

Microbial Certification of the MER Spacecraft

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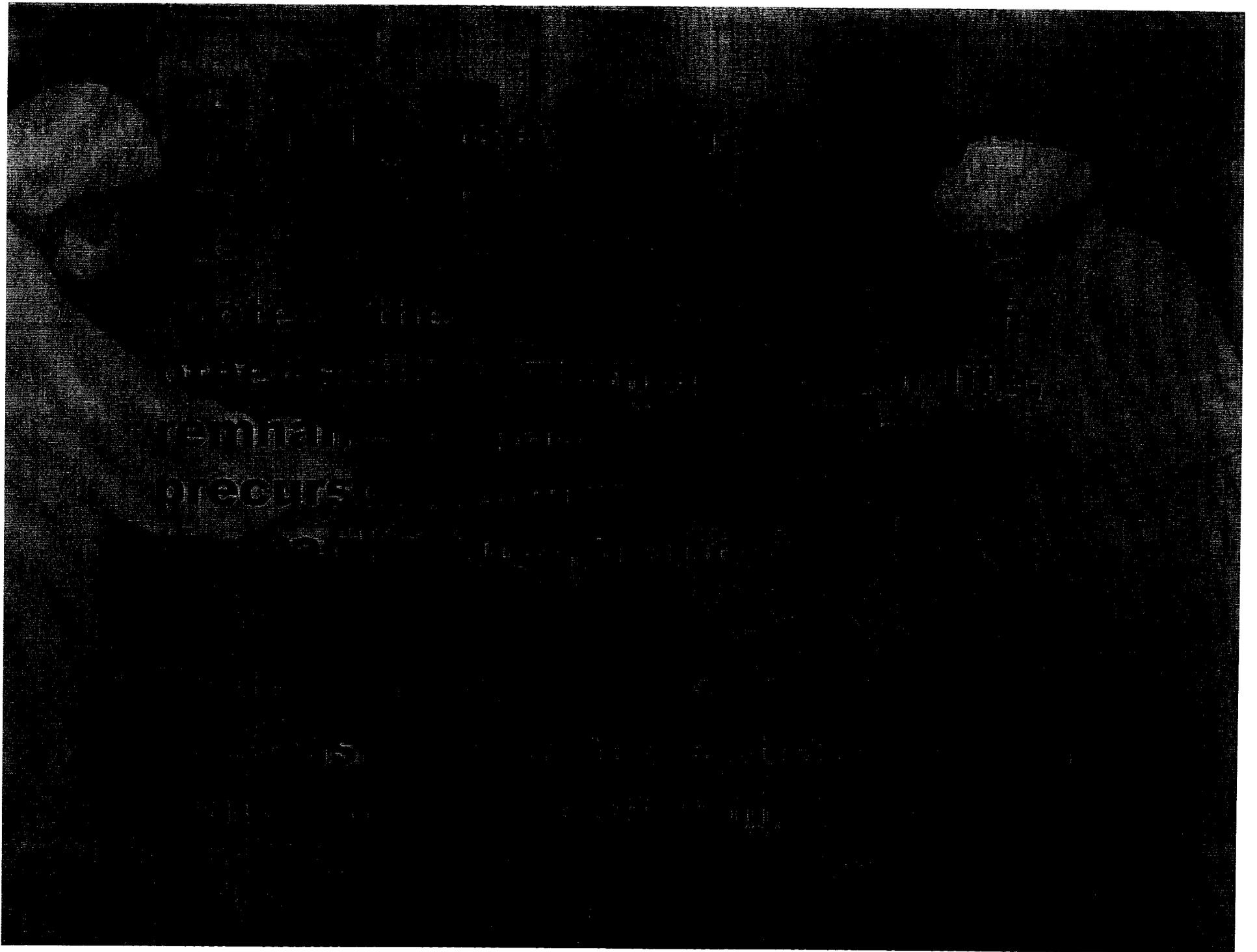
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Abstract

- Spacecraft such as the Mars Exploration Rovers (MER) must meet acceptable microbial population levels prior to launch. Sensitive parts and materials prevent any single sterilization method from being used as a final step on the assembled spacecraft. How can a flight project achieve minimal microbial populations? Our approach to meeting the low population requirements include the active destruction of viable microbes and spores by Dry Heat Microbial Reduction (DHMR), the assembly of spacecraft in cleanrooms, frequent alcohol wiping of spacecraft parts, and use of protective barriers to prevent re-contamination. Procedural controls are used for evaluating and tracking components to ensure compliance with clean assembly practices and DHMR specifications. Bioburden assessments utilize Tryptic Soy Agar (TSA) pour-plates and the enumeration of resulting colonies from aerobic spores. The use of a barcode system with a custom-designed database permits the tracking of colony counts from swab-samples, hardware parts and spacecraft zones, in order to keep a running tally of the spacecraft bioburden and carry out statistical calculations. Additional biochemical methods that detect ATP or endotoxin (Limulus Amebocyte Lysate (LAL) assay) are performed for research purposes as rapid assessment methods. Pour-plate assay results, when compared to ATP and LAL results, lead to similar conclusions regarding the levels of cleanliness. For MER, the required bioburden level for surfaces is an average density of less than 300 spores/m² and a total bioburden of less than 3 x 10⁵ spores. These levels apply to “exposed” (planetary protection accountable) surfaces only. Data from our bioassays of the spacecraft find the current estimates of the total spore population and spore density levels to be significantly less than these requirements. We conclude that a combination of DHMR and control measures during complex mechanical assembly processes can result in a total spore bioburden that meets requirements.



Introduction

- **Purpose: Prevention of the unintentional introduction of microbes to Mars.**
- **Problem: Many parts, materials, electronics and instruments cannot tolerate sterilization temperatures.**
- **Plan: Multi-Pronged approach for reducing microbial contamination.**
- **Performance: Actions taken resulted in sufficiently low microbial bioburden to allow the launch of the spacecraft**

*The research described in this presentation was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

Approaches Used to Lower Microbial Levels

- **Dry Heat Microbial Reduction (125 °C, 5 hours, <1.0 Torr)**
- **Alcohol wiping**
- **Bioassays to evaluate cleanliness, using swab or wipe samples**
- **Clean Environment for assembly**
- **Tracking Hardware for compliance**
- **Barriers to prevent re-contamination**
- **Filtered Air**
- **HEPA filters as built in vents for some spacecraft parts**
- **People wear cleanroom clothes, gloves, masks**
- **Biomolecule spot checks for cleanliness detecting ATP and lipopolysaccharide**

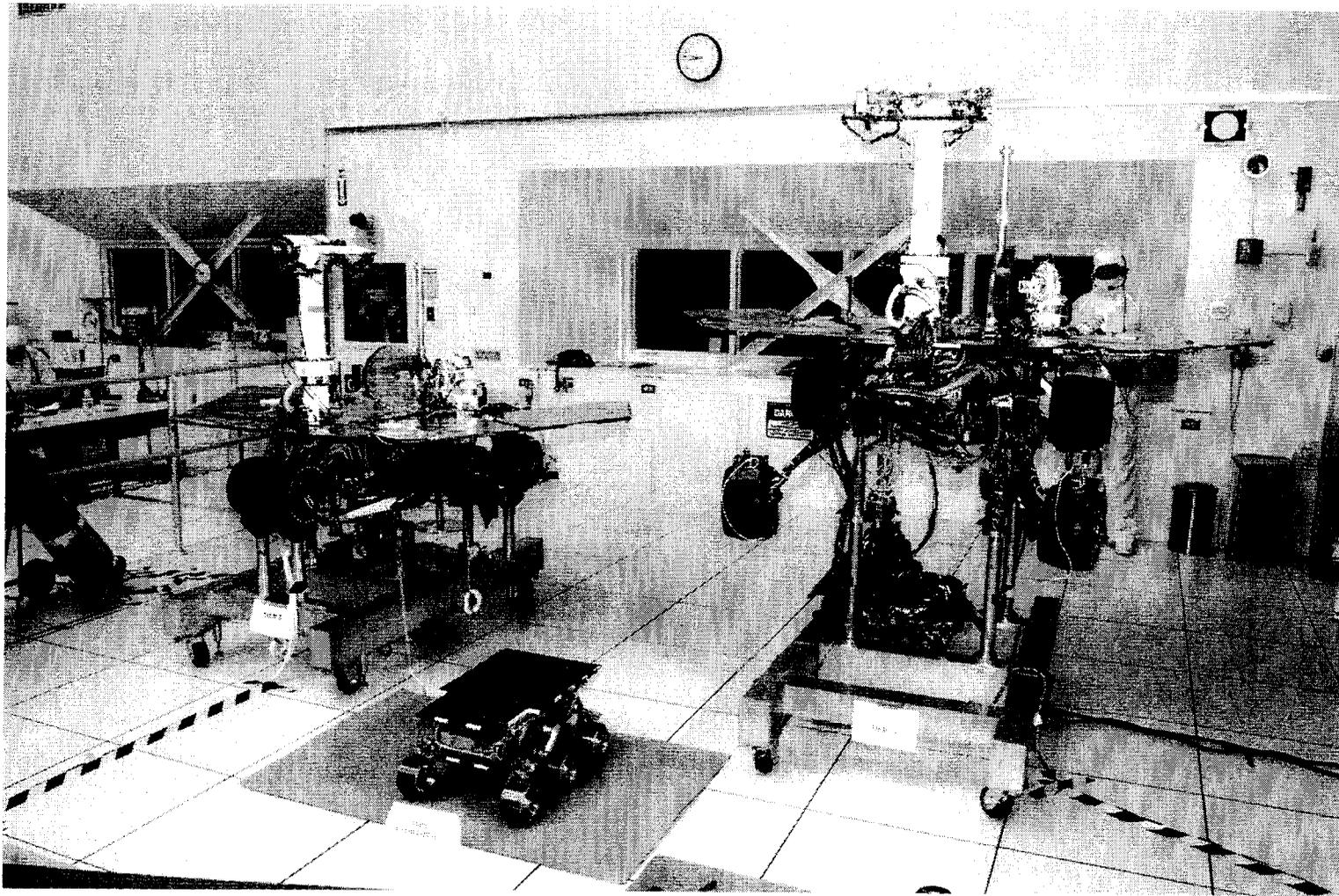
Rogues Gallery List of Sampes

Still in progress

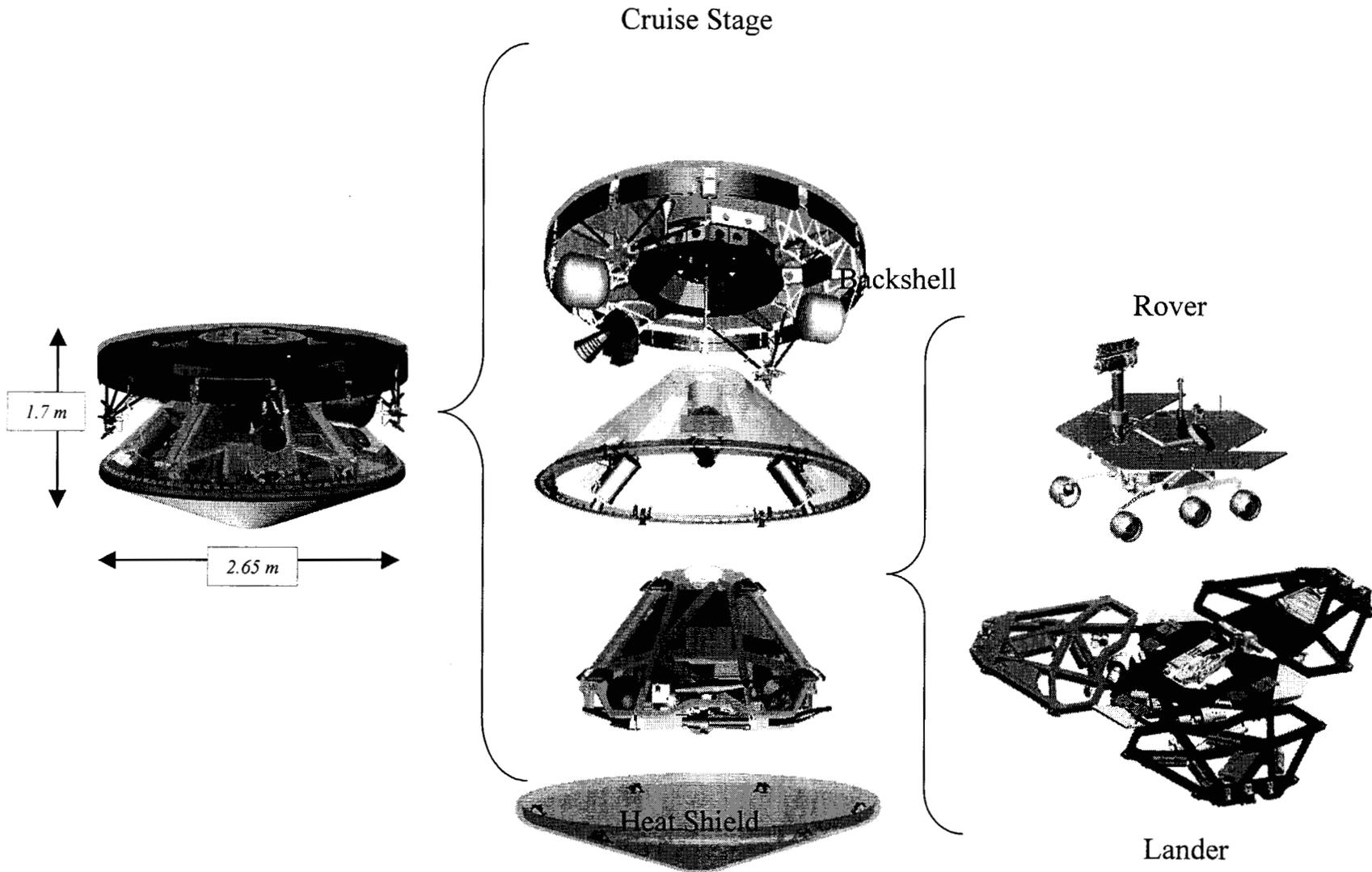
A Standard Culture-Based Method was used for Detecting Spores.

- **Collect Samples by wiping surfaces with sterile moist swabs or wipes.**
- **Vortex and sonicate to suspend cells in 10 mL DI-H₂O (swabs), or phosphate buffered, Tween-80 rinse solution (wipes).**
- **Heat shock for 15 minutes at 80°C (spore selection, kills vegetative cells).**
- **Four 2 mL aliquots were pipeted into dishes for swabs, twenty-five dishes for wipes. Tryptic Soy Agar (TSA) used for pour plates.**
- **Incubate aerobically at 32°C.**
- **Count plates at 24, 48 and 72 hours.**

MER #1, MER #2, & Sojourner Spare Rovers

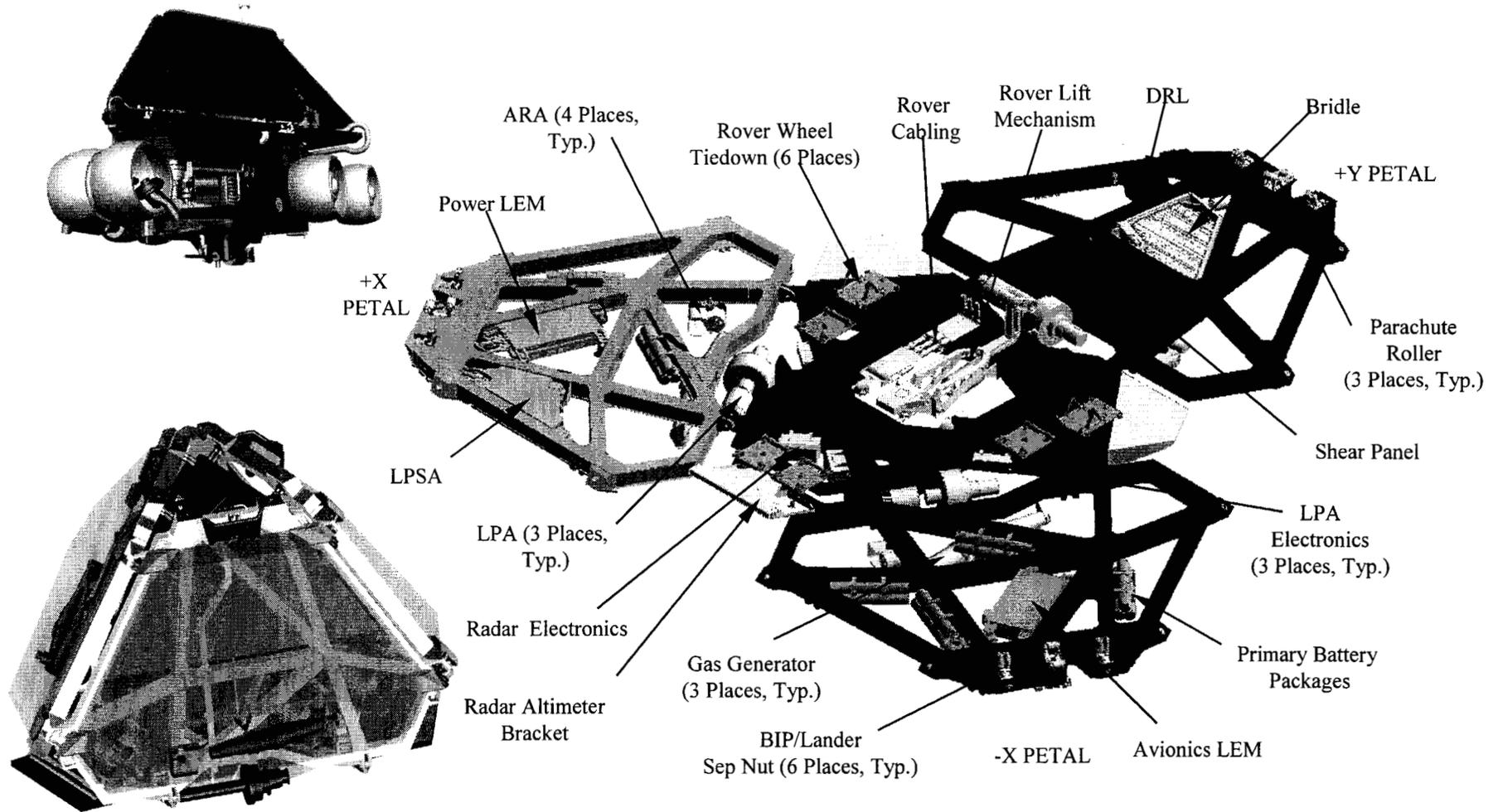


Spacecraft Configuration



Courtesy of JPL/Caltech

Lander Configuration

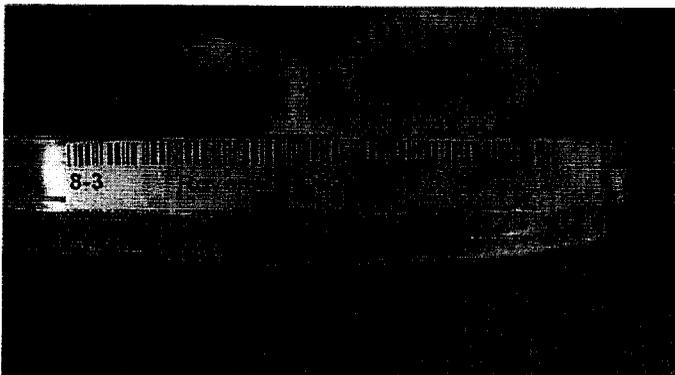
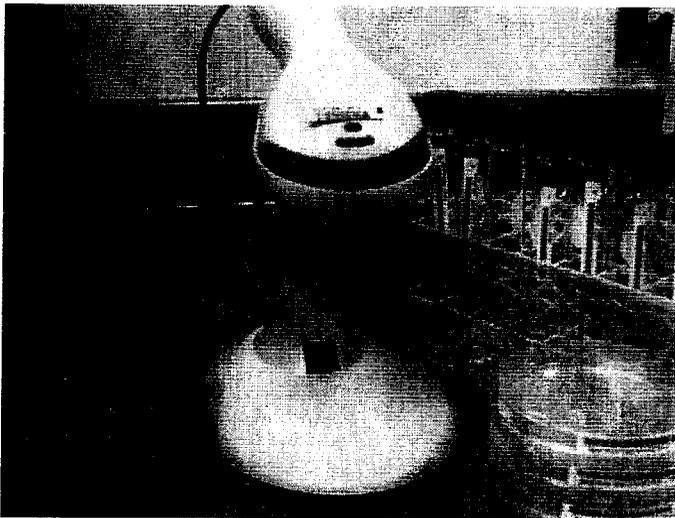


Courtesy of JPL/Caltech

Custom Barcode System Developed

Tracks Samples, Bioassay
Plates, CFU's and more

Easy Data Input for Plate
Counts



Assay Results

Plate ID: 0619021-001-4

MER 1

Cruise Stage

Date Sampled: 6/19/2002

Colony Count

	24 Hours	48 Hours	72 Hours
Time recorded	6/21/2002 1:02:34 PM	6/22/2002 1:45:56 PM	6/23/2002 2:35:31 PM
COUNT	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
TOTAL		<input type="text" value="0"/>	<input type="text" value="0"/>

Notes:

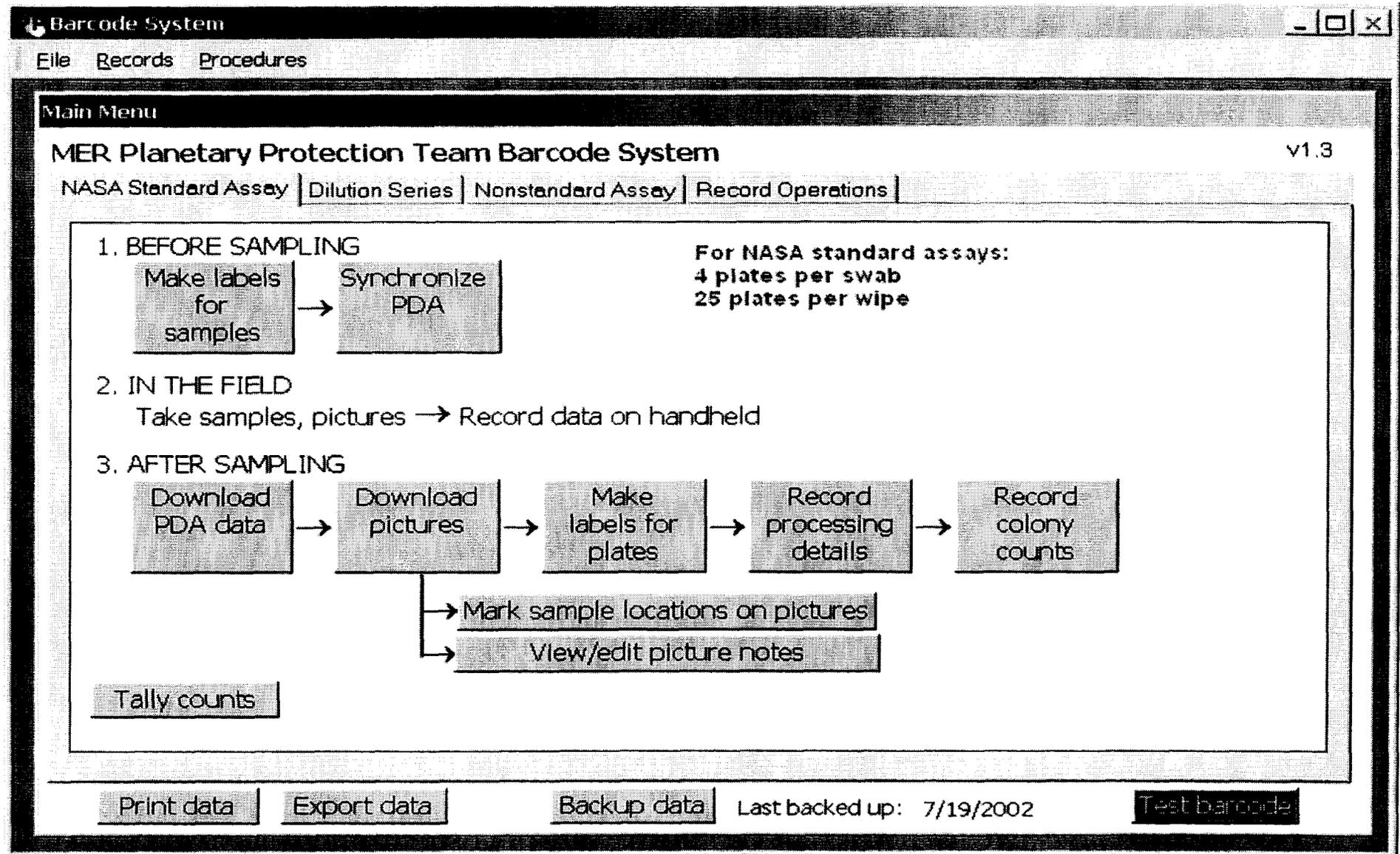
Done

Next unscannable plate

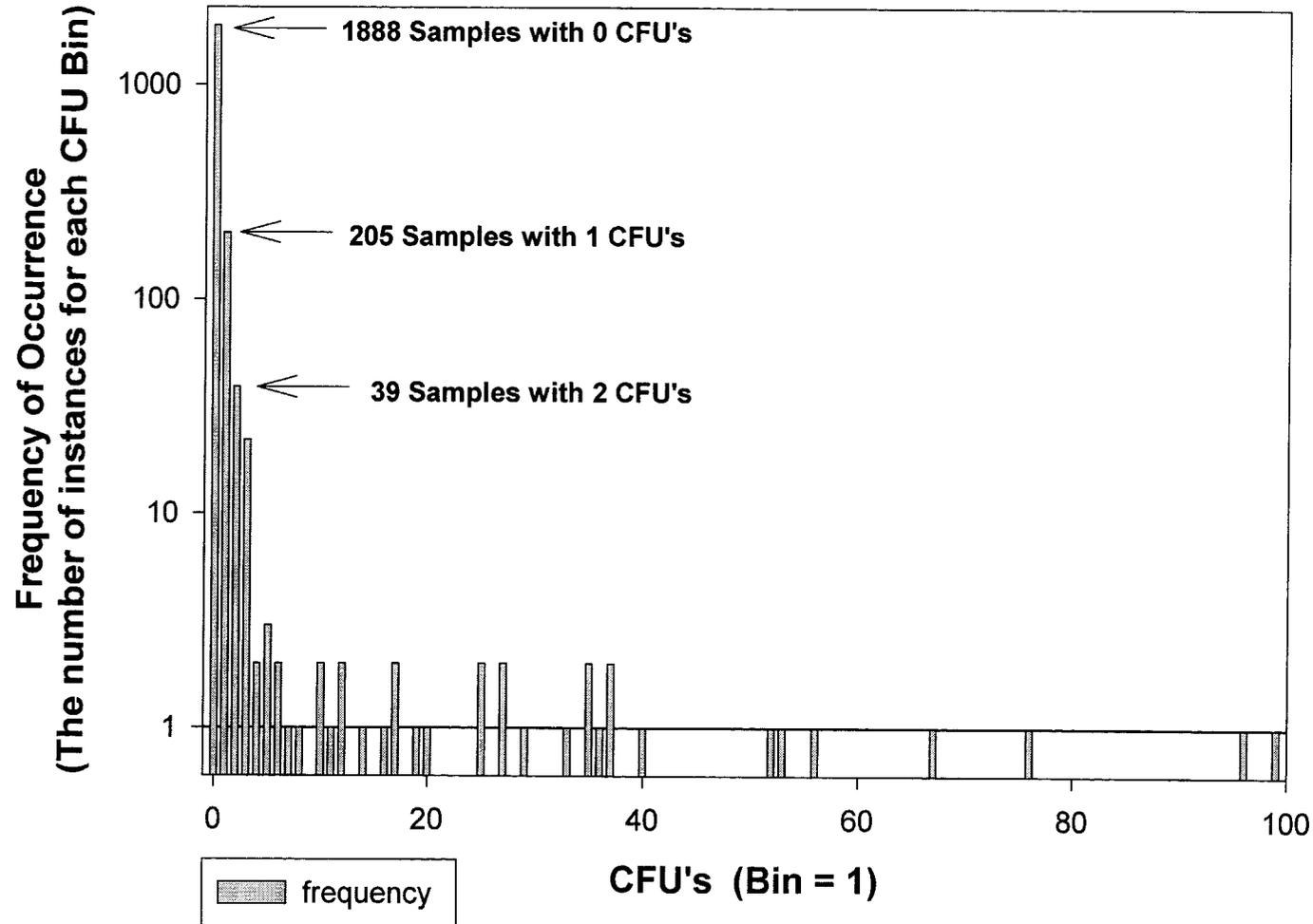
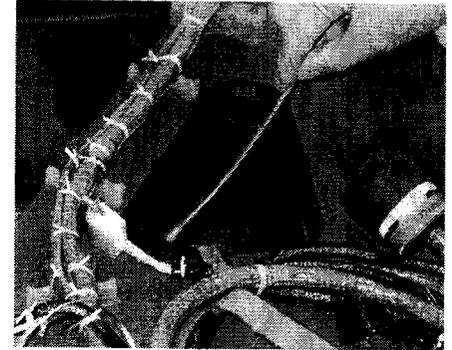
NEXT PLATE

Custom Barcode System

Merges Data & Images and Generates Reports



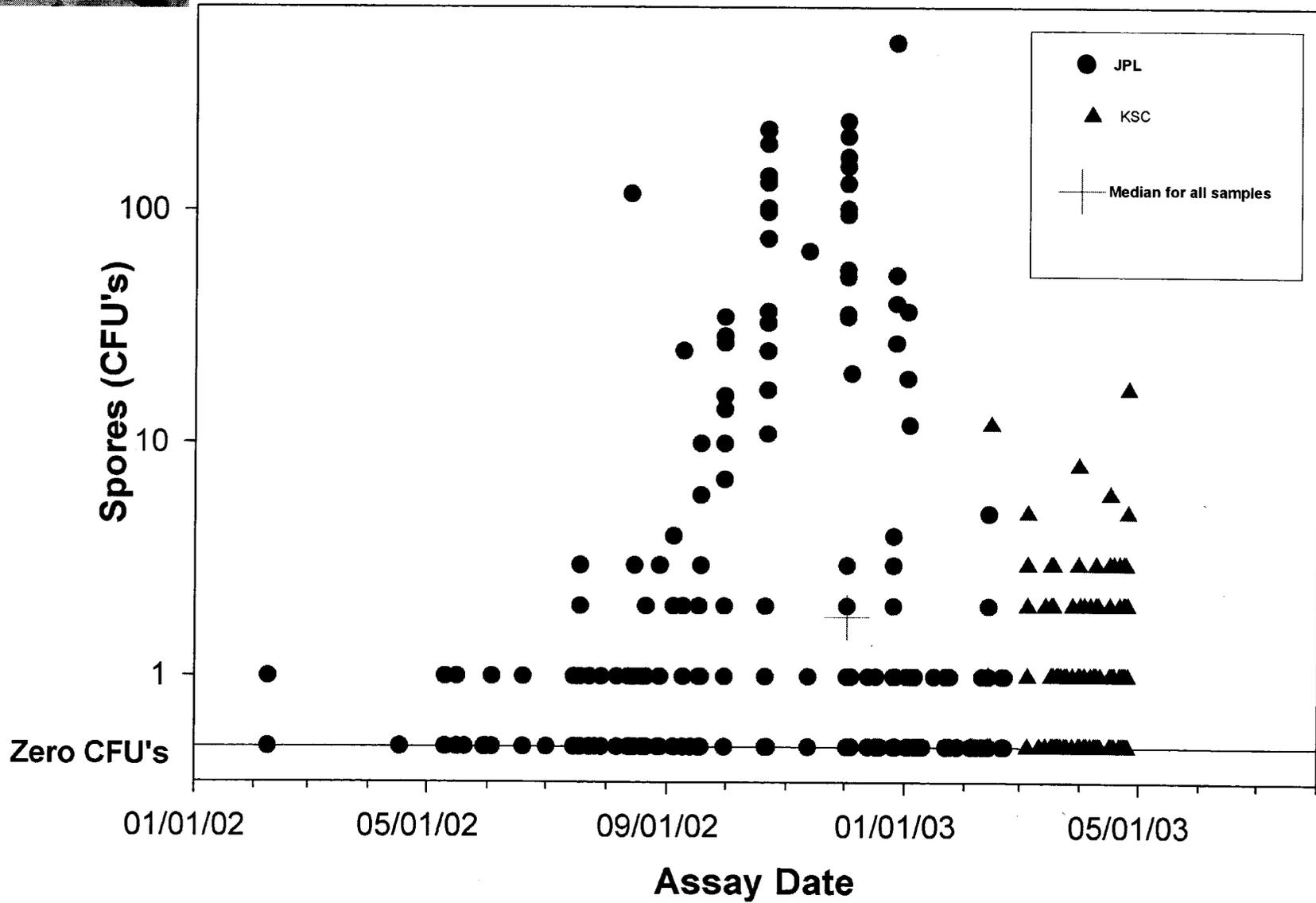
Frequency of CFU Counts Swab Samples





Colony Counts from Spacecraft

(Spores per Swab Sample)



Certification for Launch is Based on Low Spore Bioburden

Spacecraft Structure	Accountable Surface Area (m ²)	Spore Bioburden Estimate* 4/07/03		Comment
		MER-A (2) (+ Fairing)	MER-B (1)	
Lander	298	6.26E+04	7.85E+04	
Rover	72	1.32E+04	1.64E+04	
Aeroshell	405	4.82E+04	4.89E+04	
Entry Descent & Landing	2232	2.23E+04	4.13E+04	
Total		1.45E+05	1.80E+05	Certified for Launch!
Reserve Spore Allocation		1.55E+05	1.19E+05	
Requirement (maximum spores permitted)		3.00E+05	3.00E+05	

*Estimate based on bioassay results & assigned worst case high values

Bioburden Estimates for Selected Mars Missions

The following estimates are based on a statistical approach and exceed the bioburden detectable by a microbiological survey.

Mission	Mission Type	Life Detection?	Total Estimated Bioburden
Mars Mariner (1969)	Fly-by	No	10⁴
Viking (1976)	Landers (2)	Yes	< 30 spores
Mars Pathfinder (1996)	Lander	No	3.4 x 10⁴
MER	Lander (2)	No	Work in Progress

Conclusions

- **The first Mars Exploration Rover (MER) spacecraft was certified for levels of microbial contamination that were sufficiently low, allowing the justification of launch approval.**
- **Multiple microbial reduction approaches successfully resulted in a total spore bioburden that satisfied requirements.**
- **The majority of spores were found in relatively few samples. Only 2% of the samples contained 90% of the bioburden, and 15% of the samples contained 99% of the bioburden. No spores were found in 85% of the swab samples.**
- **Detectable spores decreased during final assembly.**

END of SLIDES

Note to Graphics

Make title size 8 inches high by 6 feet long, mars surface background, folded 3 or 4 places