# ATP Assay Development for Planetary Protection

Roger G. Kern
Biotechnology and Planetary Protection Group
Jet Propulsion Laboratory

Presented to the Microbial Detection and Characterization Workshop

Cocoa Beach, Florida 01/11/04

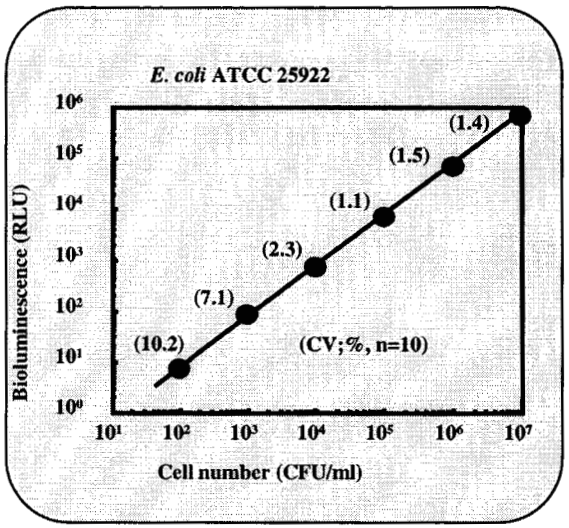
# Phase I
# Summary of Objectives

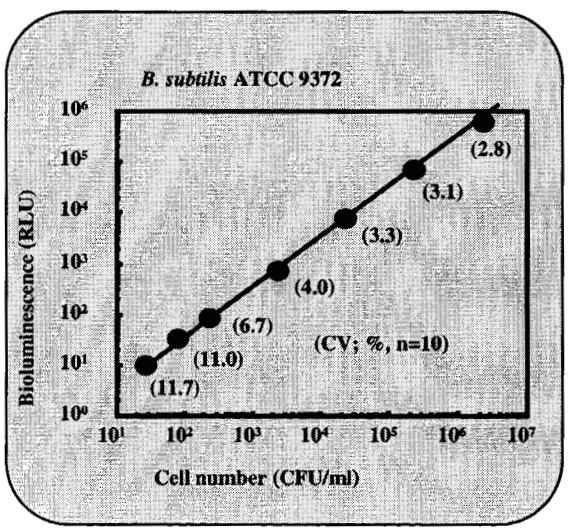
- **Experimental design**
  - Analysis of Spacecraft Sampling Data
  - Preliminary Experiments:
    - Molecular ATP Stability Studies
    - Molecular ATP Variability Studies
- **Generation of Standard Curves**
- **Validation of ATP/LAL Bioassays**
  - Environmental sample studies
    - Sample Stability Studies
    - Sample Variability Studies
  - Selection of Materials & Inoculation Methodology

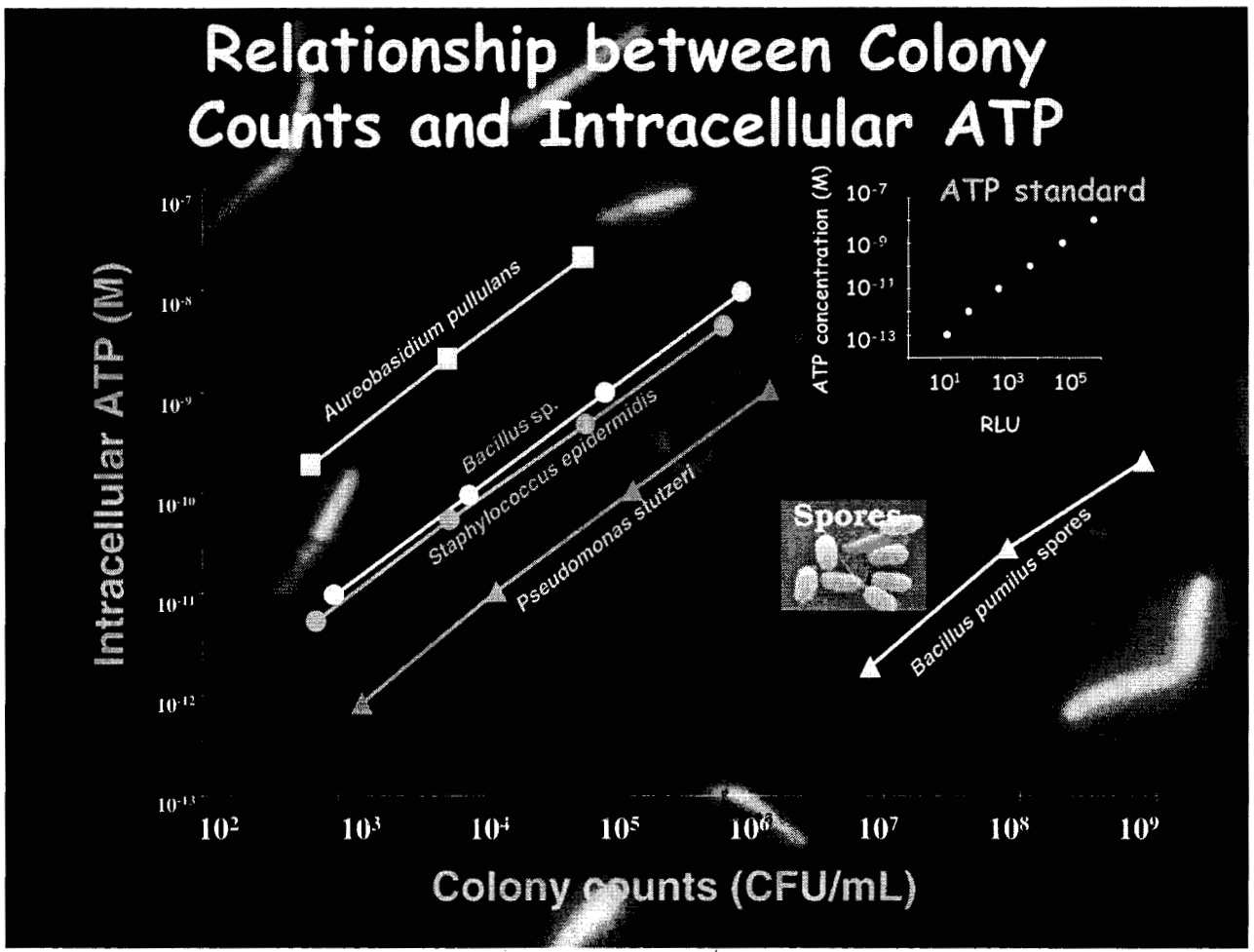
# Experimental Design

- Molecular ATP Stability Studies
- Molecular ATP Variability Studies
- Generation of ATP Standard Curves
- Environmental Sample Studies
  - Sample Stability
  - Sample Variability
- Statistical Analyses
  - Individual samples assayed in quadruplicate
  - Negative controls always included during assays
  - Extensive calibration curves carried out
    - 5 different concentrations used
      - » Ranged from $10^{-9}$ moles/L to $10^{-13}$ moles/L of ATP
      - » ATP chemical (positive control)
      - ❖ Same catalog #, lot # and frozen
      - ❖ $10^{-9}$ moles/L for consistency
  - ATP calibration curves carried out in parallel to daily assays

# Generation of standard curves



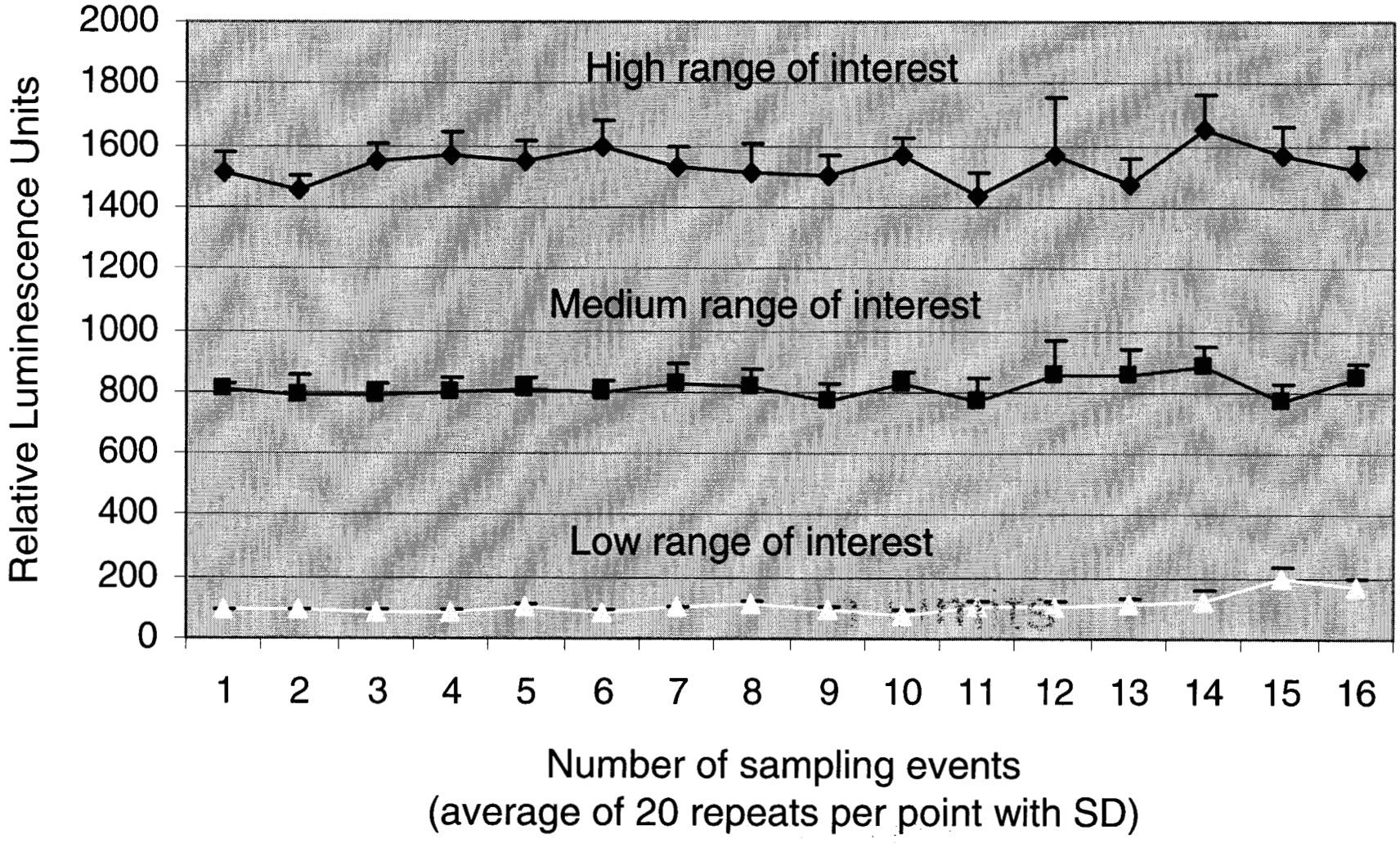




Although ATP is linear, it is proportional to the size of the microbe tested

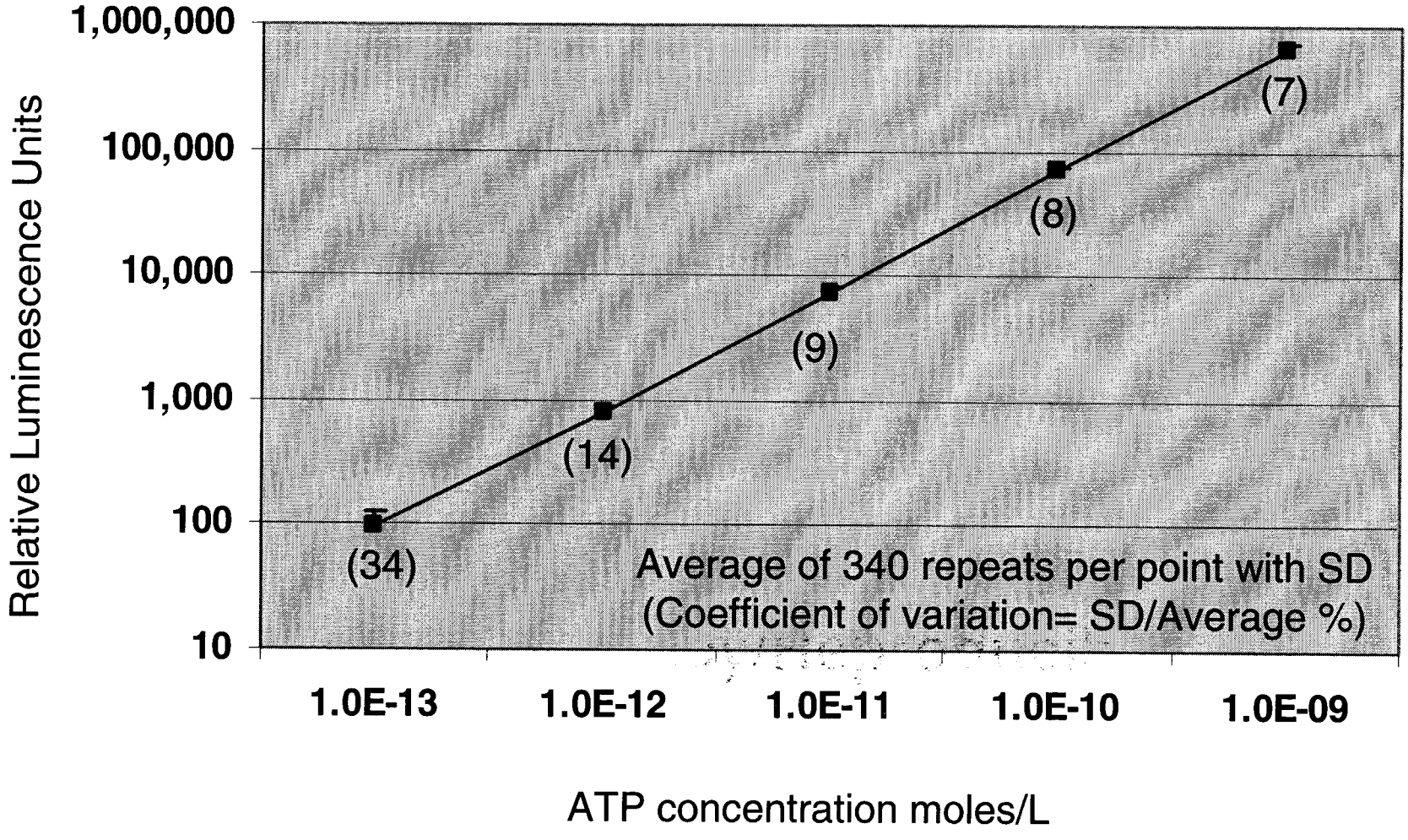
# Validation of ATP Bioassays

- Quality Assurance Test Plan for T-ATP and LAL
  - Prepared by Amy Baker and developed by Baker, Wainwright and Kern
- Quality Assurance
  - Method Detection Limits
  - Method Accuracy/Reproducibility
  - Hold time study
- Complete Procedure Assessment
  - Sampling and Sample Preservation
  - Sample Storage and Preservation
  - Quality Controls
  - Data Presentation
    - MER study

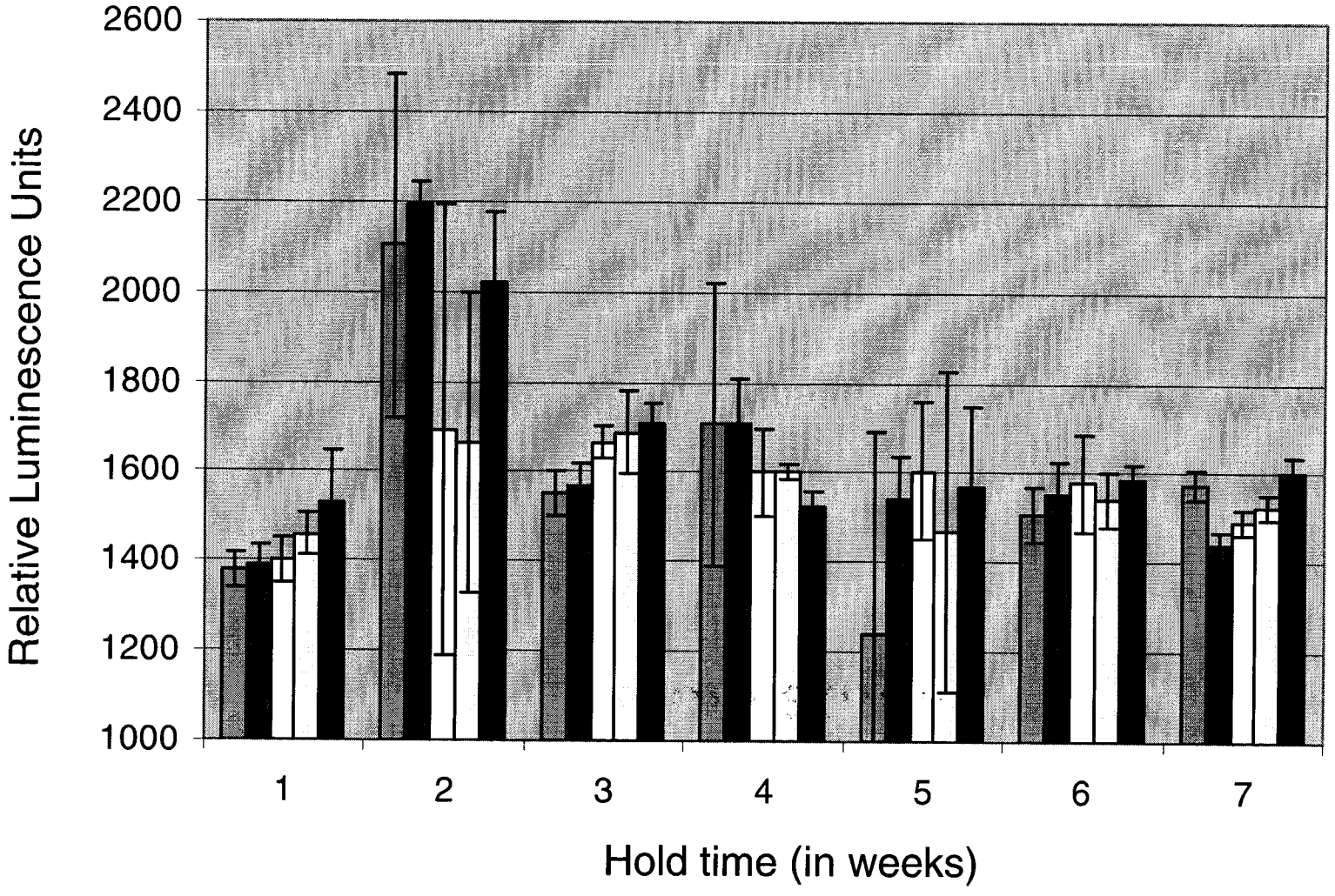
# Method Detection Limits



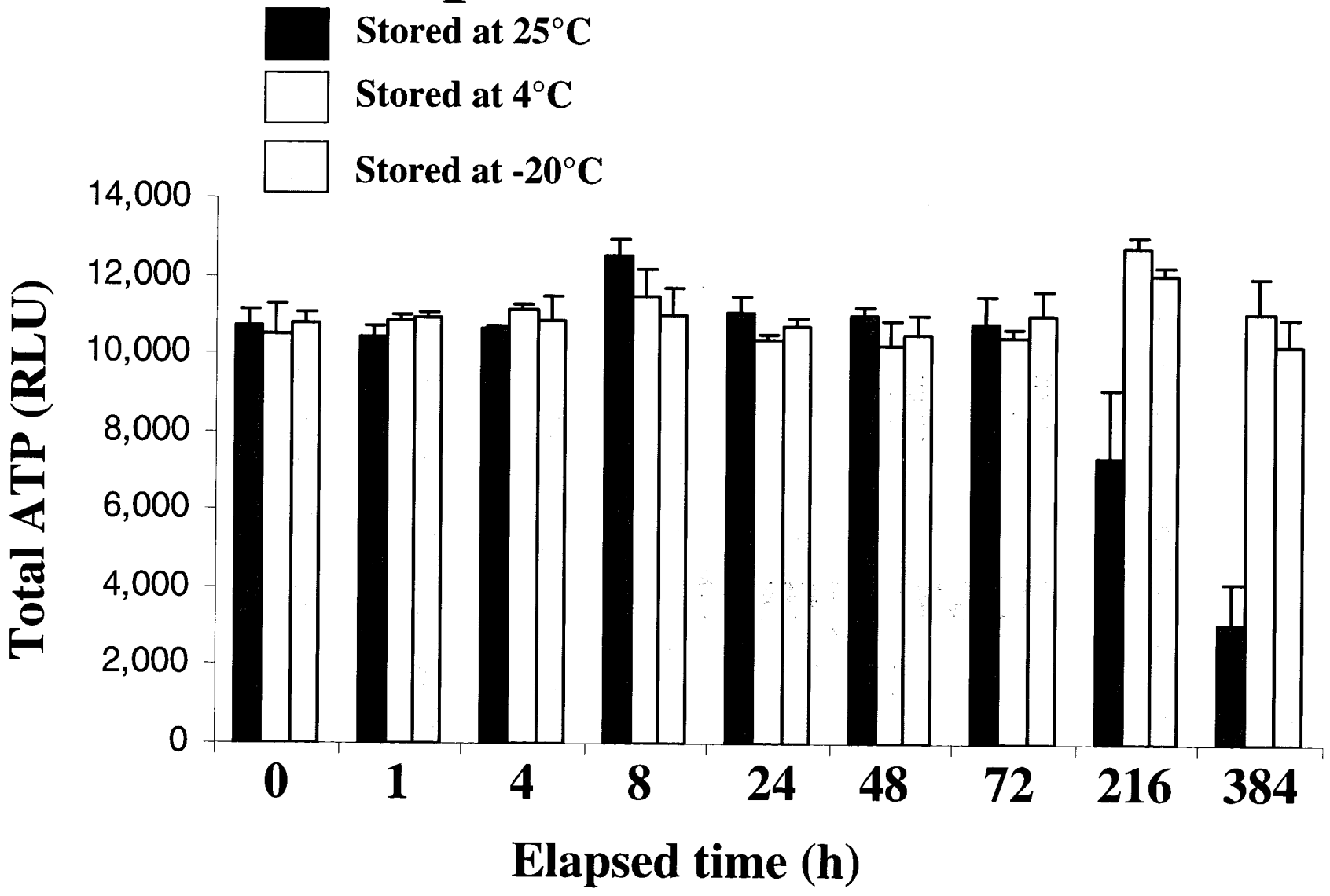
# Method Accuracy/Reproducibility



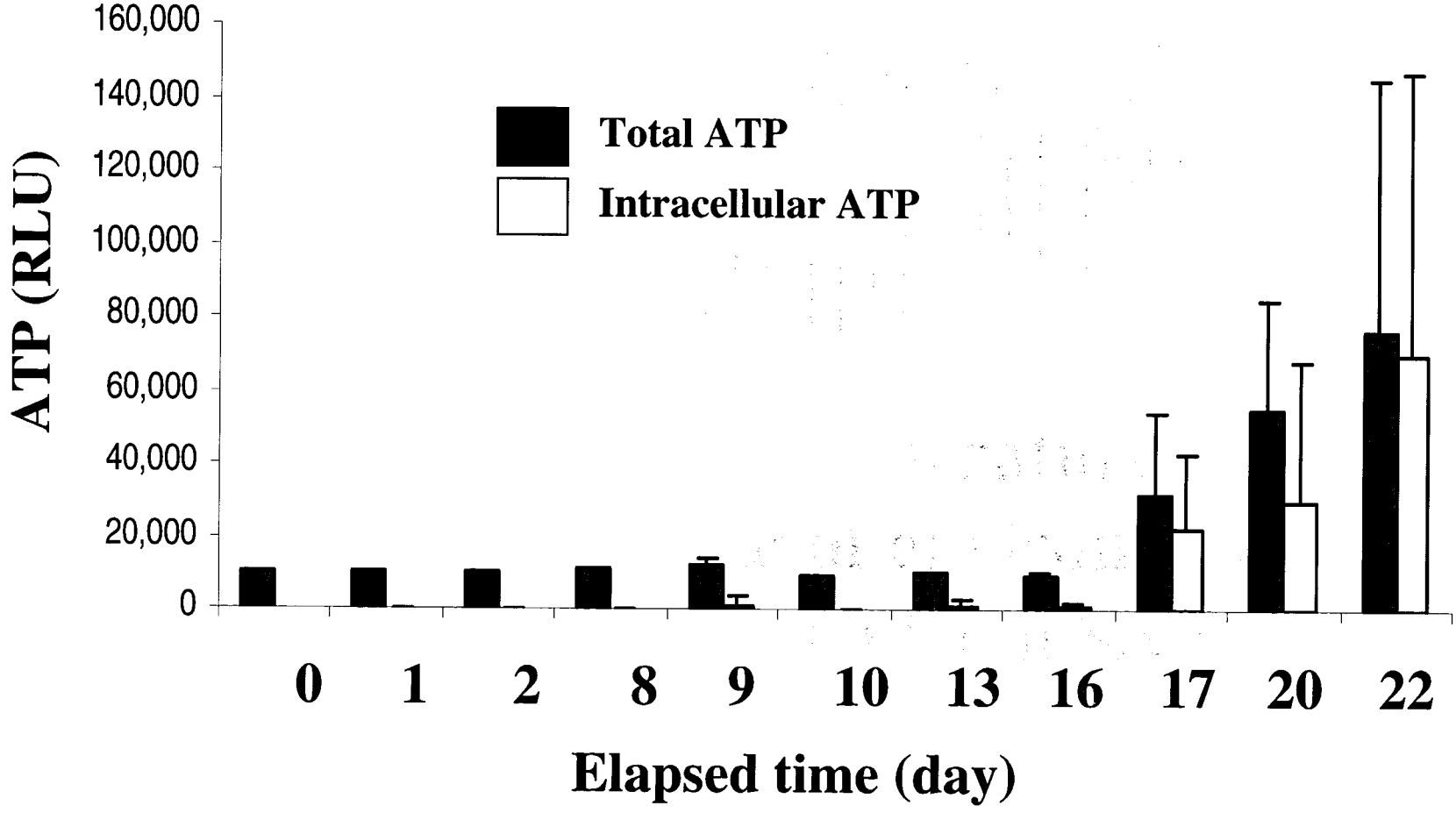
# Hold time study



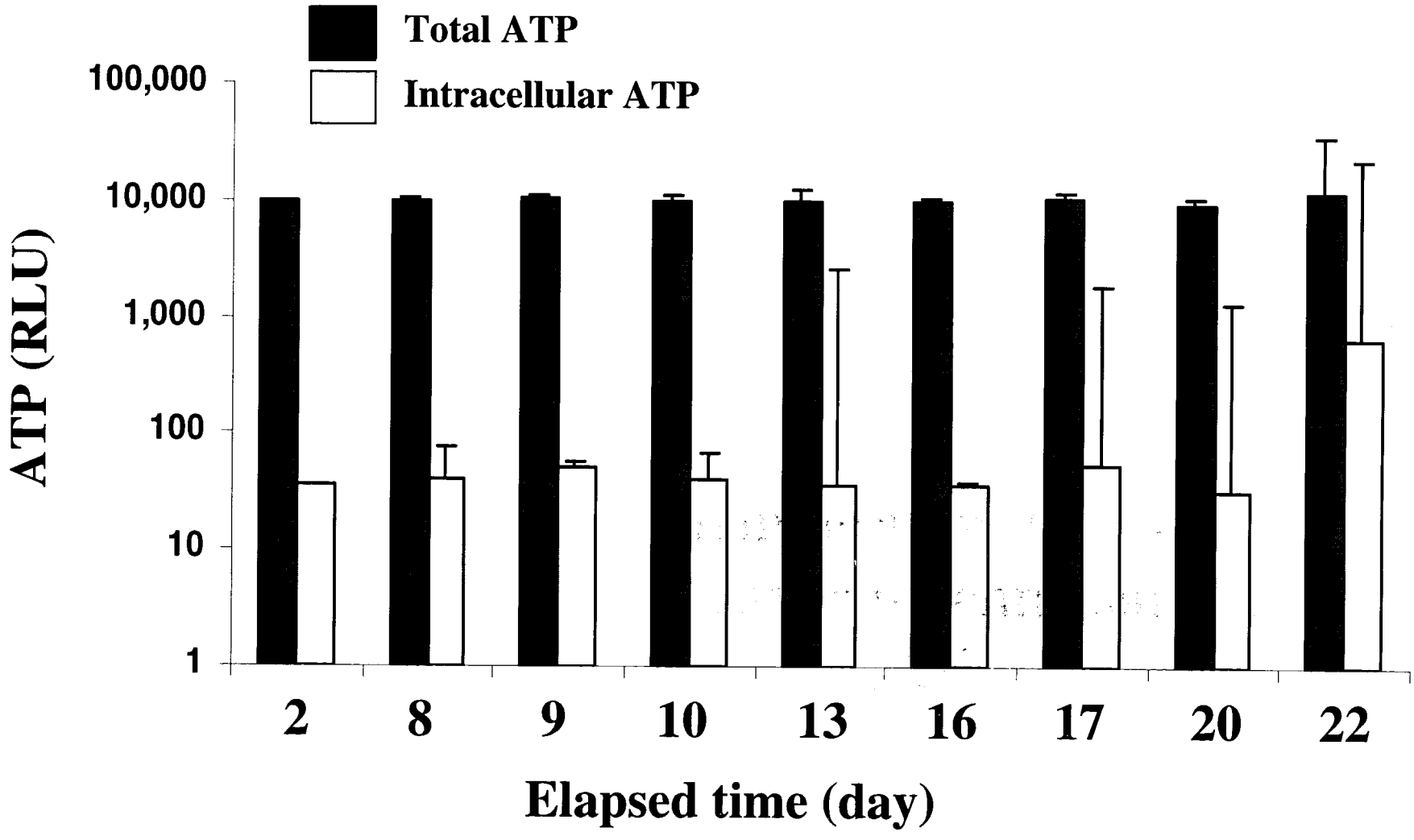
# Effect of storage temperature for purified ATP



# Influence of storage temperature (4°C) in the microbial ATP of environmental samples collected from SAF



# Influence of storage temperature (-20°C) in the microbial ATP of environmental samples collected from SAF



# MER sampling study (ATP assay)

| SAMPLE # | # of repeats for T-ATP | | | | Total ATP | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Average | SD | CV |
| 1 | 138 | 146 | 141 | 147 | 143 | 4 | 3 |
| 2 | 246 | 254 | 267 | 251 | 254 | 9 | 4 |
| 3 | 136 | 134 | 123 | 128 | 130 | 6 | 5 |
| 4 | 37 | 36 | 36 | 32 | 35 | 2 | 6 |
| 5 | 40 | 42 | 39 | 37 | 39 | 2 | 5 |
| 6 | 1104 | 1073 | 1133 | 1100 | 1102 | 25 | 2 |
| 7 | 977 | 926 | 945 | 962 | 952 | 22 | 2 |
| 8 | 475 | 438 | 451 | 486 | 462 | 22 | 5 |
| 9 | 313 | 305 | 296 | 286 | 300 | 12 | 4 |
| 10 | 834 | 784 | 846 | 841 | 826 | 29 | 3 |
| 11 | 503 | 474 | 466 | 446 | 472 | 24 | 5 |
| 12 | 815 | 860 | 848 | 905 | 857 | 37 | 4 |
| 13 | 74 | 97 | 67 | 71 | 77 | 13 | 18 |
| 14 | 238 | 231 | 237 | 242 | 237 | 5 | 2 |
| 15 | 269 | 253 | 267 | 266 | 264 | 7 | 3 |
| 16 | 23 | 18 | 23 | 24 | 22 | 3 | 12 |
| 18 | 558 | 602 | 613 | 574 | 587 | 25 | 4 |
| 19 | 6429 | 2719 | 2708 | 2704 | 3640 | 1859 | 51 |
| 20 | 150 | 118 | 125 | 124 | 129 | 14 | 11 |
| SIGMA H2O | 16 | 16 | 17 | 16 | 16 | 1 | 3 |

| SAMPLE # | # of repeats for T-ATP | | | | Total ATP | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Average | SD | CV |
| 23 | 1961 | 1885 | 1849 | 1851 | 1887 | 52 | 3 |
| 24 | 887 | 906 | 867 | 897 | 889 | 17 | 2 |
| 25 | 416 | 401 | 396 | 398 | 403 | 9 | 2 |
| 26 | 440 | 464 | 453 | 457 | 454 | 10 | 2 |
| 28 | 456 | 481 | 467 | 470 | 469 | 10 | 2 |
| 29 | 177 | 175 | 168 | 164 | 171 | 6 | 4 |
| 30 | 2232 | 2236 | 2229 | 2237 | 2234 | 4 | 0 |
| 31 | 1413 | 1411 | 1465 | 1442 | 1433 | 26 | 2 |
| 32 | 173 | 173 | 179 | 177 | 176 | 3 | 2 |
| 33 | 100 | 95 | 102 | 104 | 100 | 4 | 4 |
| 34 | 180 | 130 | 101 | 100 | 128 | 38 | 29 |
| 36 | 477 | 453 | 456 | 437 | 456 | 16 | 4 |
| 37 | 232 | 230 | 230 | 229 | 230 | 1 | 1 |
| 38 | 306 | 272 | 256 | 535 | 342 | 130 | 38 |
| 39 | 798 | 867 | 791 | 803 | 815 | 35 | 4 |
| 40 | 507 | 445 | 506 | 480 | 485 | 29 | 6 |
| 41 | 1974 | 1114 | 1181 | 1151 | 1355 | 414 | 31 |
| 42 | 1 | 23 | 8 | 5 | 9 | 10 | 104 |
| 43 | 0 | -1 | -2 | -1 | -1 | 1 | -82 |
| 44 | 1 | 0 | 1 | 0 | 1 | 1 | 115 |
| SIGMA H2O | 21 | 19 | 18 | 18 | 19 | 1 | 7 |

Amongst 164 repeats, 4 times technician error was suspected

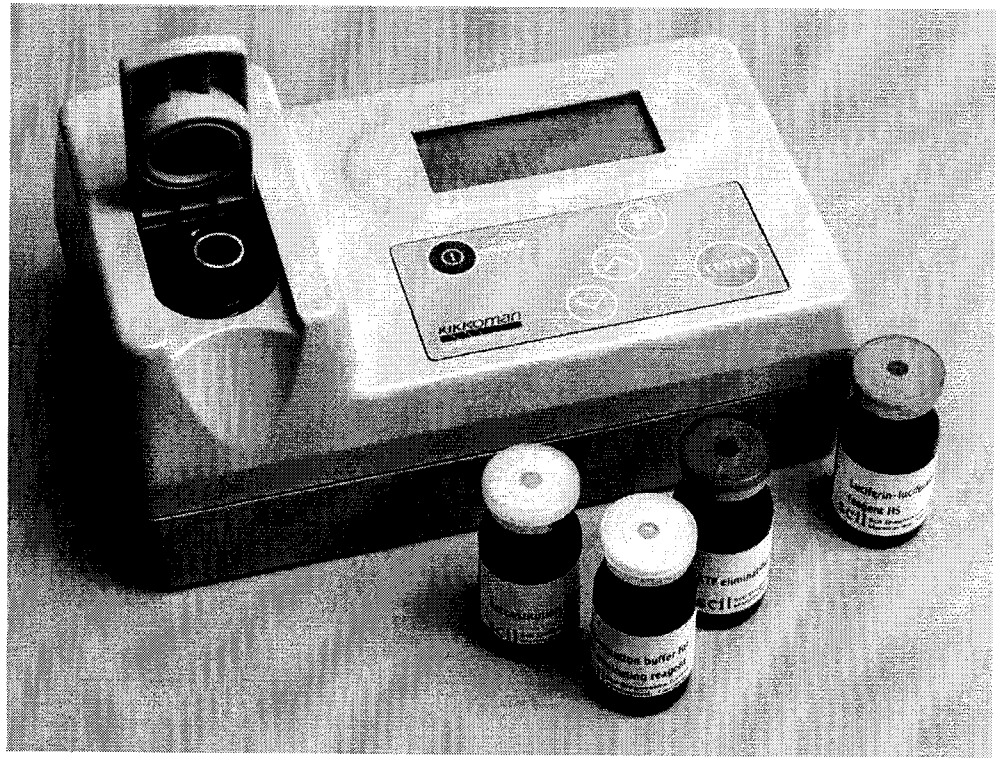
# Phase II
# Summary of Objectives

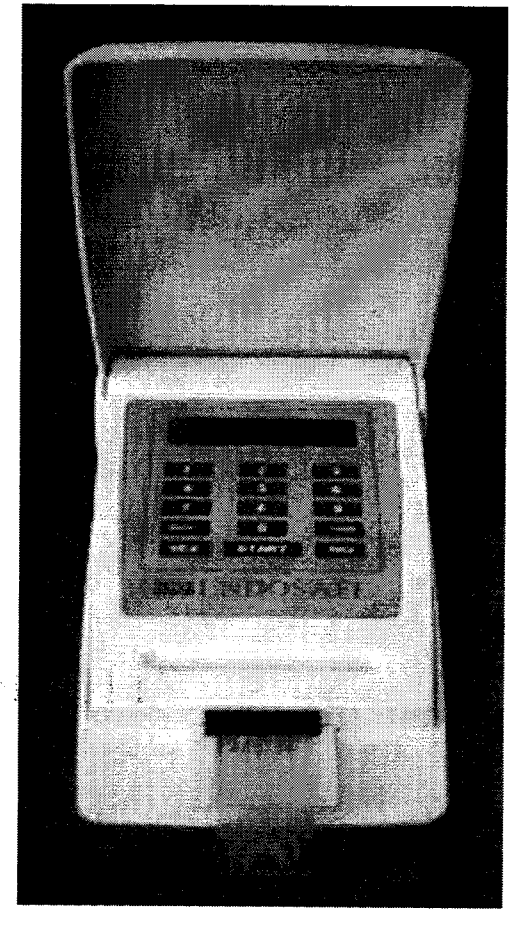
- Demonstrate reproducibility of the enzyme based methods by studying MER ATLO
- Demonstrate Hand Held/ portable detection units
- Evaluate swab/wipe method
- Prepare final report and presentation for review committee with recommendation to accept or reject inclusion of methods as a

# Accomplishments

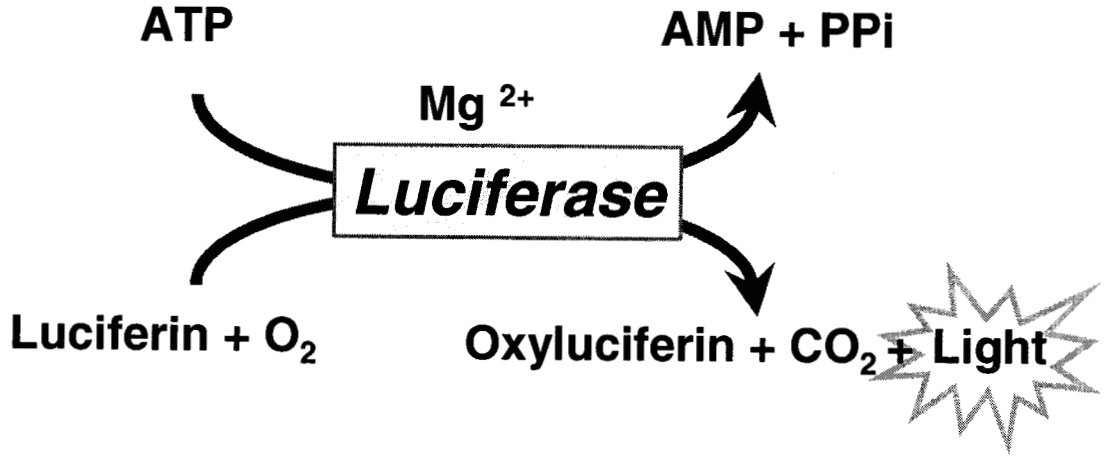
- JPL MER ATLO samples (total 300)
  - Collection, 100%
  - Assayed, 100%
  - Analysis, 99%
- KSC MER ATLO samples (total 200+)
  - Collection, 100%
  - Assayed, 100%
  - Analysis, 99%

# Portable and hand held units for ATP determination onsite



## *Firefly Luciferase Bioluminescent Reaction*




**Light decays with the consumption of ATP**

# MER ATLO Sampling

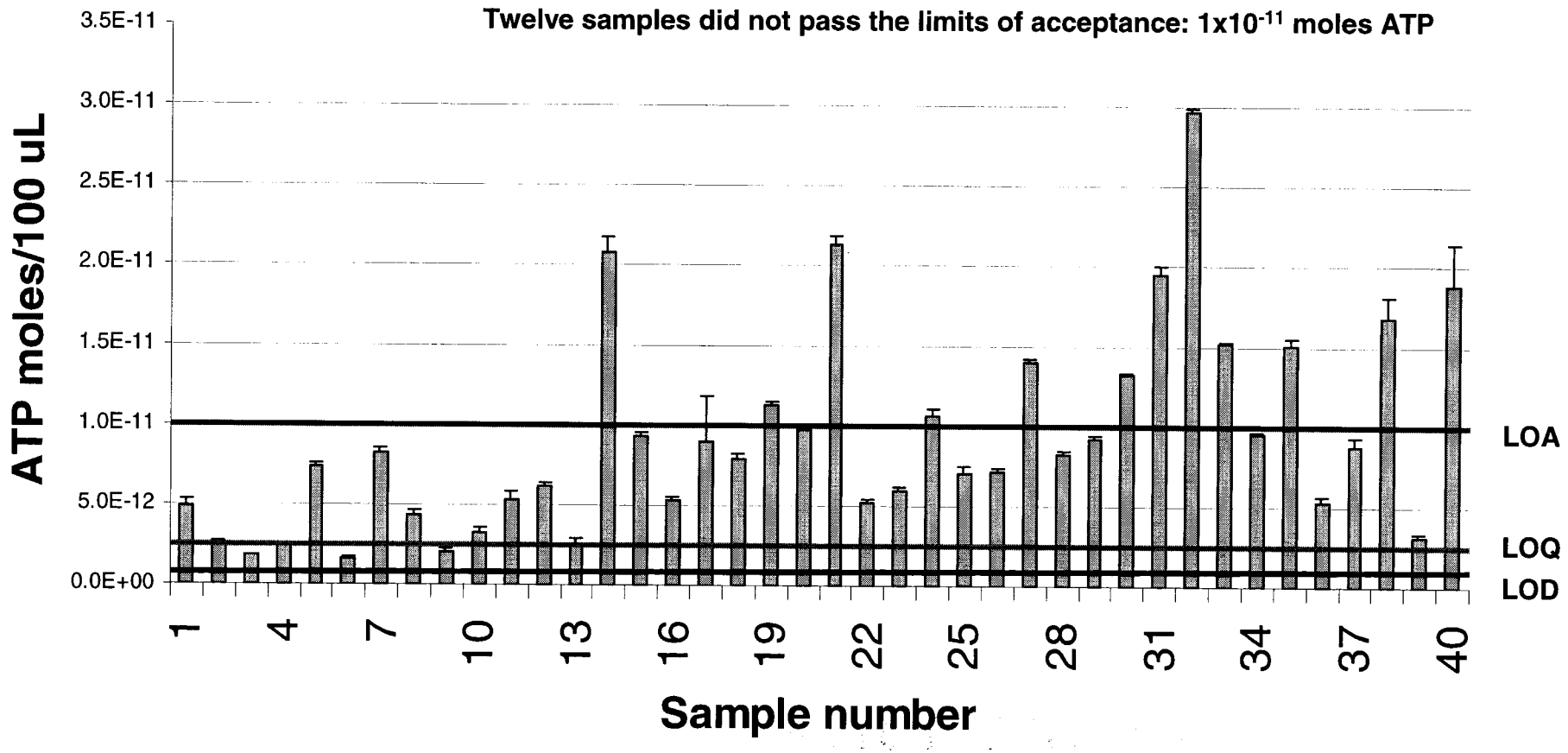
**JPL**

| | | |
|---|---|---|
| • 4-17-02 | MER cruise stage | 45 samples |
| • 6-19-02 | MER cruise stage | 39 samples |
| • 7-19-02 | MER cruise stage | 44 samples |
| • 10-21-02 | MER lander petals | 28 samples |
| • 11-12-02 | MER base petals and rover | 35 samples |
| • 12-19-02 | MER cruise stage segments | 8 samples |
| • 12-26-02 | MER cruise stage | 25 samples |
| • 12-27-02 | MER cruise stage | 34 samples |
| • 1-15-03 | MER cruise stage | 14 samples |
| • 1-16-03 | MER backshell | 19 samples |
| • 1-22-03 | MER lander petals | 16 samples |

**KSC**

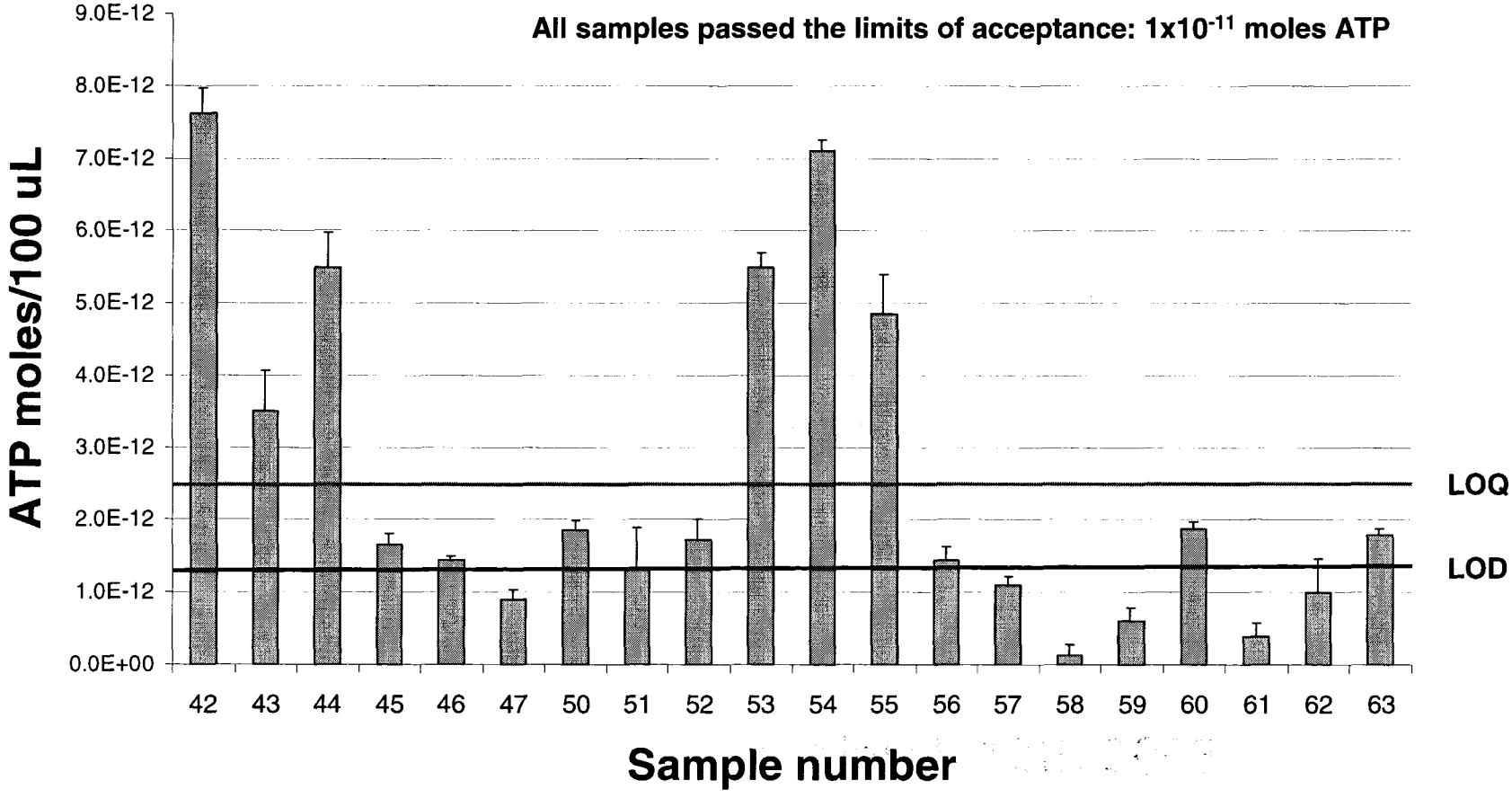
| | | |
|---|---|---|
| • 3-11-03 | MER lander petals, rover and cruise stage | 40 samples |
| • 3-13-03 | MER lander petals, rover and cruise stage | 60 samples |

# MER-PHSF sampling March 13th 2003




LOD: Limit of detection; LOQ: Limit of quantification; LOA: Limit of acceptance

# MER-PHSF Cruise Stage (March 13th 2003)

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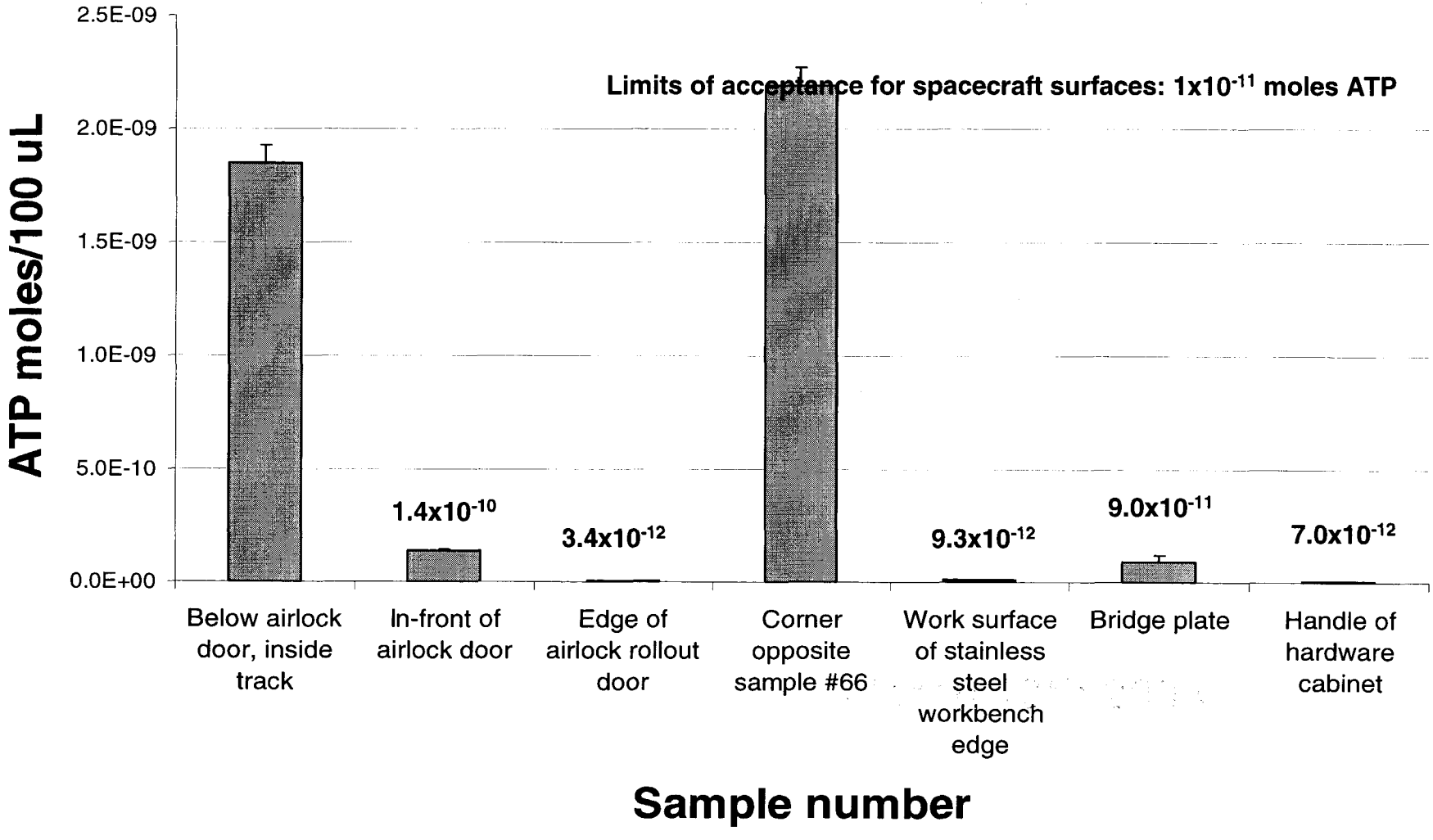
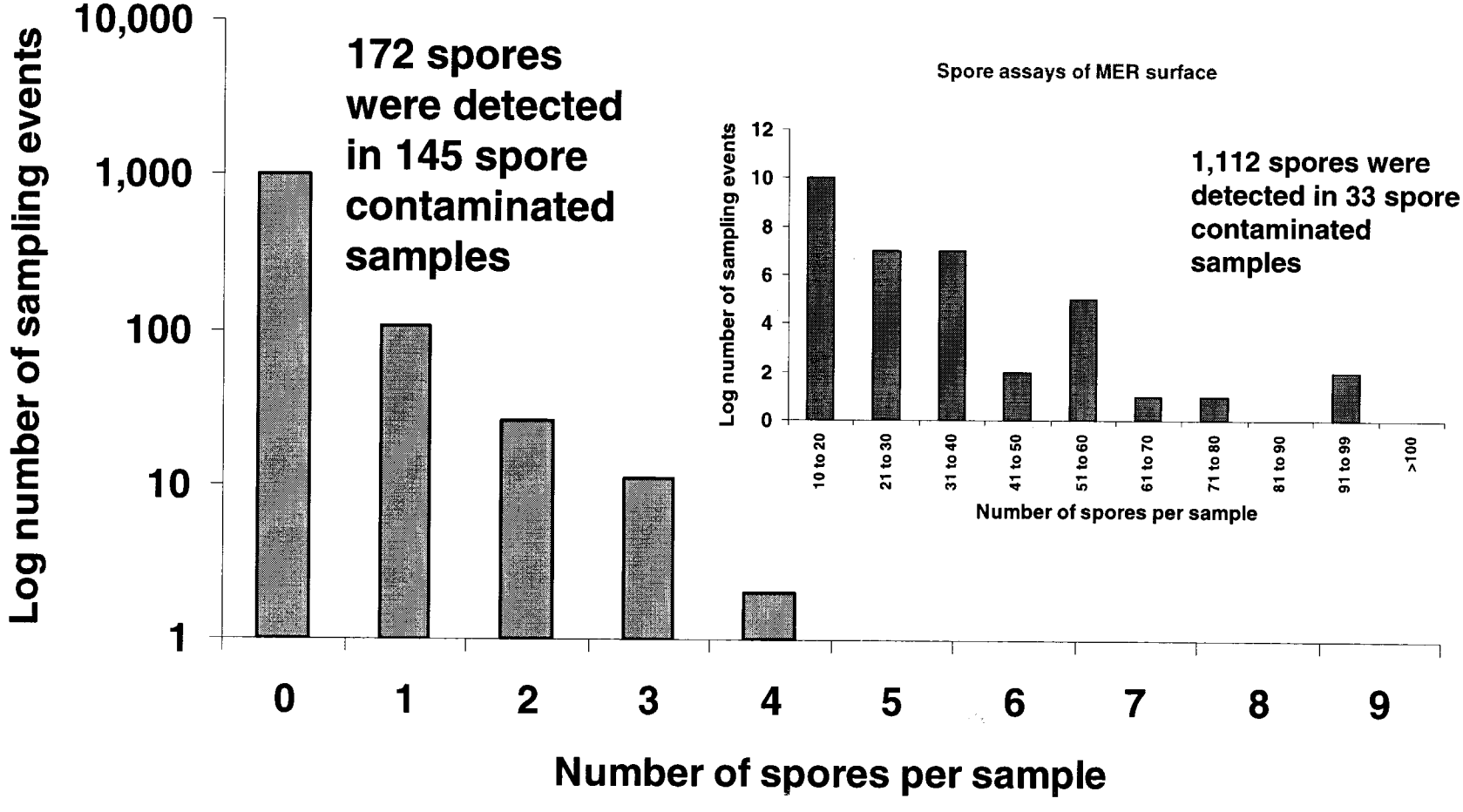
# MER-PHSF Facility Samples (March 13th 2003)



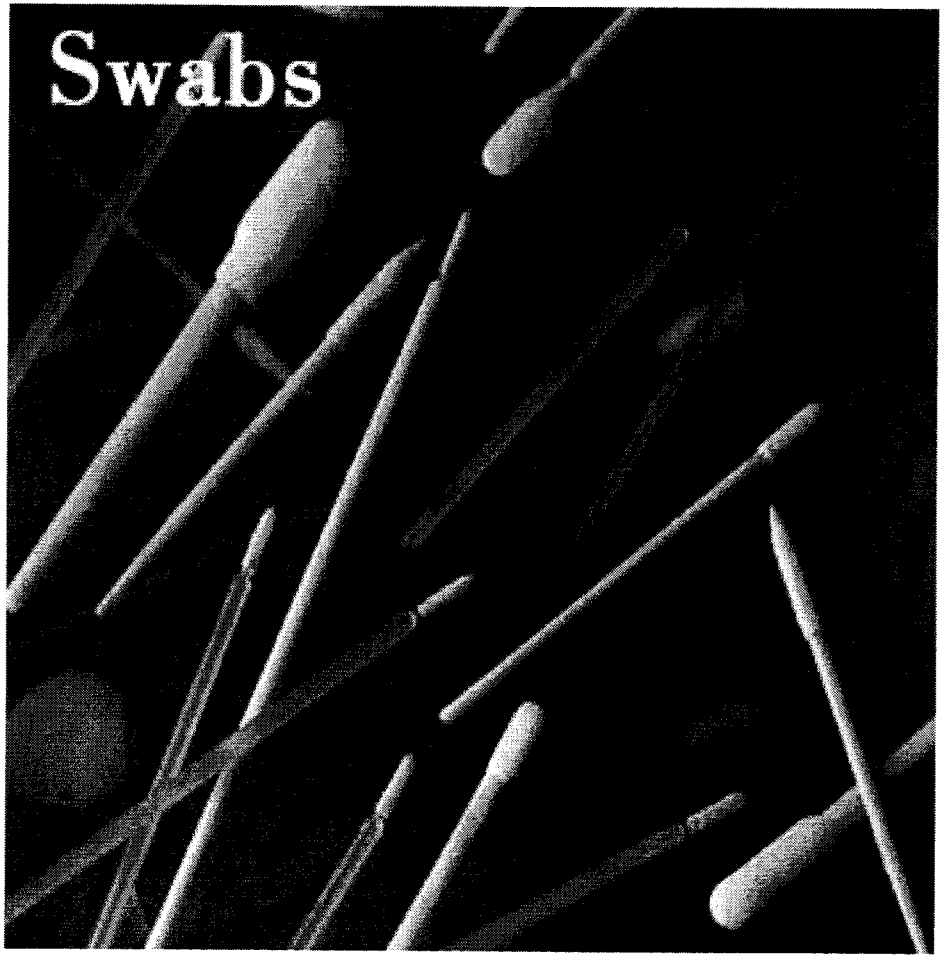
# Spore assays of MER surface



# Relative performance of the bio-burden assays

| Assays | Number of samples that are: | |
|---|---|---|
| | Total | Positive |
| Spores | 100 | 5 |
| ATP | 100 | 11 |
| LAL | 100 | 21 |
| Spores, ATP | 100 | 1 |
| Spores, LAL | 100 | 0 |
| ATP, LAL | 100 | 3 |
| Spores, ATP, LAL | 100 | 3 |
| Total contamination events: | | 44 |

# Evaluation of Swab Method

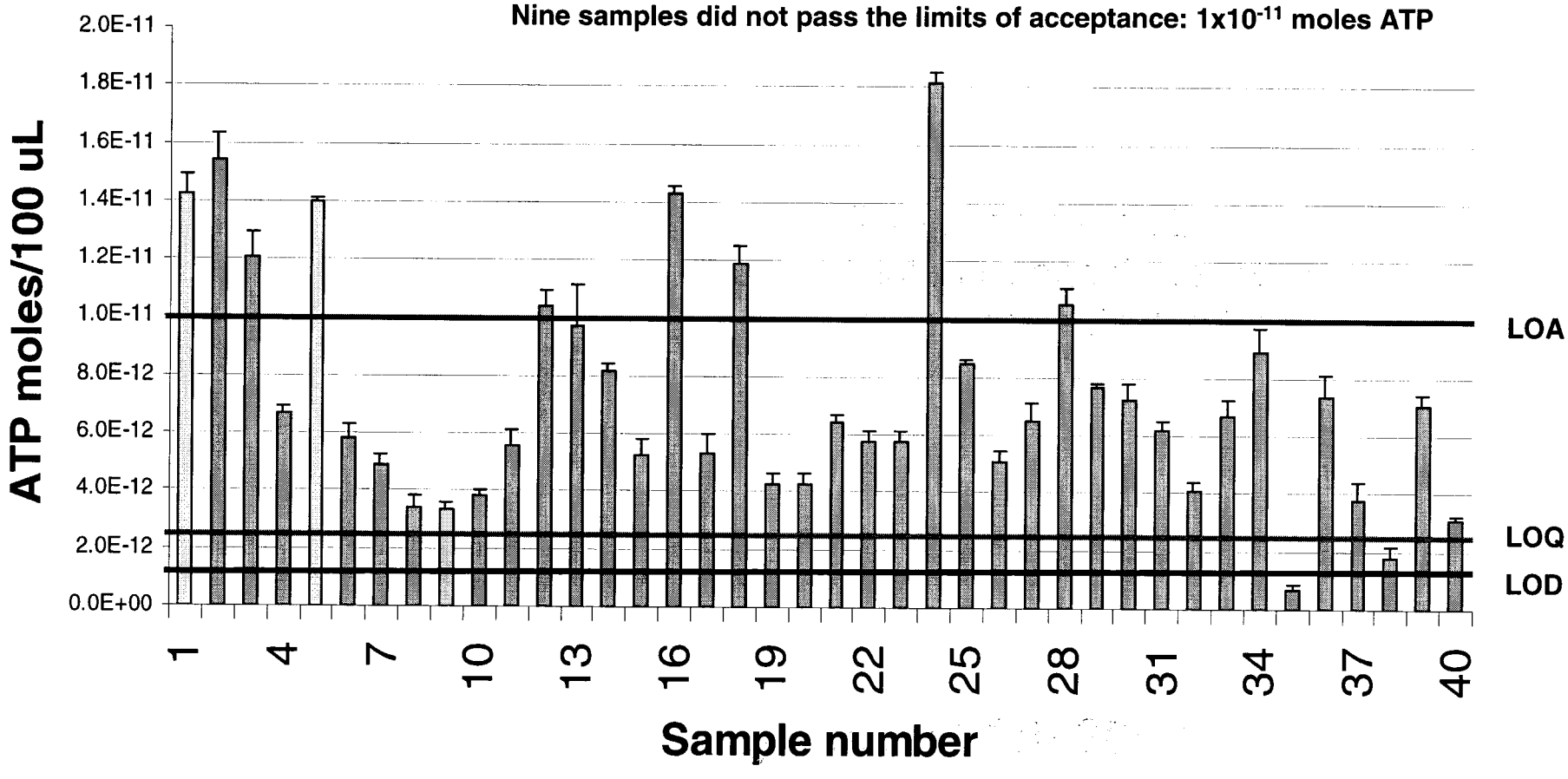



Standard 5.7 cm square coupons have been inoculated with a mixture of bacterial spores and gram negative bacterium

Three representative spacecraft surface materials and three swab materials have been selected for a comparative study

Preliminary results show low level of LPS and ATP recovery

# MER-PHSF sampling March 11th 2003

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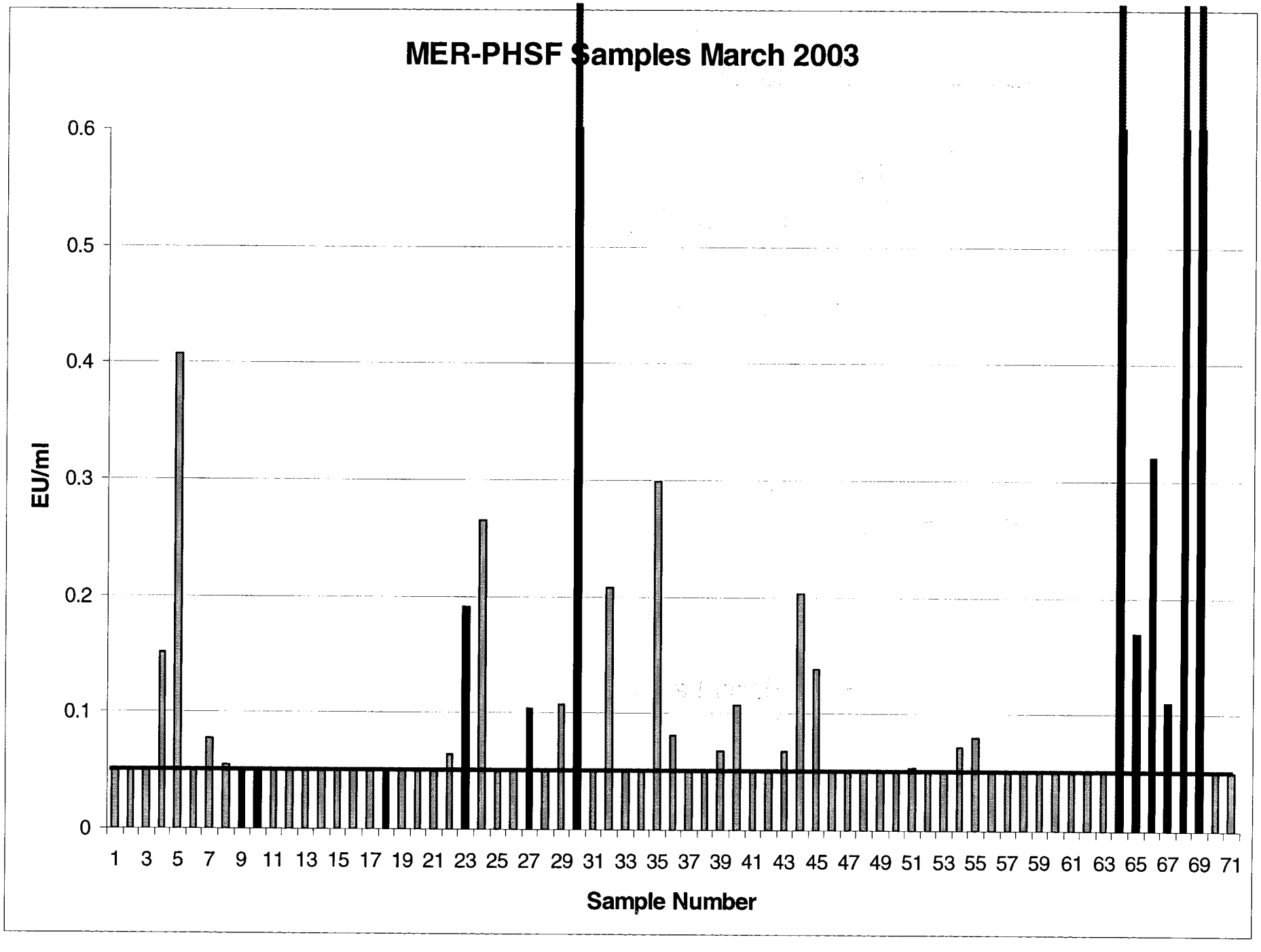
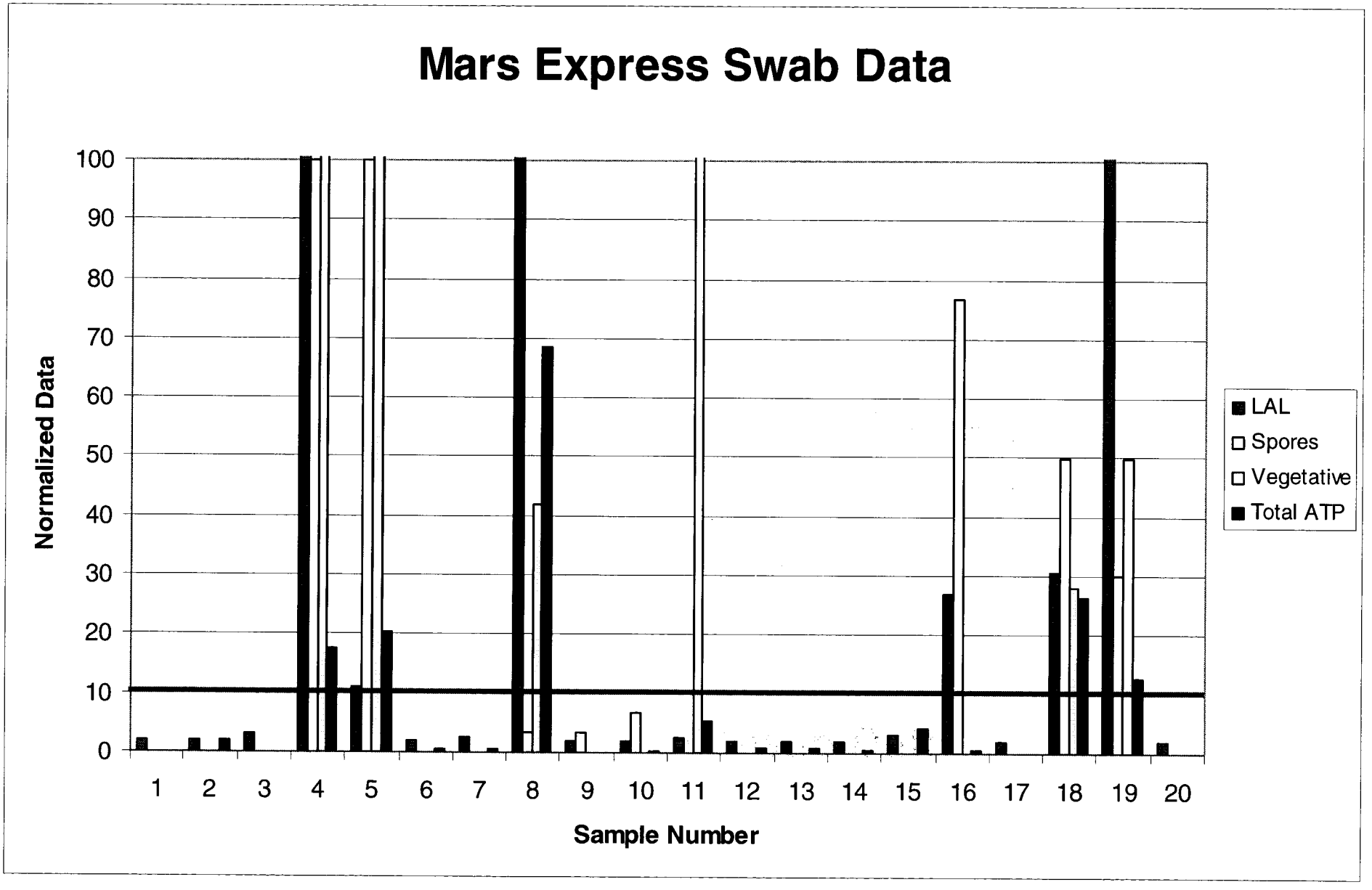

MER-PHSF Samples March 2003
EU/ml
0.6
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71
Sample Number


Mars Express Swab Data
Normalized Data
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Sample Number
LAL
Spores
Vegetative
Total ATP

# Summary

- Good correlation of ATP and LAL when data normalized to tentative limits of acceptance
- Examples of spores being negative and LAL and/or ATP detecting contamination
- Model for future studies
- Establish relevance of EU/ml and moles of ATP values
- Compare to Standard Methods

# Alignment with Mars Program Goals

- Present
  - Enable rapid identification of likely contamination "hot spots"on exposed surfaces
  - Amend NHB 5340.1C , "The Microbiological Examination of Space Hardware"
- Future
  - Enable a more complete assessment of bioburden contamination for future missions
  - Provide useful bioburden tools for MRSH

# Comparison of assays

| Assay that measured | Total number of spores/$m^2$ | % samples that showed a contamination event | Time taken for the analysis | Hand-held |
|---|---|---|---|---|
| Spores | 112 | 9[1] | 3 days | No |
| ATP | 60 | 27[2] | 15 min | Yes |
| LAL | 88 | 36[3] | 30 min | Yes |

[1]At least one spore per sample
[2]$>1.0 \times 10^{-11}$ ATP moles per sample
[3]>0.05 pg endotoxin per sample

# Phase III
# Summary of Objectives

- Demonstrate reproducibility of the enzyme based methods by studying MER ATLO
- Demonstrate Hand Held/ portable detection units
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# Relative performance of the bio-burden assays

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[3]>0.05 pg endotoxin per sample

# Final Conclusions

- ATP detection is by itself a useful method of monitoring bioburden
- ATP can be used in a complementary fashion with the LAL assay monitoring bioburden
- ATP and LAL results used together can be a good indirect predictor of the presence of spores on a spacecraft surface

An Approach to the Use of Modern Biological Assay Methods as a Fast Criterion for the Surface Cleaning/Assay Decision (LAL and ATP)

Roger Kern, Norman Wainwright, Gayane Kazarians, Greg Kuhlman, Micheal Kempf
Fei Chen, and Kasthuri Venkateswaran

NASA has an ongoing research effort to introduce new methodologies to evaluate trace levels of biological contamination on spacecraft outbound from Earth to the surface of Mars. Present NASA regulations call for the evaluation of bioburden on spacecraft surfaces by the determination of aerobic spore-forming bacteria as a proxy for the total bioburden actually present. We are currently investigating molecular based methodologies that assess bioburden in a more rapid manner than the NASA standard technique. These are expected to find initial use, not for regulatory purposes, but for assisting the engineering team during the assembly of spacecraft by providing a rapid indicator of bioburden. We have evaluated two complementary methodologies and found they correlate to a significant degree with the presence of bacterial spores. A bioluminescence based detection method is capable of rapidly monitoring ATP levels from $10^{-13}$ M to $10^{-10}$ M. Similarly a Limulus amoebocyte lysate assay is capable of rapidly monitoring levels of bacterial lipopolysaccharide (LPS) from 0.005 to 50 endotoxin units (approx. $5x10^{-13}$ to $5x10^{-10}$ g *E. coli* LPS). Both methods have been tested during the course of the Mars Exploration Rover's assembly with a total of over 500 samples taken. Not only do these methods give one an independent measure of bioburden, they can also reasonably predict the presence of spores. There appears to be a significant correlation between samples containing ATP, LPS and spores.