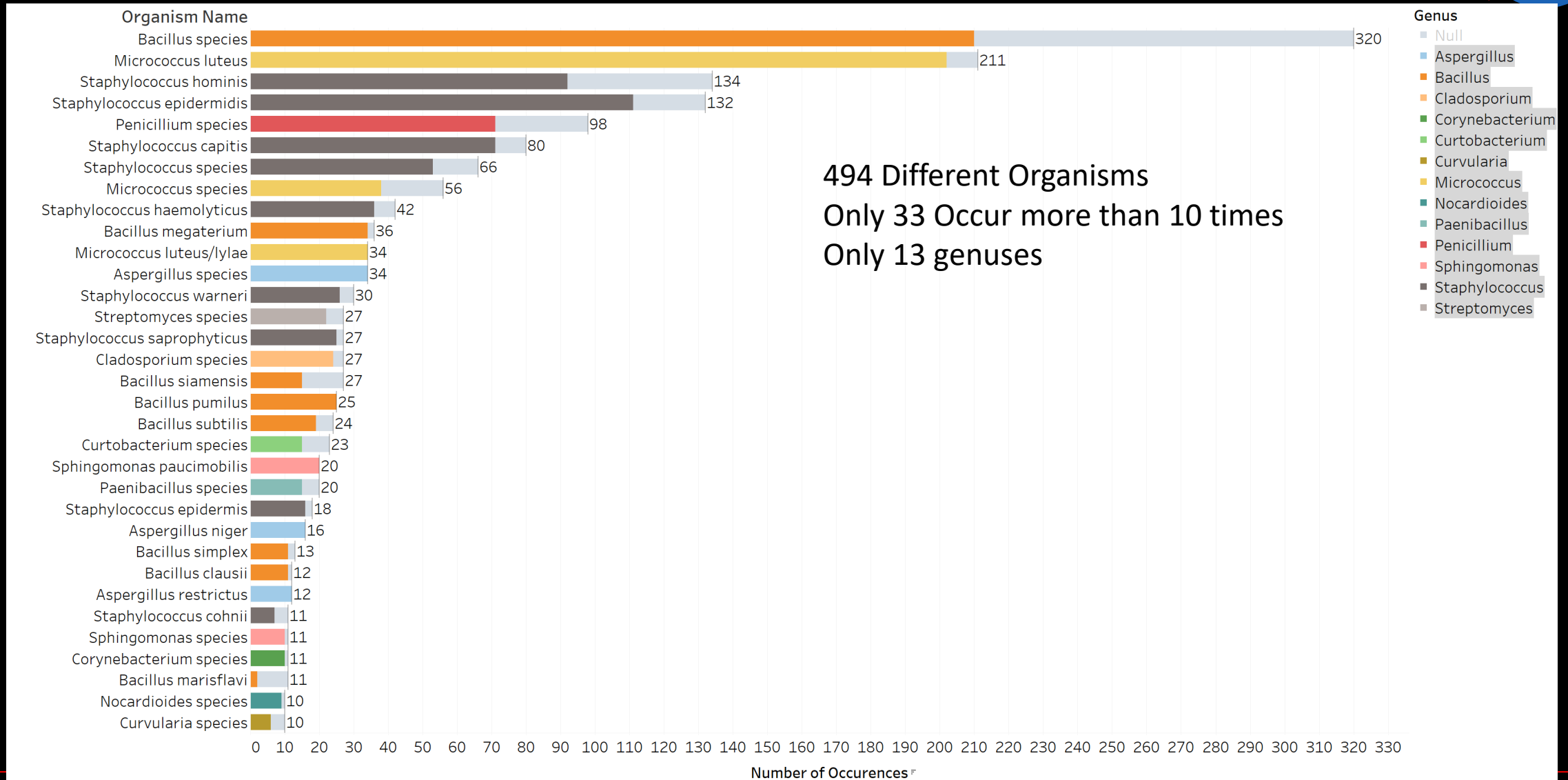
# WE REPEATEDLY OBSERVE THE SAME ORGANISMS



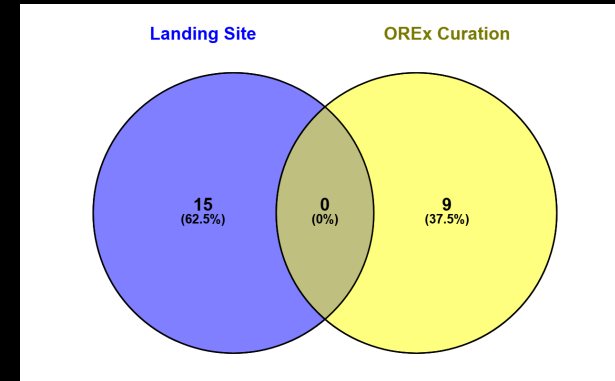
# WE REPEATEDLY OBSERVE THE SAME ORGANISMS



# WE REPEATEDLY OBSERVE THE SAME ORGANISMS



# WE DID NOT OBSERVE ANY OF THE RECOVERY SITE BACTERIA OR FUNGI IN THE CURATION CLEANROOM



UTTR Landing Site	OREx Curaton
<i>Alternaria alternata</i>	<i>Bacillus aryabhatai</i>
<i>Bacillus glycinifermentatus</i>	<i>Brevibacillus species</i>
<i>Bacillus licheniformis</i>	<i>Micrococcus luteus</i>
<i>Bacillus mojavensis</i>	<i>Staphylococcus aureus</i>
<i>Bacillus siamensis</i>	<i>Staphylococcus capitis</i>
<i>Bacillus subtilis spizizenii</i>	<i>Staphylococcus epidermidis</i>
<i>Didymella maydis</i>	<i>Staphylococcus haemolyticus</i>
<i>Hypocrea / Trichoderma species</i>	<i>Staphylococcus hominis</i>
<i>Hypocrea jecorina</i>	<i>Streptomyces species</i>
<i>Microbacterium hydrocarbonoxydans</i>	
<i>Montagnea candollei</i>	
<i>Nigrospora sphaerica</i>	
<i>Pseudomonas aeruginosa</i>	
<i>Pseudomonas luteola</i>	
<i>Roseomonas aquatica</i>	

# RESEARCHING NEW GLOVEBOXES



DWI Breadboard TRR April 2018  
ID Technical Landmarks

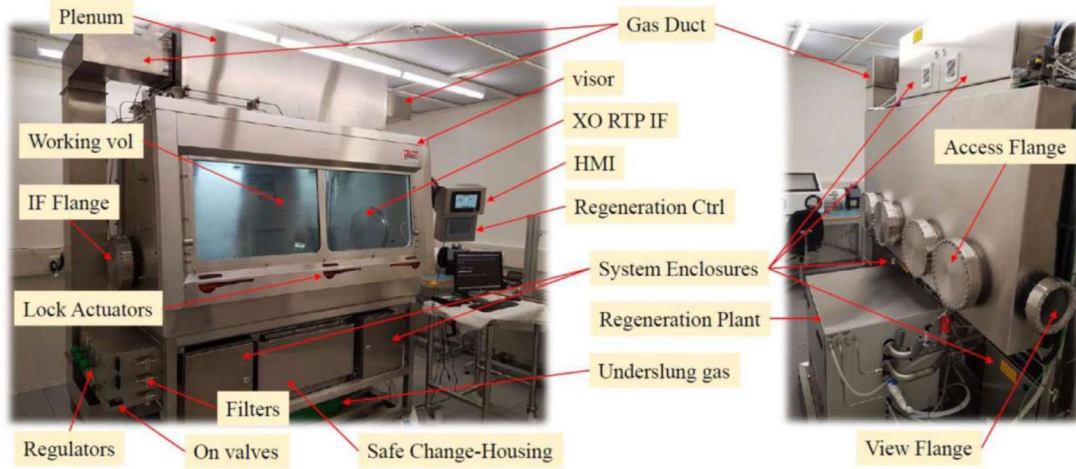


Figure 3.2-1. Double-walled isolator breadboard. [Source: University of Leicester]

- Mars Sample Return needs a new kind of glove box
- A Double Walled Isolator combines:
  - positive pressure to protect the samples from terrestrial contamination
  - a negative pressure biobarrier to protect earth from biological contamination from Mars

Mattingly, R. L., Smith II, A. L., Calaway, M. J., and Harrington, A. D. (2020). Tours of High-Containment and Pristine Facilities in Support of mars Sample Return (MSR) Sample Receiving Facility (SRF) Definition Studies. Available at: <https://ntrs.nasa.gov/citations/20210009183>

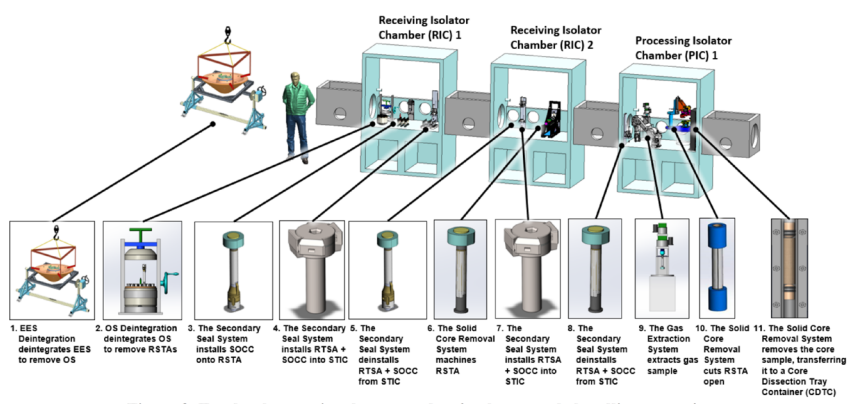


Figure 3. Top-level operational concept showing key sample handling operations.

Younse, P., Chesin, J., Phelps, P., Gerdts, S., Perez, O. R., Plourde, J., et al. (2024). Mars Sample Return Sample Handling Technology Development., in 2024 IEEE Aerospace Conference, 1–26. doi: 10.1109/AERO58975.2024.10521426

# RESEARCHING STERILIZATION METHODS THAT MINIMIZE ORGANIC AND INORGANIC CONTAMINATION

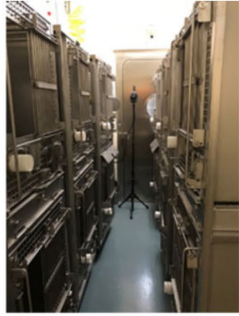
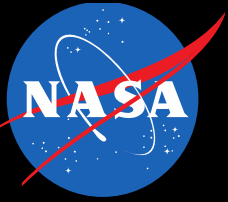


Figure 1a: Fumigation Room Decontamination Setup.



Figure 1b: Portasens II - Parametric testing of O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> concentrations during decontamination.

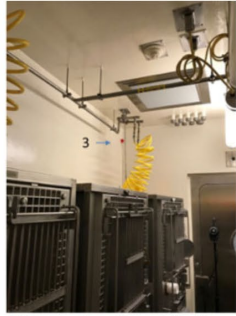


Figure 1c: Sample 3 located behind the iHP application nozzle.

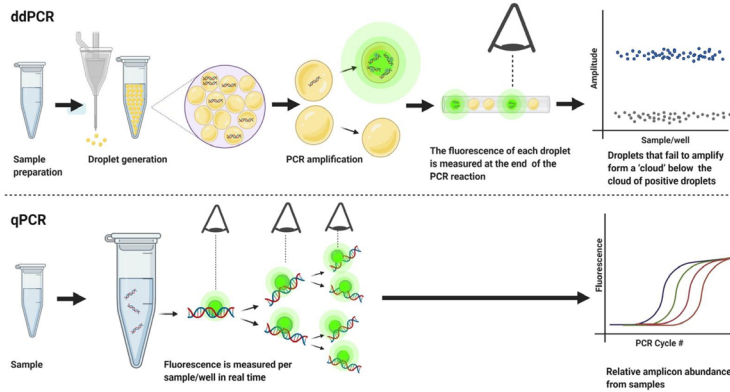
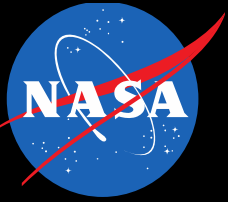


Figure 1d: Sample 4 located behind the iHP application nozzle. Also, view decontamination equipment.

- Vaporized 30 wt% H<sub>2</sub>O<sub>2</sub> to sterilize surfaces and isolators
- Ionized 7.5 wt% H<sub>2</sub>O<sub>2</sub> to sterilize more sensitive materials

Grimaldo, M. A., Bouyer, D. H., and de Siqueira, C. L. M. (2020). Determining the Effectiveness of Decontamination with Ionized Hydrogen Peroxide. *Appl Biosaf* 25, 134–141. doi: [10.1177/1535676020935405](https://doi.org/10.1177/1535676020935405)

# RESEARCHING IMPROVED MONITORING METHODS



**Figure 1.** Droplet digital PCR (ddPCR) versus quantitative PCR (qPCR). In ddPCR, samples are partitioned into thousands of droplets, each of which undergo amplification. After PCR cycling, the fluorescence of each droplet is measured at the end of the PCR reaction. As an output, droplets are grouped as positive or negative based on their fluorescence amplitude levels (y axis). In contrast, qPCR, fluorescence gives one measure per sample, at the end of each PCR cycle. Due to such fundamental differences in the approach, guidelines for standardizing qPCR assays such as the Minimum Information for publication of Quantitative real-time PCR Experiments (MIQE) [10] are not appropriate for ddPCR, and users are left to develop their own quality control protocols. Overall, ddPCR does not require sample replication, does not require to generate a standard curve for absolute quantification, and has increased sensitivity in target detection. As such, ddPCR represents a great option for analyzing environmental samples. The figure was created with BioRender (BioRender.com; 9 April 2021).

Kokkoris, V., Vukicevich, E., Richards, A., Thomsen, C., & Hart, M. M. (2021). Challenges Using Droplet Digital PCR for Environmental Samples. *Applied Microbiology*, 1(1), 74–88. <https://doi.org/10.3390/applmicrobiol1010007>

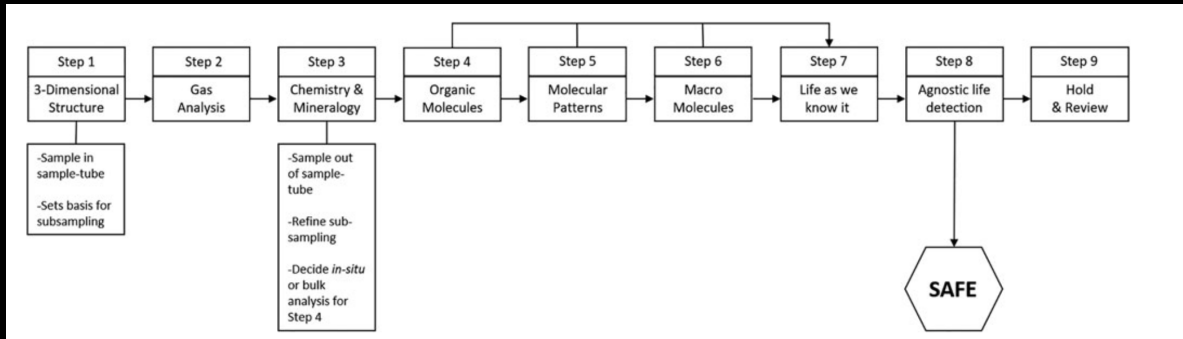
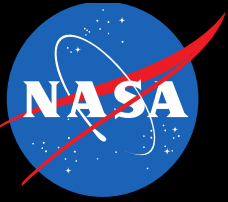
- Viable Particles
- Non-Viable Particles
- Microbial Collection

BioTrak® Real-Time Viable Particle Counter provides complete environmental monitoring of all in cleanroom spaces. Monitoring for both total and viable particles can be performed with this one instrument using a single isokinetic probe located within the test area.

- 1 Total Particle Counter**  
A 1 CFM (28.3 LPM) total particle counter provides the same trusted measurements users expect from all TSI particle counting instruments. Fully compliant to ISO 21501-4, it is suitable for use in all GMP applications.
- 2 Biofluorescent Particle Counter (BFPC)**  
Viable particles, also referred to as autofluorescence units (AFU), are detected using laser induced fluorescence (LIF). LIF works by detecting the fluorescence of metabolites in viable microorganisms that are excited as they pass through a laser beam. This requires no growth or reagents and makes viable results available in real-time.
- 3 Particle Filter**  
A highly efficient gelatin filter can be installed to capture the particles that pass through the BFPC. The filter can be transferred to growth media for an opportunity to identify cultureable contaminants that were present in the sample.

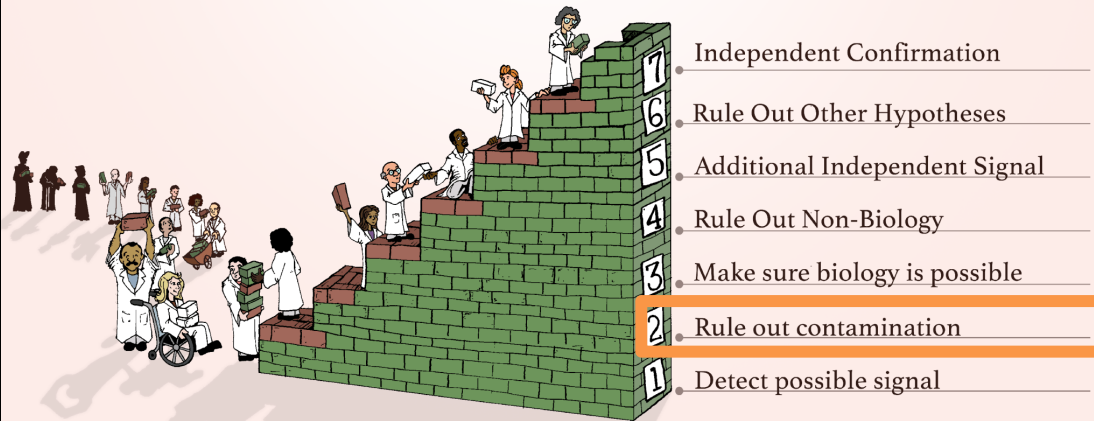
- Collaborating with planetary protection to develop fast and reliable culture-independent microbial monitoring methods
  - Digital PCR to quantify biomass
  - Metagenomics methods for extremely low biomass samples
- Testing FDA and EU approved biological particle counters for automated monitoring
- Need better surface sampling methods

# TESTING METHODS TO ASSESS MARS SAMPLE SAFETY



- We need collaborators!
- Sample safety assessment may rely on mass spectrometry-based identification, quantification, and sequencing
  - DNA
  - RNA
  - Peptides
  - Proteins
- Proposed Instruments
  - X-ray tomography
  - Hyperspectral imaging
  - Electron microscopy
  - Fourier transform infrared spectrometry
  - Gas or liquid chromatography mass spectrometry
  - Spatially resolved or mass spectrometry
  - Raman spectroscopy

## CoLD SCALE



# CONCLUSIONS



- Recent sample return missions like OSIRIS-REx and Hayabusa 2 have taught us a lot about how to curate biologically sensitive samples
- We will learn more from the MMX sample return mission
- More research is needed to be ready more mars sample return
  - High containment cleanrooms
  - Novel cleaning, sterilization and monitoring techniques
  - Workflows for safety assessment and life detection verified and validated on analog samples