
Taguchi Statistical Design and Analysis of Cleaning Methods for Spacecraft Materials

Y. Lin, S. Chung, G. A. Kazarians, J. O. Blossiu, R. A.
Beaudet, M. S. Quigley, and R. G. Kern

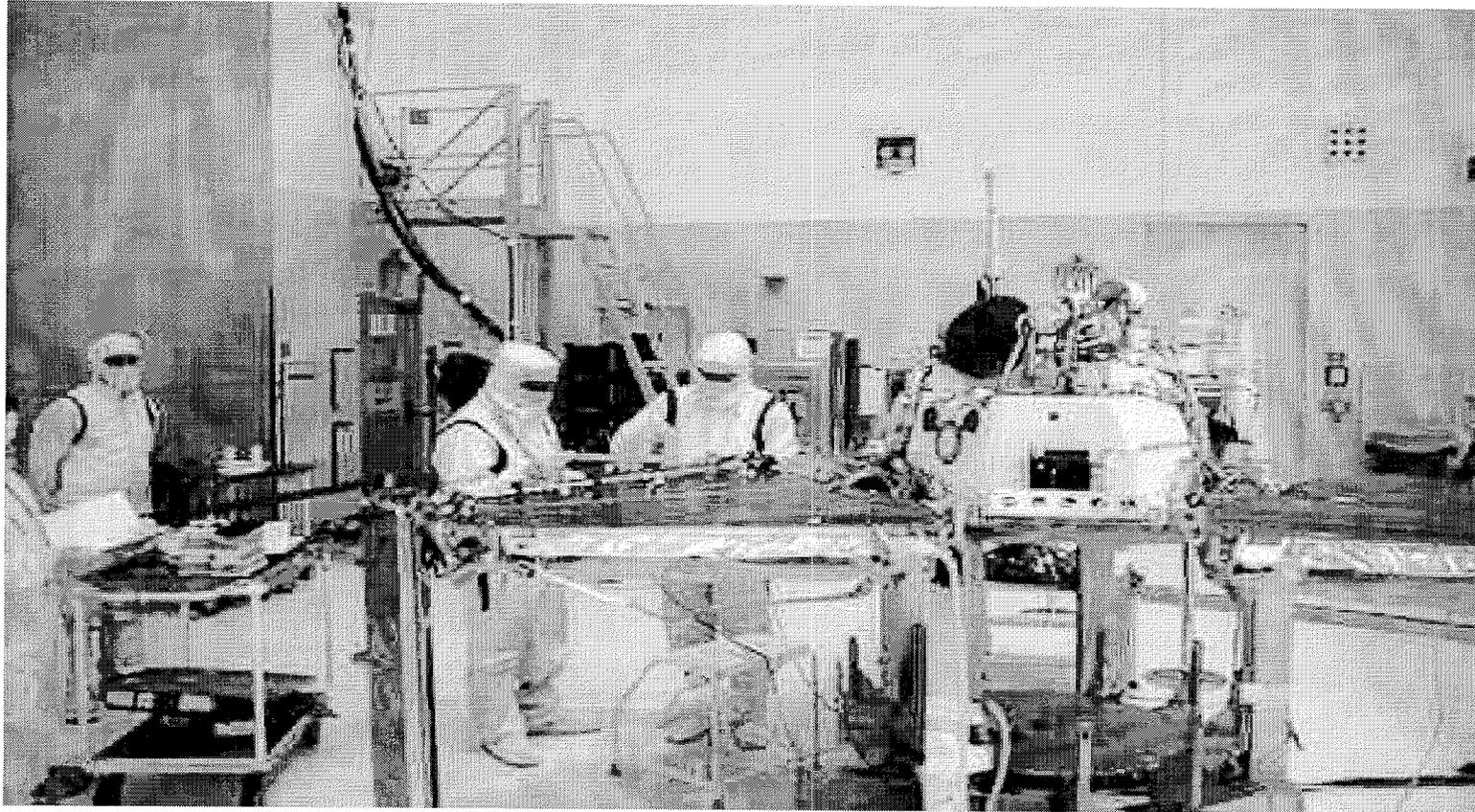
Jet Propulsion Laboratory
California Institute of Technology
Pasadena, CA 98119



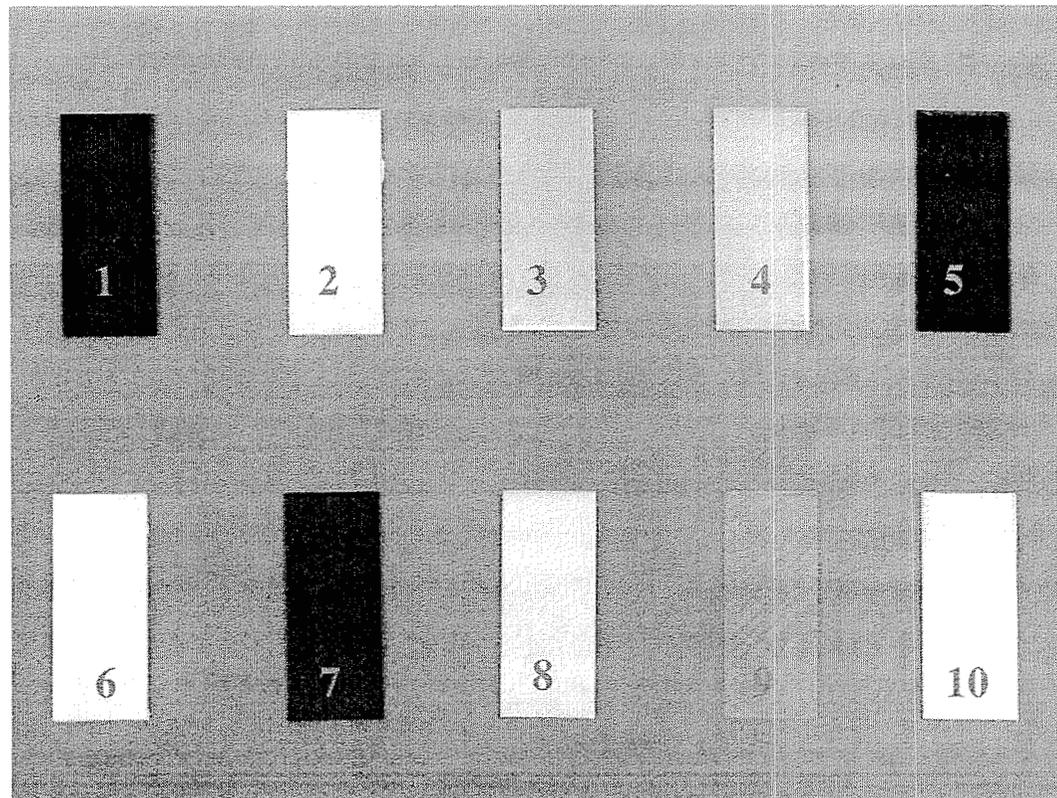
Abstract

To minimize terrestrial microorganisms being transported to other planets via a spacecraft, the spacecraft components must be cleaned and maintained within specific planetary protection bioburden requirements. The current cleaning procedure adopted by spacecraft engineers at NASA's flight hardware facilities is to clean with wipes saturated with 100% isopropyl alcohol (IPA). In this study, we have extensively tested various cleaning protocols. The variant parameters included the type and concentration of solvent, type of wipe, pre-treatment conditions, and various rinsing systems. Taguchi statistical method was used to design and evaluate various cleaning conditions on ten common spacecraft materials. The cleanliness of the material surfaces was evaluated by two enzyme-based assays to quantify the amount of bioburden before and after the cleaning process. The results of the cleaning study were analyzed using the Taguchi statistical method. The optimal cleaning condition was predicted for each material and a separate confirmation run was conducted. The results showed that the best solvent system for effectively cleaning gram-negative *Pseudomonas stutzeri* is 50% IPA instead of the current 100% IPA. The best pre-treatment method, the most effective wipes, and the final rinsing solutions have also been identified.

Sampling of Spacecraft

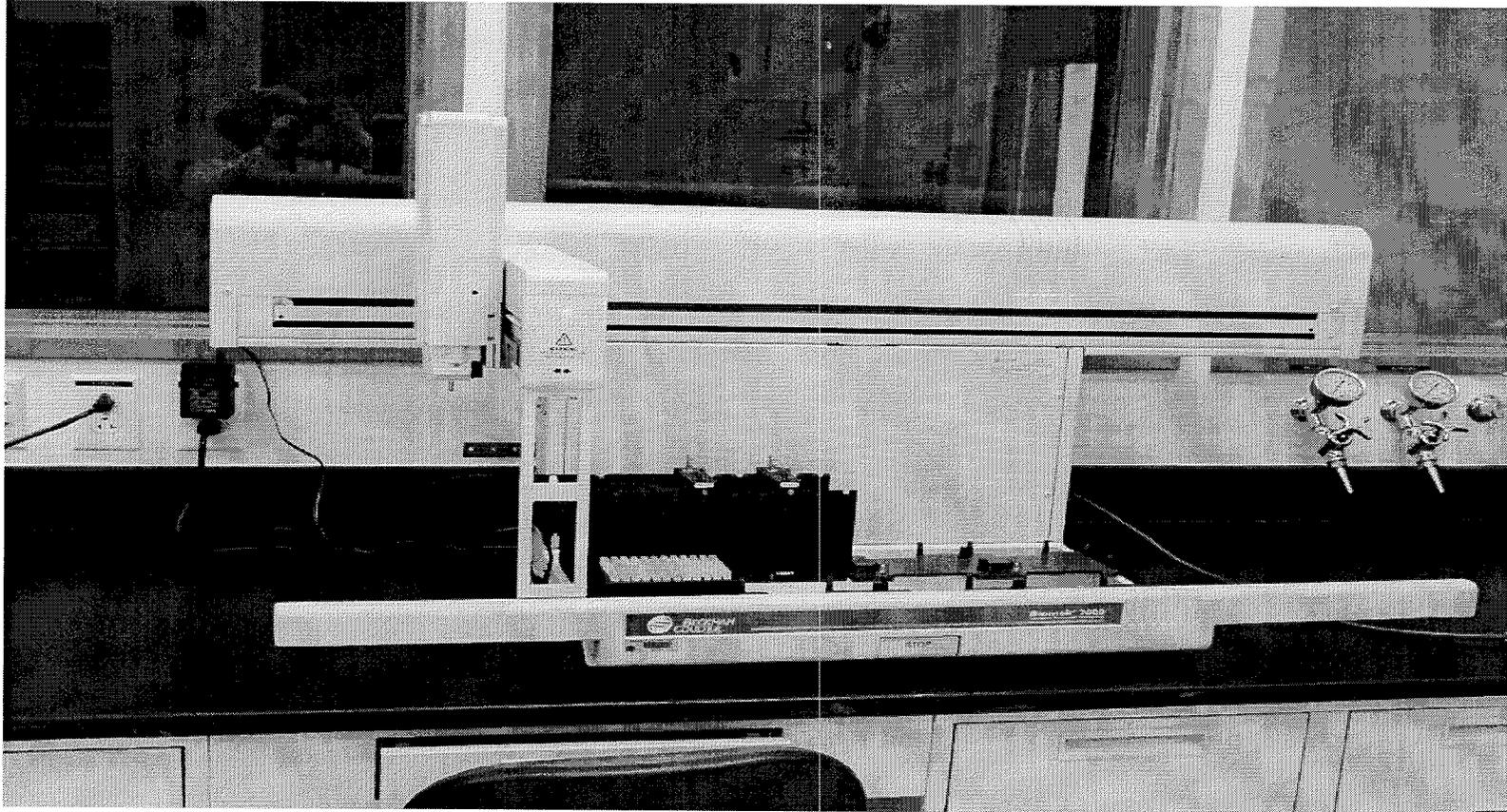


Spacecraft Materials Used for the Cleaning Study



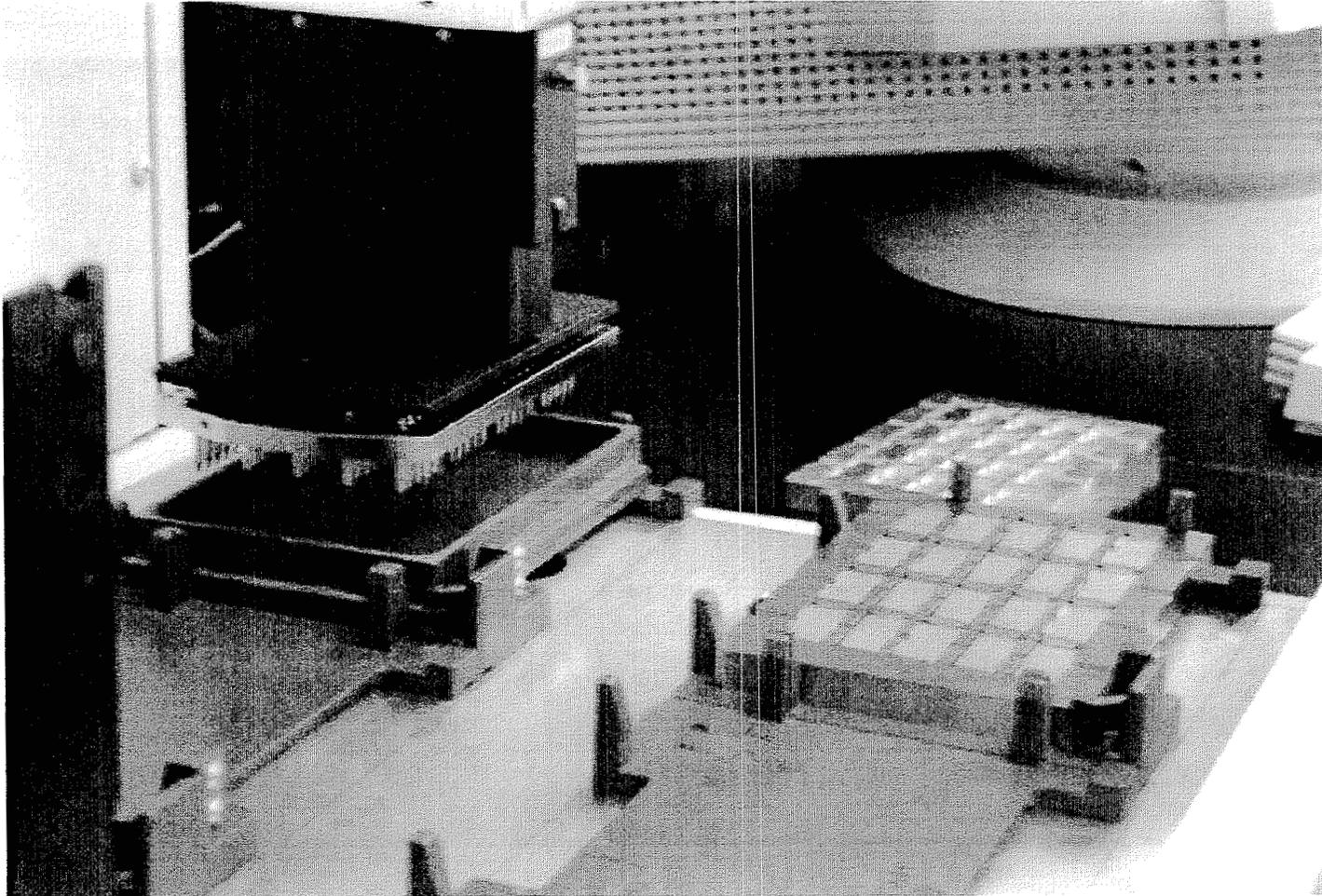
1. Anodized Black
2. NS43G Paint
3. Chemfilmed Aluminum 6061T6
4. Stainless Steel
5. Graphite Composite
6. White Epoxy Paint (446-21-7925)
7. Black Paint (463-3-8)
8. Anodized Clear
9. Astroquartz
10. Bare Aluminum (6061)

Automated Coupon Stamping Machine



Gram negative cells of *Pseudomonas stutzeri* were inoculated onto 1x2.3 cm coupons using robotic Biomek 2000 from Beckman Coulter, Inc.

Close-Up View of the Cell Deposition Process



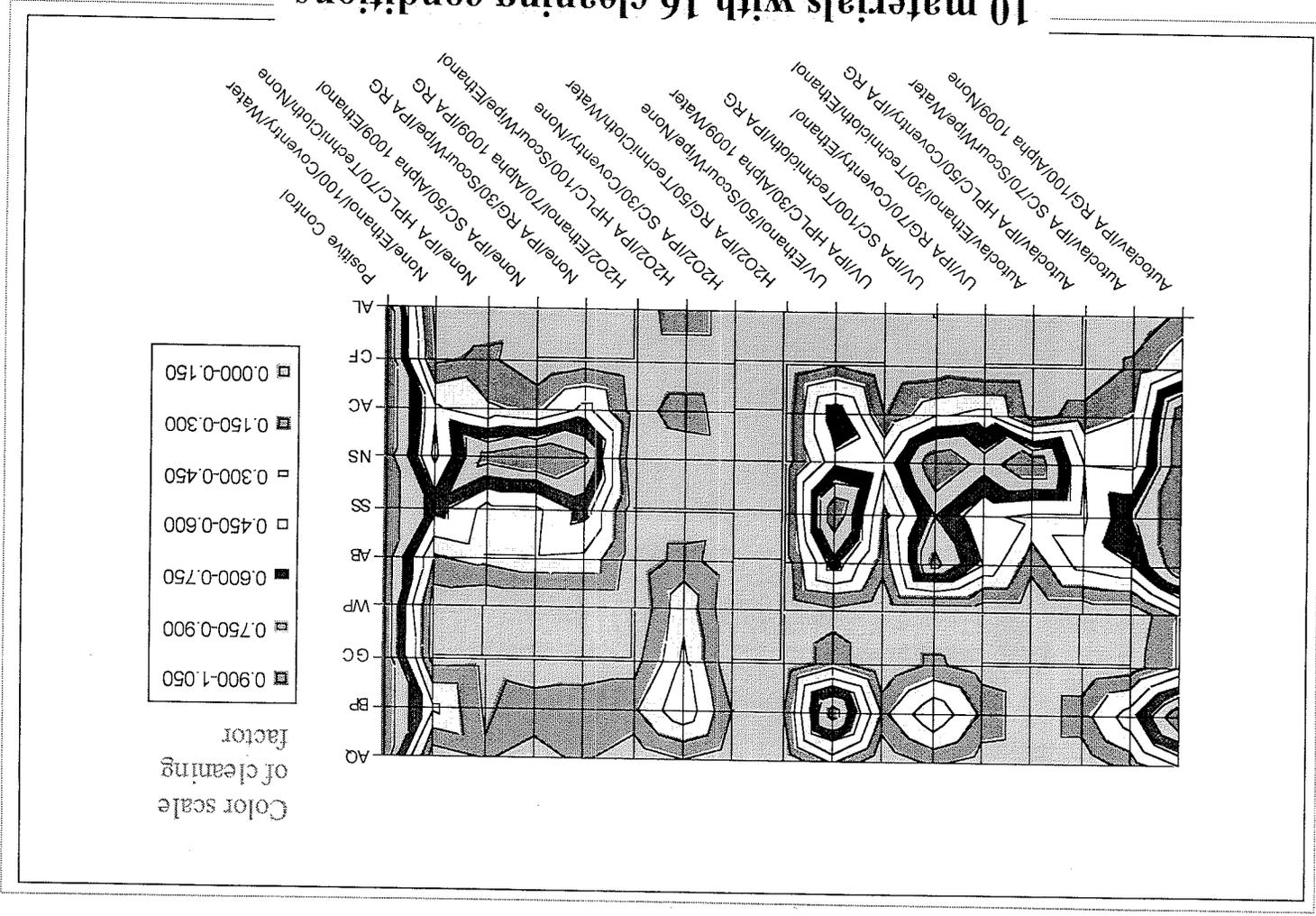
Statistical Analysis of the ATP Method

	Relative error (s_y)	Experiment parameters	Remarks
ATP_{total} measurement (Positive control coupons, ATP_{average} = 4000)	15.3%	Derived from 27 pairs of ATP measurements on stamped coupons, each pair has exactly the same condition.	This gives the ATP measurement (in)consistency independent of coupons.
ATP_{total} measurement (negative control coupons, ATP_{average} = 80)	28.9%	Derived from 90 pairs of ATP measurements on pre-cleaned unstamped coupons, each pair has exactly the same condition.	This has a higher percentage error due to smaller ATP values, indicating non-trivial error behavior.
Stamping and sonication	9.1%	The average of the tri-set data of 30 positive control coupons, yielding 17.8% error.	The direct data error includes stamping, sonication, and ATP measurement combined. Uncorrelated errors sum quadratically.
Cleaning (wiping)	11%	The average of 27 tri-set of the cleaned coupons.	This is derived based on the assumption $\sigma_{total} = (\sum \sigma_i^2)^{1/2}$. Since the ATP values are closer to low value ends, this may be an over-estimated value.
Total error (on cleaned coupons)	25%	The average of 27 tri-set of the cleaned coupons.	This is the total measurement error for the cleaning study. The measured ATP values range from tens to hundreds.

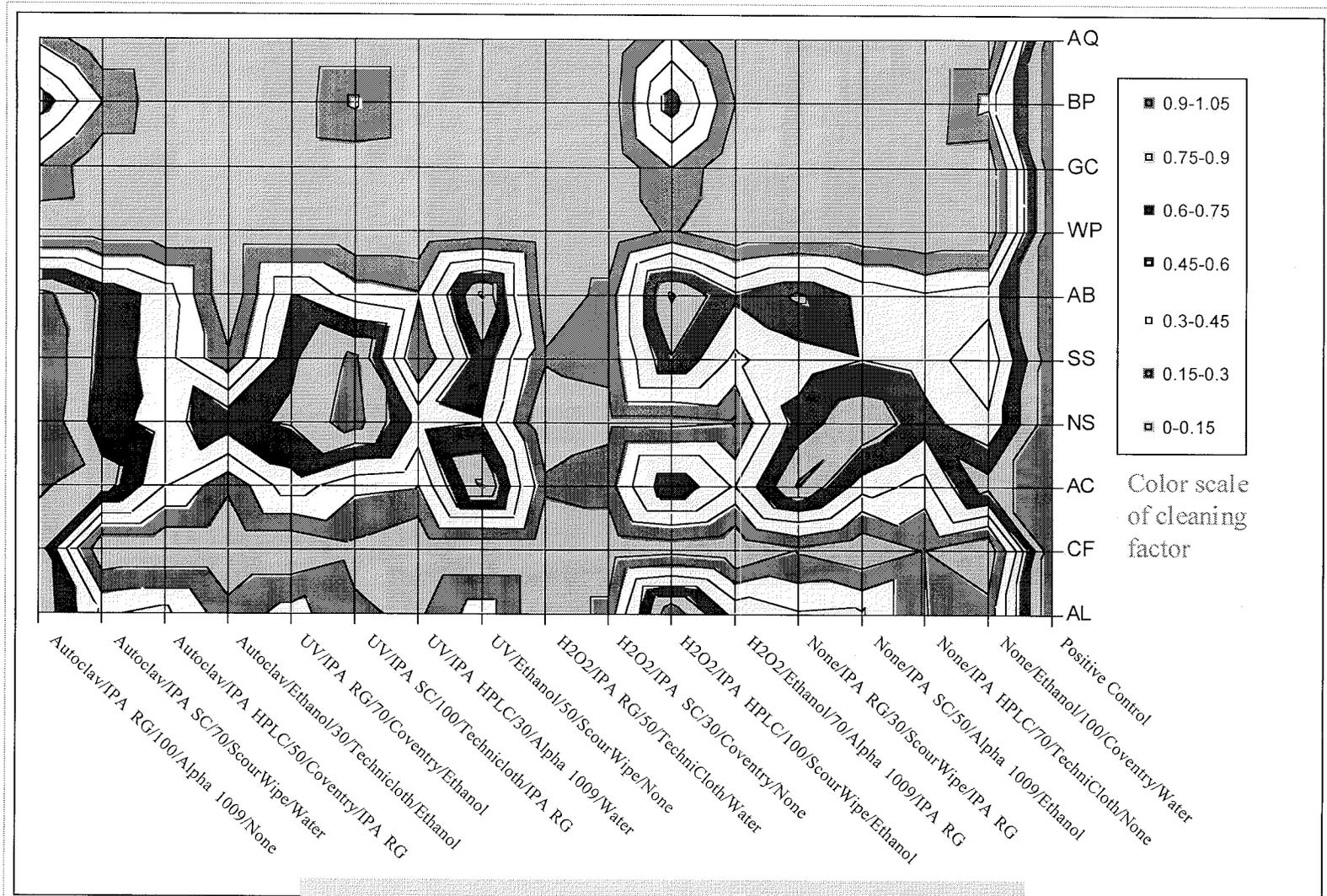
Statistical Analysis of the LAL Method

	Relative error (σ_y)	Experiment parameters	Remarks
LAL measurement (Positive control coupons, $LAL_{average} = 17$)	5.92%	Derived from 27 pairs of LAL measurements on stamped coupons, each pair has exactly the same condition.	This gives the LAL measurement (in)consistency independent of coupons
LAL measurement (negative control coupons, $LAL_{average} = 0.1$)	73.2%	Derived from 50 pairs of LAL measurements on pre-cleaned unstamped coupons, each pair has exactly the same condition, and excluding those with instrument limit readings.	This has a much higher percentage error due to smaller LAL values, indicating non-trivial error behavior.
Stamping and sonication	30.0%	The average of the tri-set data of 30 positive control coupons, yielding 30.6% error.	The direct data error includes stamping, sonication, and ATP measurement combined. Uncorrelated errors sum quadratically.
Cleaning (wiping)	61.2%	The average of 27 tri-set of the cleaned coupons.	This is derived based on the assumption $\sigma_{total} = (\sum \sigma_i^2)^{1/2}$. Since the LAL values are closer to low value ends, this may be an over-estimated value.
Total error (on cleaned coupons)	68.4%	The average of 27 tri-set of the cleaned coupons.	This is the total measurement error for the cleaning study. The measured LAL values range from tens to hundreds.

Cleaning Results verified using LAL Assay

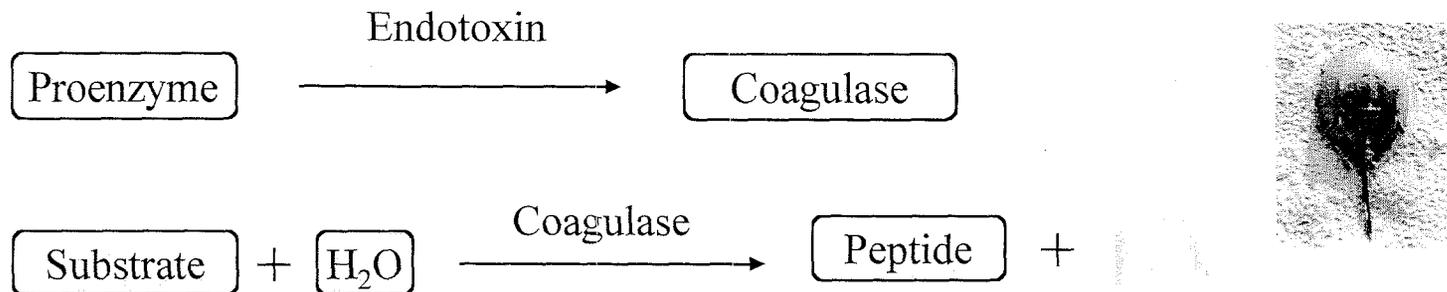


Cleaning Results Verified Using ATP Assay



10 materials with 16 cleaning conditions

LAL (Limulus Amebocyte Lysate) Assay



Bacterial endotoxin catalyzes the activation of a proenzyme in the Limulus Amebocyte Lysate (LAL). The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme catalyzes the splitting of p-nitroaniline (pNA) from the colorless substrate Ac-Ile-Glu-Ala-Arg-pNA. The pNA release is measured photometrically at 405 nm continuously throughout the incubation period. A log/log correlation between the time required for the appearance of color (Reaction Time) and the endotoxin concentration is linear from 0.005 to 50 EU/mL. The concentration of endotoxin in a sample is calculated from its Reaction Time by comparison to the Reaction Time of solutions containing known amounts of endotoxin standard.

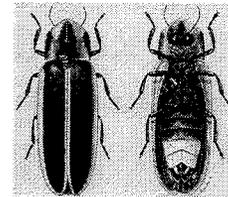
ATP Bioluminescence Assay

The bioluminescence reagent contains firefly luciferin and luciferase. Luciferase specifically react with ATP and catalyzes the following reaction.



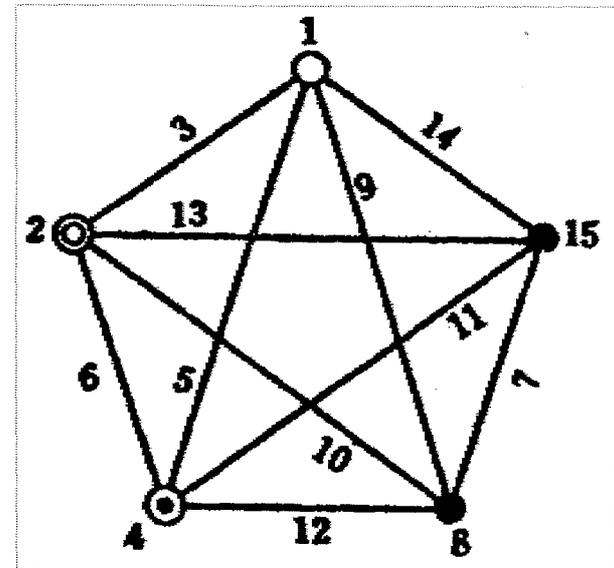
The amount of bioluminescence produced in the reaction above is in direct propotion to the amount of the ATP in the sample

CheckLite-HS set is from Kikkoman corp. Japan.



Taguchi Test Matrix

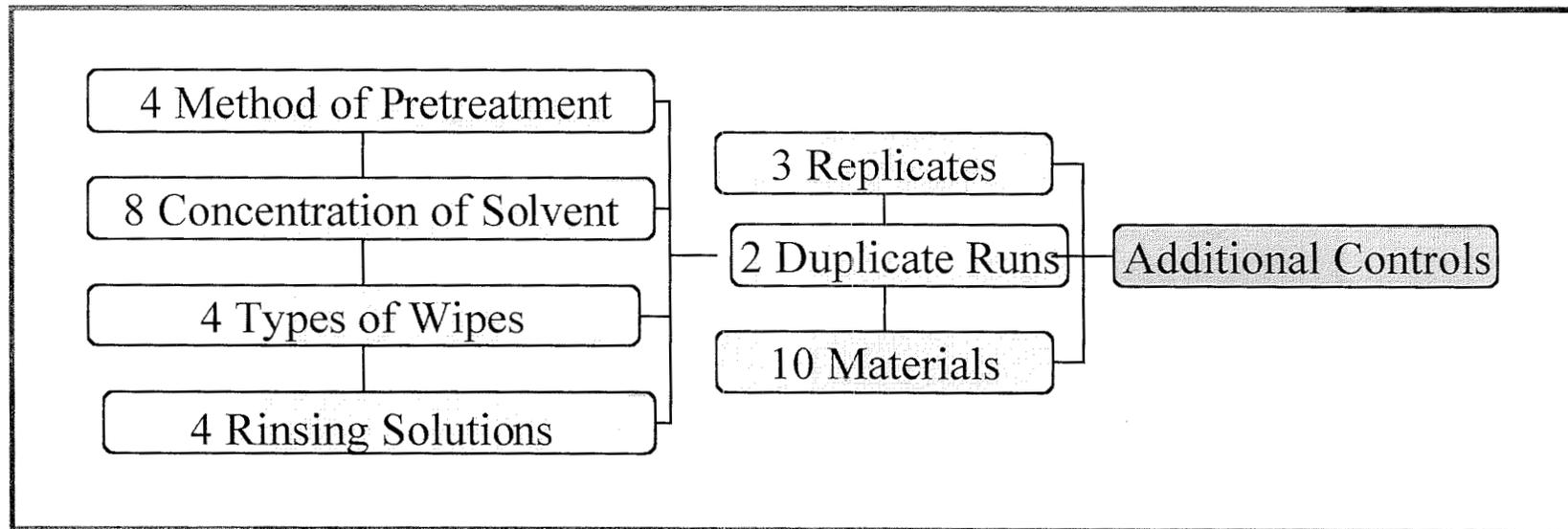
Run	Pre-sterilization	Cleaning solution	Concentration	Wipe type	Rinse
1	Autoclave	IPA RG	100	Alpha 1009	None
2	Autoclave	IPA SC	70	ScourWipe	Sigma H2O
3	Autoclave	IPA HPLC	50	Coventry	IPA
4	Autoclave	Ethanol	30	TechniCloth	Ethanol
5	UV	IPA RG	70	Coventry	Ethanol
6	UV	IPA SC	100	TechniCloth	IPA
7	UV	IPA HPLC	30	Alpha 1009	Sigma H2O
8	UV	Ethanol	50	ScourWipe	None
9	H2O2	IPA RG	50	TechniCloth	Sigma H2O
10	H2O2	IPA SC	30	Coventry	None
11	H2O2	IPA HPLC	100	ScourWipe	Ethanol
12	H2O2	Ethanol	70	Alpha 1009	IPA
13	None	IPA RG	30	ScourWipe	IPA
14	None	IPA SC	50	Alpha 1009	Ethanol
15	None	IPA HPLC	70	TechniCloth	None
16	None	Ethanol	100	Coventry	Sigma H2O



Taguchi Method of Robust Design

Taguchi Method of Robust Engineering Design is an engineering analysis process tool that can improve product or process performance. This engineering design tool makes full use of design control parameters and their selected values. The methodology starts with an implementation of the design of experimental concept by using the orthogonal arrays, first developed by a mathematician, Leonard Euler, in the seventeenth century. Using the linear algebra concept of Latin square rules, the orthogonal array methodology is limited to a minimum, but sufficient, number of experimental design to be performed. More recently, Dr. Genichi Taguchi extended the orthogonal array application of the design of experiment practice by including the use of the signal-to-noise ratio concept. Using of this approach, an optimization of the process or product performance is obtained by performing only a limited number of experiments and tests. The process of process/product optimization is performed through the measured output performance derived from the contribution of each selected value of the control parameters. To determine how much each chosen parameter value contribute to the output performance/quality characteristic is an important element in establishing the process or product optimum performance.

Developing a Robust Cleaning Evaluation Methodology



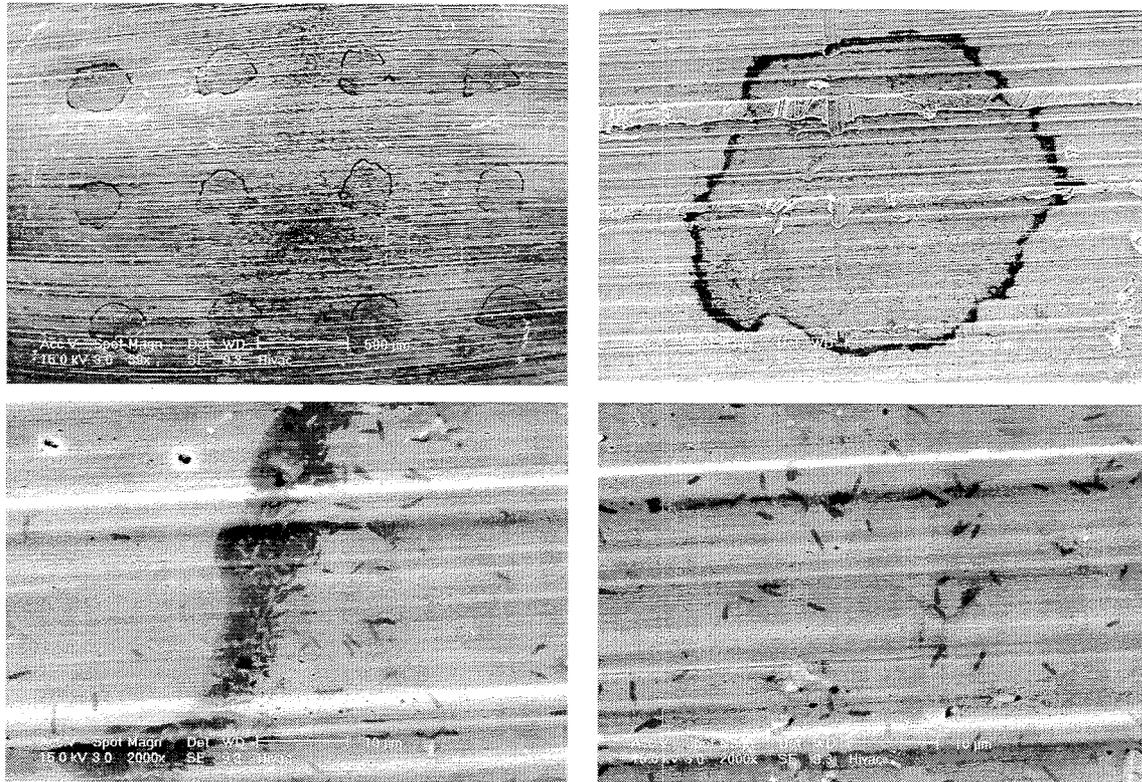
$$4 \times 8 \times 4 \times 4 \times 3 \times 2 \times 10 \times 2 = 61440$$

Running a full factorial cleaning matrix needs about 60,000 coupons!

Impossible to try out every combination, and not necessary either.

ESEM Pictures

Bare Aluminum – positive control



Deposition of the cells on bare aluminum at different magnification

Conclusions

- We have studied the cleaning effectiveness on 10 common spacecraft materials at 16 cleaning conditions. Taguchi statistical design was used to construct the cleaning matrix and to search for the optimal cleaning procedure. The matrix suggested overall trend of effective cleaning, but failed to find the most effective procedure, as one would expect.
- We found a large variation in cleaning effectiveness among different materials at different conditions. Out of the ten material studied, we found that epoxy white paint (446-21-7925) is the easiest to clean, while stainless steel the most difficult. The best experimental cleaning condition is to pre-treat the coupons with H₂O₂, wipe with pre-saturated TechniCloth with 50% IPA, and then rinse with de-ionized water.
- The statistic analysis shows no significant variation in cell deposition, sonication, and cleaning (wiping) among coupons at the same cleaning condition. This indicates a reasonable consistence in cleaning as well as in validation.
- The cleaning study suggested some future direction and improvement.