Alternate Protocol for Detecting Biological Contamination on Sensitive Hardware

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Abstract

The purpose of this project is to develop a sterile water based rapid bioburden test. Contamination engineers use two tests to assess the level of biological contamination on hardware: the rapid five minute bioburden test, which is a molecular screening for Adenosine triphosphate (ATP), a molecule found in all cells on the hardware, and a slower colony growth test, which is used to give a more accurate representation of the amount of microbes on the hardware. However, the rapid bioburden test has limited application because it leaves a residue that can be detrimental to sensitive hardware. This can cause project delays while waiting for the results from the three day colony growth test. We address this problem by adapting the commercial germicide based ATP system to a sterile water based system. The test works by reacting ATP with D-Luciferin and Luciferase protein to yield light. The light is then detected by a luminometer that outputs a Relative Light Unit (RLU) amount depending on how much ATP is present. To analyze the effectiveness of the new test, we developed a correlation between amounts of ATP and the RLU produced using the germicide based system. From these experiments, we've generated a consistent relationship between the two in the form of a power curve. From there, we developed a correlation curve between the amount of colonies and the RLU they produced. Initial tests of the new protocol have shown that the water based system isn't as sensitive as the germicide based test.

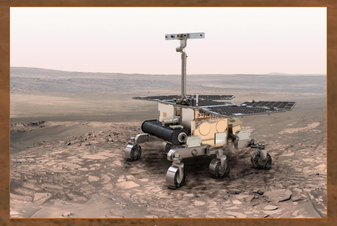

Figure 1 - ESA's ExoMars Rover (Credit: ESA)

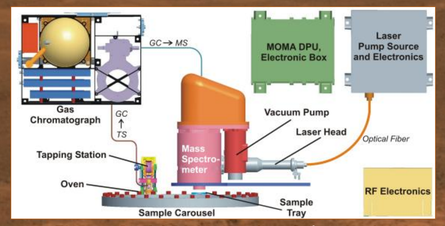

Figure 2 - MOMA Design Schematic (Credit: NASA)

Introduction and Background

Engineers at NASA's Goddard Space Flight Center are currently working on an international project called the Mars Organic Molecule Analyzer (MOMA). One of the requirements of the engineering process is to maintain the cleanliness and sterility of the hardware. Biologicals on the hardware could interfere with any results that MOMA obtains. The standard test used to detect biological contamination is a three day colony growth test: surface samples from the hardware are plated onto a nutrient agar and monitored for three days while microbes in the sample grow into visible colonies. The rapid ATP test takes only a few minutes, and allows hardware engineers to continue working with a higher level of confidence while the three day test is underway. This test currently involves sampling a surface using a commercial swab with a germicidal solution. The test works by reacting ATP in the sample with D-Luciferin and Luciferase protein to yield light by the reaction shown below.

D-Luciferin + ATP → Luciferyl Adenylate (intermediate) + Pyrophosphate
Luciferyl + Oxygen → Light + Oxyluciferin + Adenosine Monophosphate

A luminometer is used to detect the light produced and gives a result. However, the germicide is a molecular contaminant and cannot be used on sensitive hardware, like MOMA. The goal of my project is to develop a test that uses NASA approved swabs with sterile water instead of the commercial swabs.

Methods and Approach

- Commercial swabs were seeded with known concentrations of ATP and the luminometer was used to develop a correlation curve from the results
- Commercial swabs were seeded with calculated amounts of microbes and the results were used to develop a correlation curve
- To test whether an alternate swab would work, we cut off the swab heads of the old test system, pipetted ATP onto a Texwipe swab and a FLOQSwab and placed them into the cut off swab's tube. We then ran the swabs through the same analysis process as the original swabs and compared the results to the correlation curve developed for ATP versus RLU with commercial swabs
- Then we tested if the swabs would work without germicide. To do this, we seeded calculated concentrations of microbes onto Texwipe swabs and processed the swabs with the luminometer. The results were then compared to the correlation curve of microbes to RLU with commercial swabs.

Discussion and Conclusion

- The relationship between Adenosine Triphosphate (ATP) and the amount of Relative Light Units (RLU) produced a power relationship in the 1 x 10^{-15} to 2.5 x 10^{-12} moles of ATP range
- At higher concentrations, the amount of ATP seems to be reach the luminometer's range of detection limit and because of this, the curve begins to level off
- The relationship between the amount of Colony Forming Units (CFU) and RLU is also a power relationship
- The Texwipe swabs do allow for detection using the Hygiena luminometer, though a higher background is observed than the commercial swabs.
- Preliminary tests suggest that the swabs can be used to detect for the presence of microbes, but may be less sensitive.

Results and Graphs

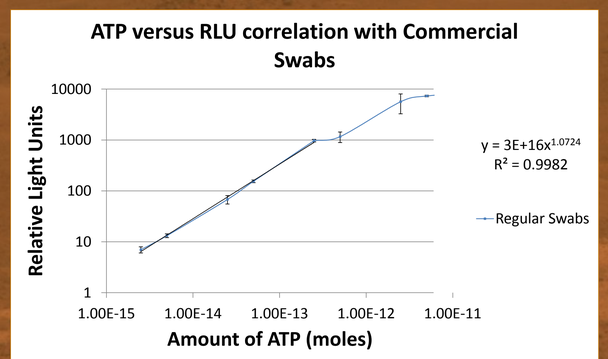

Figure 3 – The correlation curve between the amount of RLU that ATP produces with commercial swabs

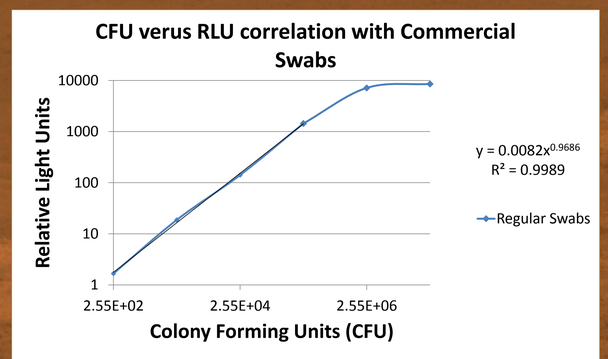

Figure 4 – The correlation curve between the amount of RLU that CFU produces with commercial swabs

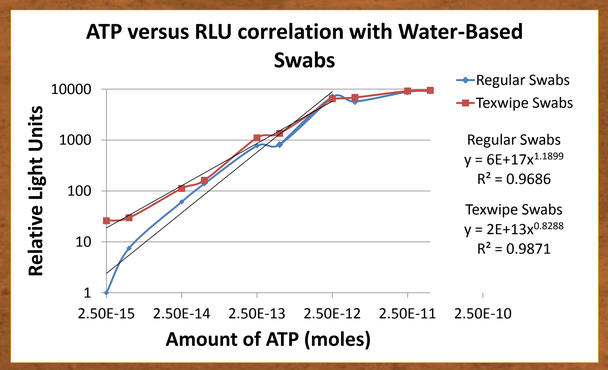

Figure 5 – The correlation curve between the amount of RLU that ATP produces with water-based swabs compared to the commercial swabs

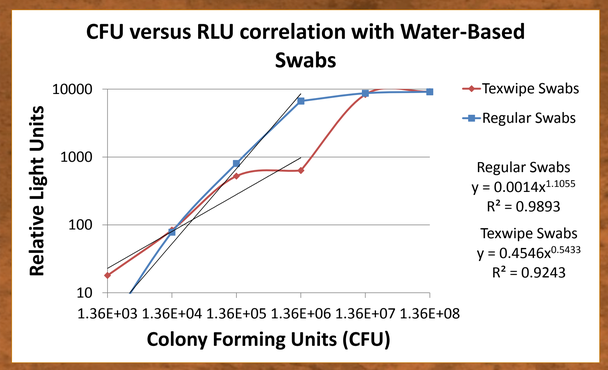

Figure 6 – The correlation curve between the amount of RLU that CFU produces with water-based swabs

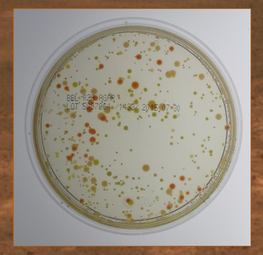

Figure 7 – Colony growth on an agar plate

Figure 8 - Hygiena SuperSnap Swab (Credit: Erin Lalime)

Figure 9 – Hygiena enSure Luminometer (Credit: Erin Lalime)

References and Acknowledgements

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