Heat Inactivation of Microbes Embedded in Aerospace Materials

Emily Klonicki

Jet Propulsion Laboratory (JPL) / California Institute of Technology

Jet Propulsion Laboratory
California Institute of Technology

Planetary Protection (PP) ensures that spacecraft meet stringent cleanliness requirements to prevent forward and backward biological contamination

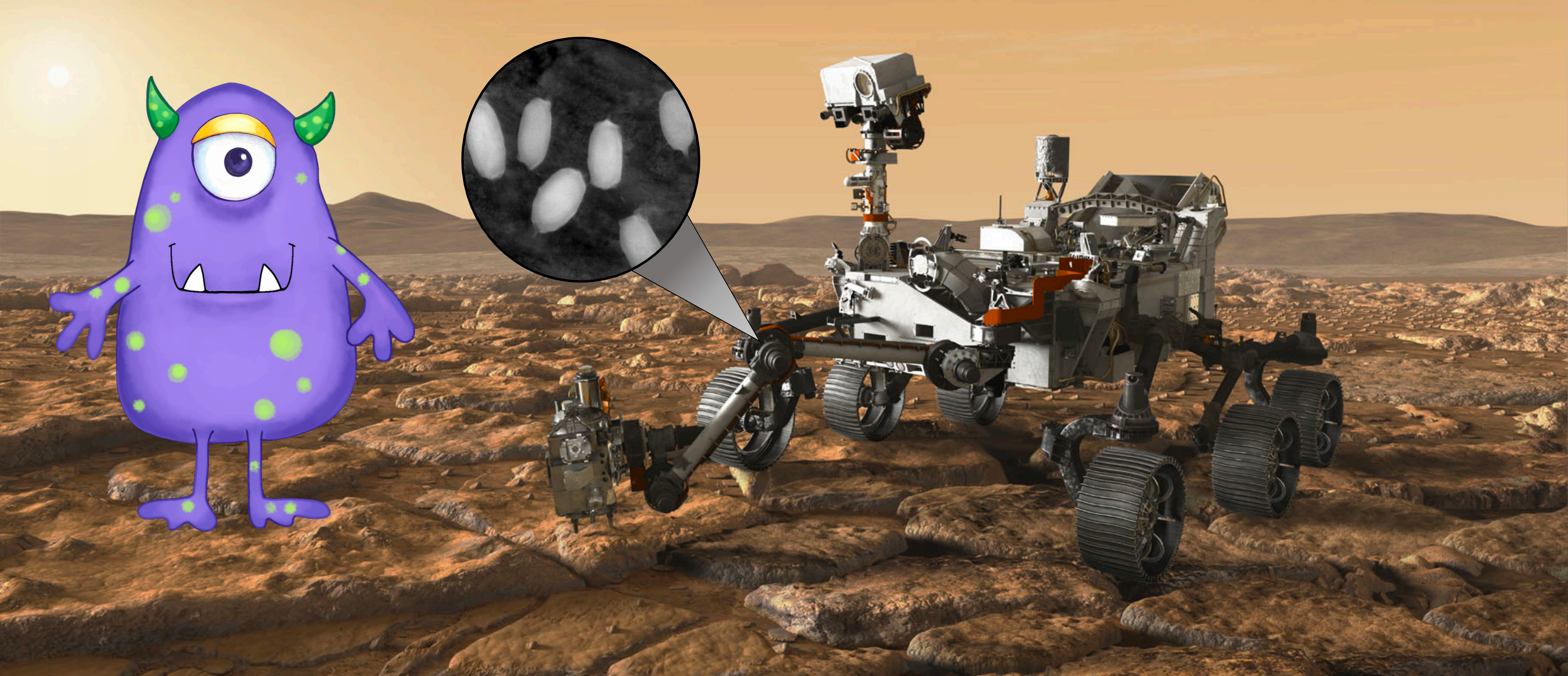


Image Credit: NASA/NASA JPL

JPL PP Group seeks to advance spacecraft cleanliness, sterilization, and validation technologies for NASA's solar system exploration missions

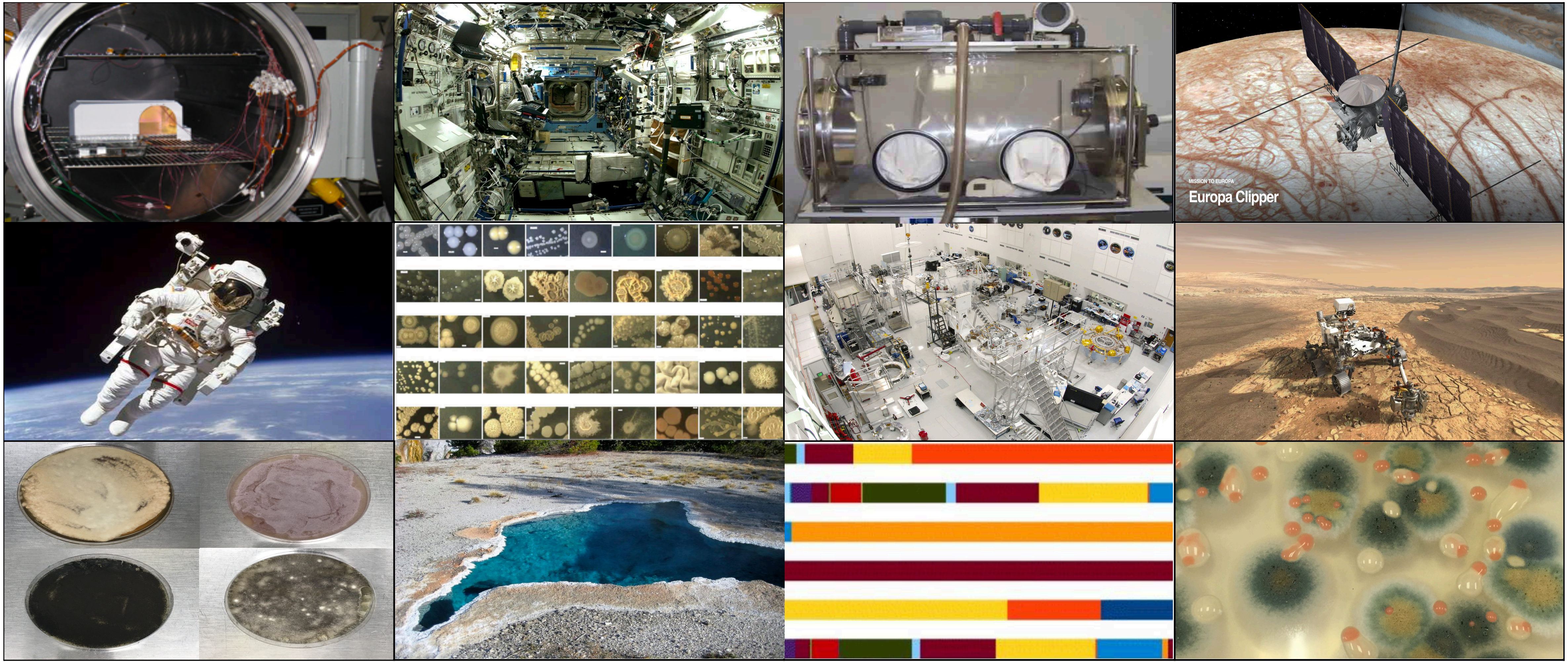
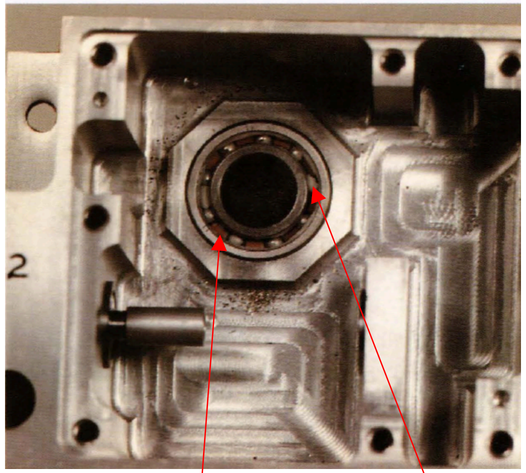


Image Credit: NASA/NASA JPL

Heat Microbial Reduction (HMR) is considered the "Gold Standard" of sterilization techniques at NASA

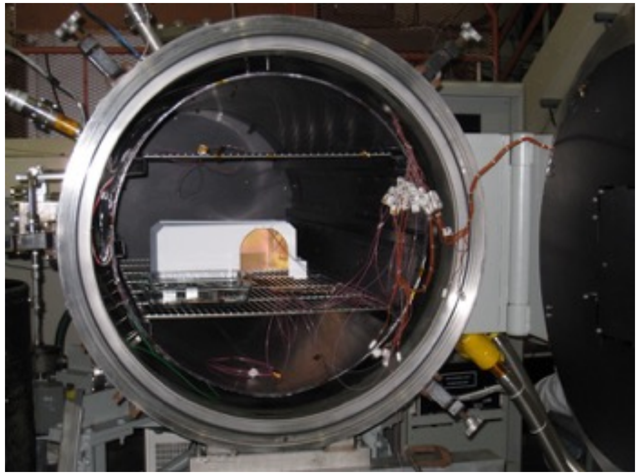
- NASA PPO approved standard process used for spacecraft surface microbial reduction
- Employed on assemblies with large surface areas, difficult to clean, or with limited access, and on assemblies with encapsulated bioburden in bulk materials
- Time-temp requirement based on lethality curves
  - (Developed from ATCC 29669 JPL data generated by JPL & ESA)
- D-value
  - Decimal reduction value
  - Specifies the dose (time-temp) required for reducing spore populations
    - 1D-value = 1-log reduction = 90% reduction of initial population

For **encapsulated**, the *D*-value is 10 times the *D*-value for a free surface, or 5 times a mated surface



Oil-impregnated polymeric material

Perfluorinated polyether extreme-pressure grease



Small part vacuum 'bake out chamber' for HMR at JPL

Image Credit: NASA/NASA JPL

Jet Propulsion Laboratory
California Institute of Technology

There is limited data on to the effect of HMR on embedded spore survival

1. **Are the current heat microbial reduction standards well positioned?**
2. **Are there any “unknown” effects of encapsulation/embedding?**

2. Heat sample under vacuum in Thermal Spore Exposure Vessels (TSEVs) with oil bath

1. Embed *Bacillus atropheus* spores

3. Process material (Liquid-liquid extraction and cryogrinding)

Clockwise from top left: Arathane 5750 conformal coat, Braycote 600 lubricant, Apiezon H lubricant

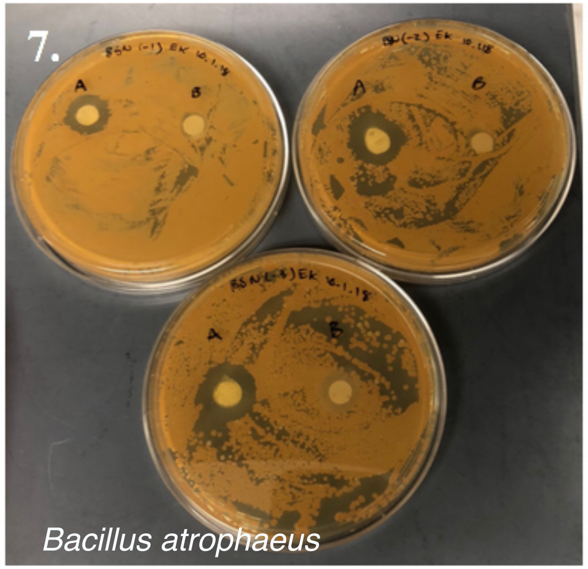
Image Credit: Apiezon, Krayden, Castrol

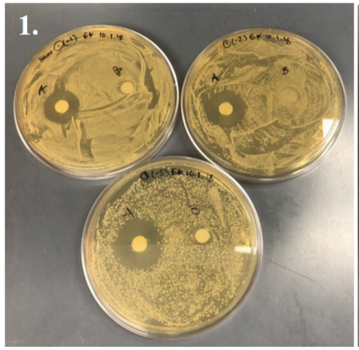
Jet Propulsion Laboratory
California Institute of Technology

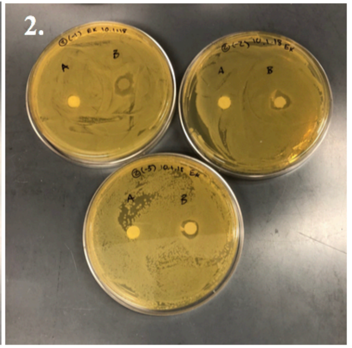
Arathane (Part A) inhibits the growth of *Bacillus atropheus* spores

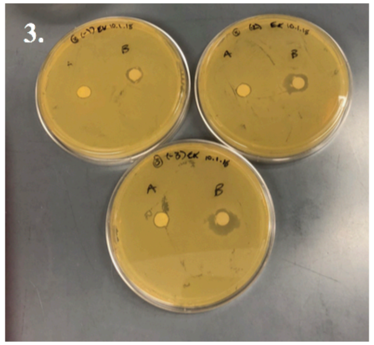
Organism	Component	10^{-1}	10^{-2}	10^{-3}
1. Staphylococcus epidermidis ATCC 12228	A	19 mm	25 mm	25 mm
2. Escherichia coli 11775	No effect	--	--	--
3. Stenotrophomonas ATCC 13637	No effect	--	--	--
4. Staphylococcus xylosus OB 205 (Viking Mission Isolate)	A	17 mm	19 mm	15 mm
5. Deinococcus radiodurans	A	15 mm	23 mm	17 mm
6. Acinetobacter radioresistens OB 305 (Viking Mission Isolate)	No effect	--	--	--
7. Bacillus atrophaeus (Lot 1092572,1x10^9/mL)	A	17 mm	23 mm	19 mm

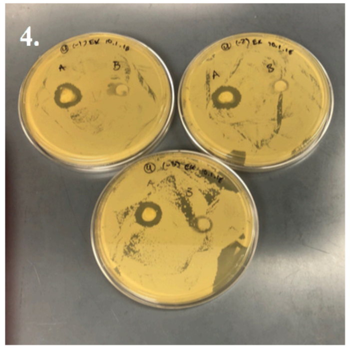
Inhibitory Component: Part A

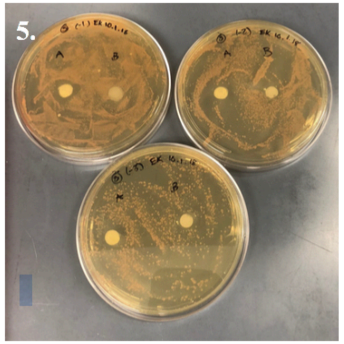


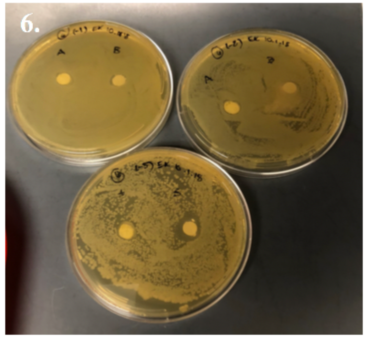






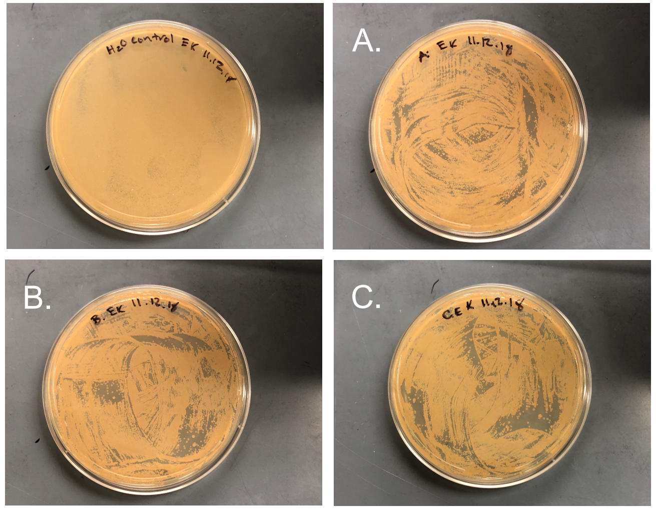






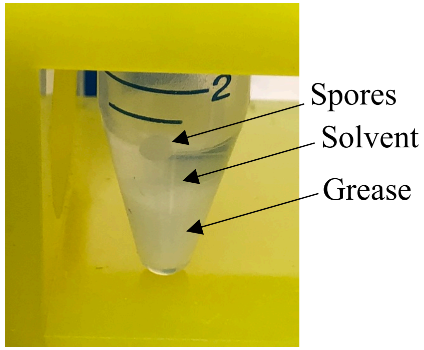
A liquid-liquid extraction protocol was developed to remove spores from vacuum greases

Solvent Toxicity Study → Preliminary Exposures → Extraction protocol development

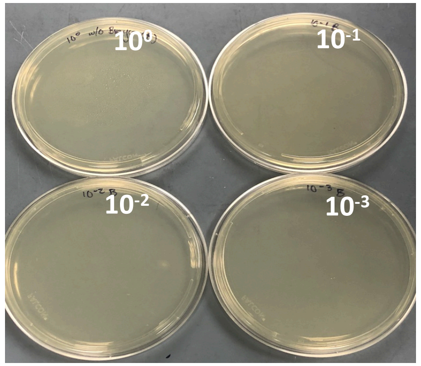


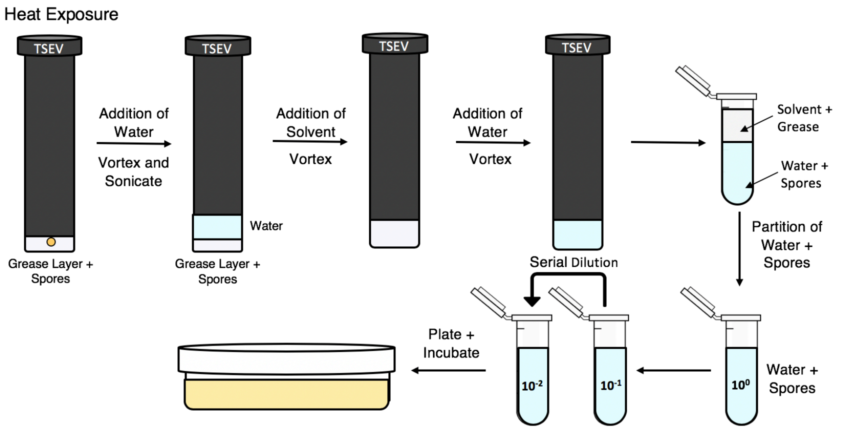
Negative toxicity results for Freon TF (B), Castrol Brayco IC X-100 (C), and Dupont Vertrel XF (D) compared to the deionized water positive control (A)

1. Spores mixed into dissolved grease (then partitioned)



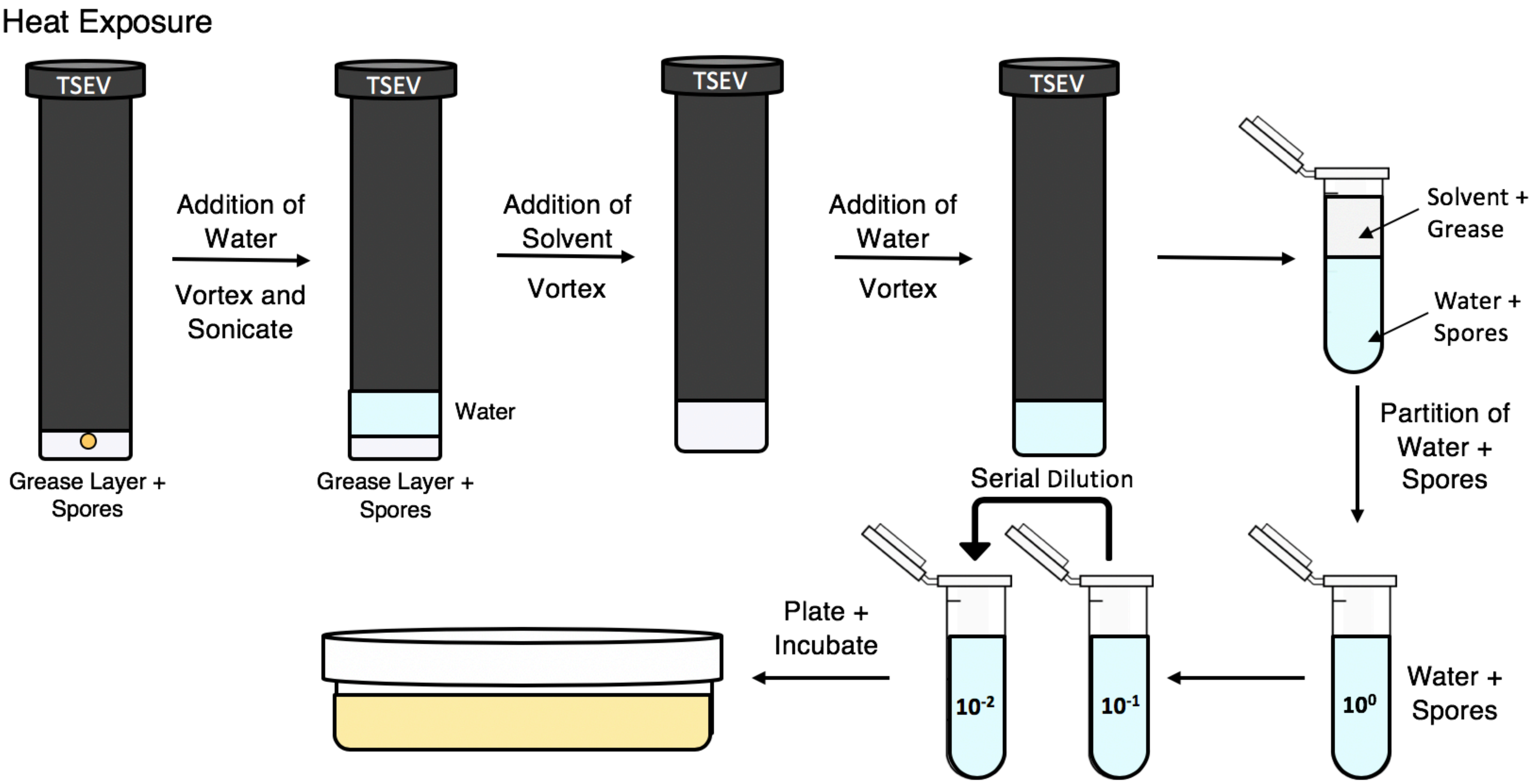
2. Spores mixed directly into Brayco
3. Spores dried before mixed into solvent



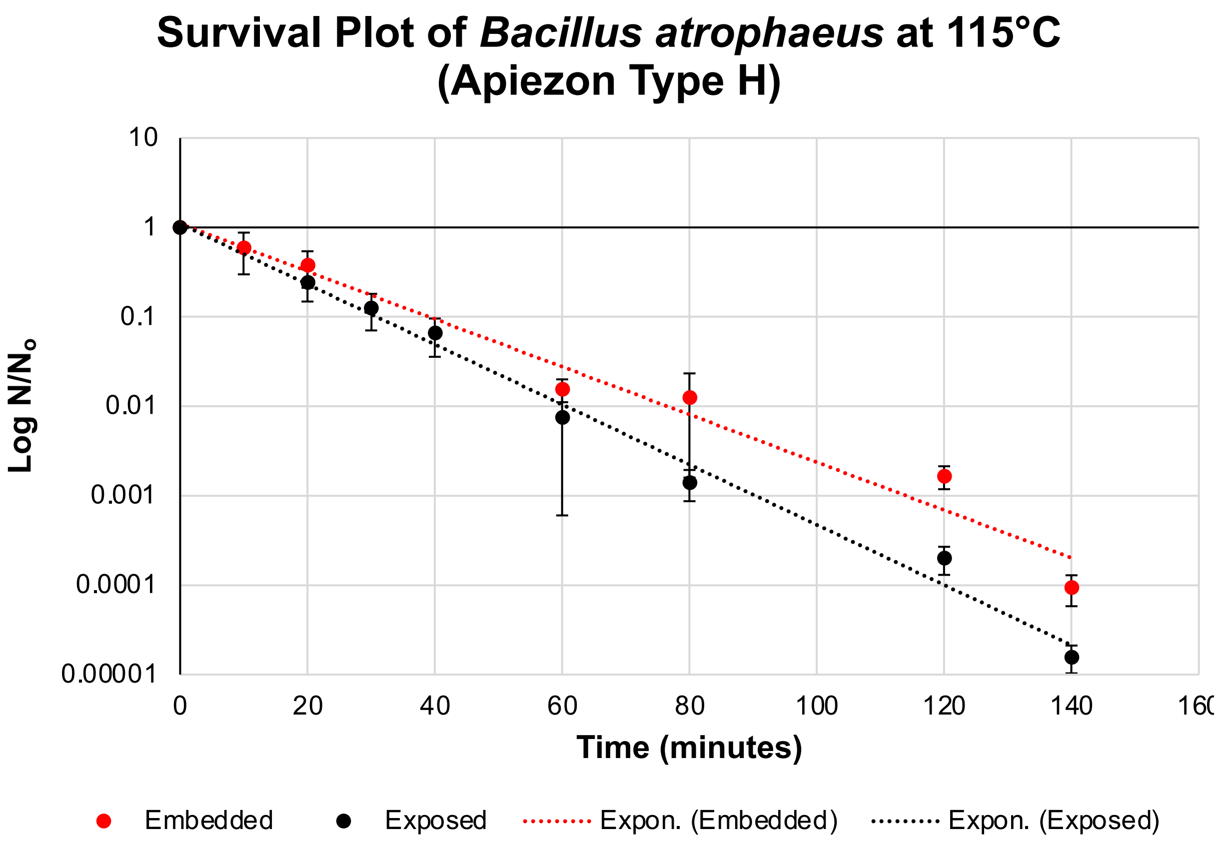


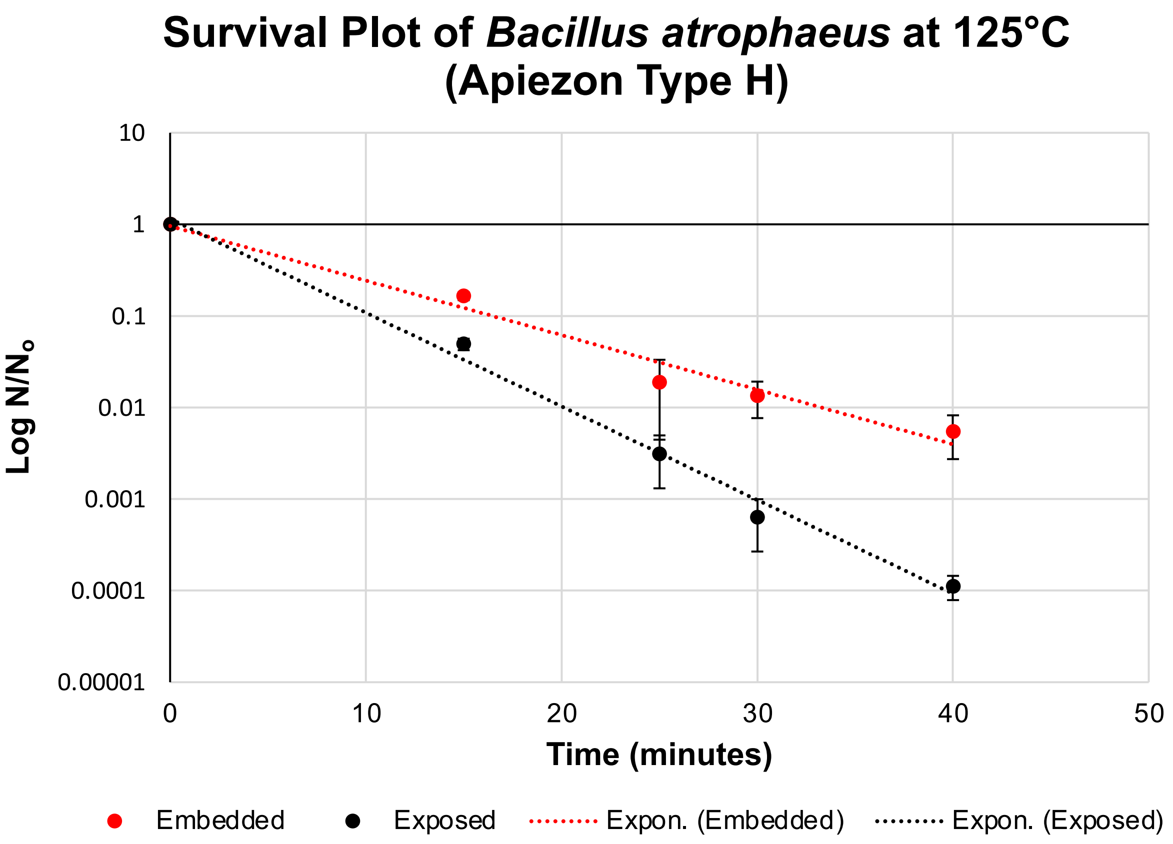
Jet Propulsion Laboratory
California Institute of Technology

A liquid-liquid extraction protocol was developed to remove spores from vacuum greases



Spores encapsulated in lubricants have a higher survivability then exposed spores

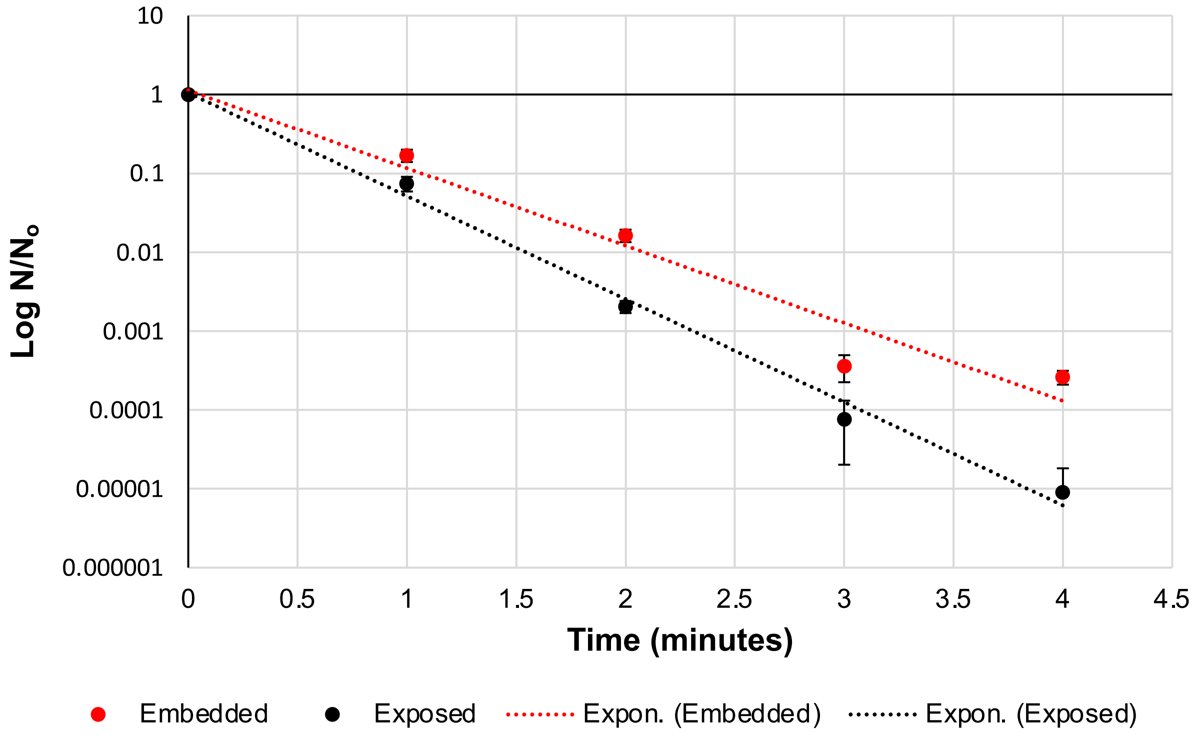




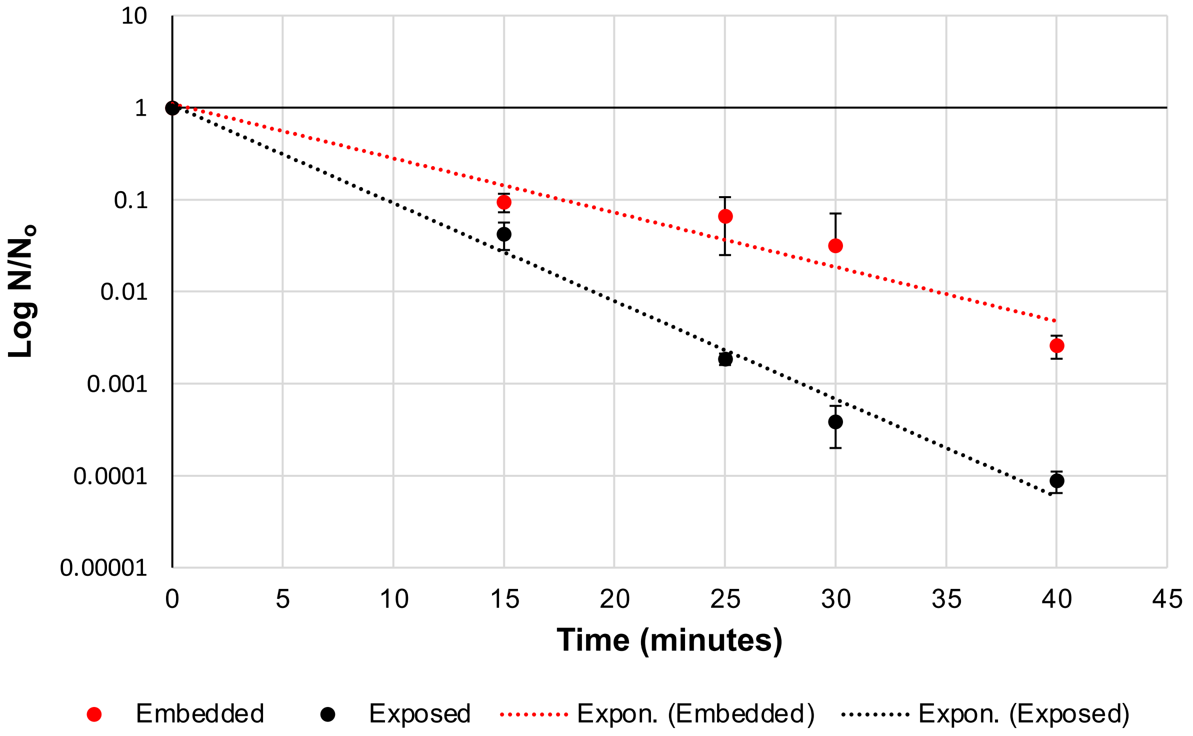
Jet Propulsion Laboratory
California Institute of Technology

Spores encapsulated in lubricants have a higher survivability then exposed spores

Survival Plot of *Bacillus atrophaeus* at 150°C (Apiezon Type H)



Survival Plot of *Bacillus atrophaeus* at 125°C (Braycote 600)



Jet Propulsion Laboratory
California Institute of Technology

The D-values for exposed spores were always found to be shorter than those embedded

Location	D-Value (minutes)	Temperature (°C)	Material	Protection Factor
Surface	29.76	115	Apiezon Type H	---
Encapsulated in Lubricant	37.45	115	Apiezon Type H	1.26 x Surface
Surface	9.78	125	Apiezon Type H	---
Encapsulated in Lubricant	16.78	125	Apiezon Type H	1.72 x Surface
Surface	0.77	150	Apiezon Type H	---
Encapsulated in Lubricant	1.02	150	Apiezon Type H	1.32 x Surface
Surface	9.43	125	Brayco 600	---
Encapsulated in Lubricant	16.92	125	Brayco 600	1.79 x Surface

*Protection factor: the difference in D-Value between embedded and exposed spores

Results in context with Mars 2020 bioburden reduction standards for Mars 2020 Rover

Conformal Coatings

- Spore toxicity observed in Arathane
- Bioburden specification can be assigned a low cell value

Lubricants

- Greases have a "protective" effect on embedded spores
- The increase in time required to kill (protective factor) is up to 1.8x
- Embedding in greases may effect core water mobility and exchange with the surrounding environment
- D-values at 125°C with Braycote and Apiezon grease were similar (16.78 and 16.92)

Bacillus atropheus

- Lethality rates lower than practical 4 – Log Mars 2020 bake-out specifications
- No concern for the need to modify the working requirement

4-log surface reduction: 112 ºC ± 2 ºC for 132.2 hrs

4-log encapsulated reduction: 116 ºC ± 6 ºC for 582.6 hrs

ENCAPSULATED

Temperature (ºC)	Time (hrs)		
	3-log	**4-log**	**6-log**
116	74.6	**582.6**	-
125	18.75	442.9	-
150	1.4	40.4	121.3
200	0.01	0.3	1.0

*Times include a 25% margin as required for Mars 2020

Thank you to the Biotechnology and Planetary Protection Group

Wayne Schubert

Jet Propulsion Laboratory
California Institute of Technology