National Aeronautics and Space Administration

Comparison of the Promega and Kikkoman ATP Devices

Ryan Hendrickson[1,2], Kasthuri Venkateswaran[2], James N. Benardini[2]

[1] Cal State University Northridge, 18111 Nordhoff Street, Northridge, CA 91330

[2] Jet Propulsion Laboratory, California Institute of Technology 4800 Oak Grove Drive, Pasadena, CA 91109

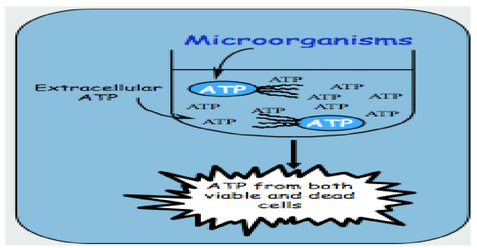
Introduction

Planetary Protection (PP) is a scientific discipline that aims to protect other celestial bodies from microorganisms found on Earth by preventing forward and backward contamination. Forward contamination is bringing life from Earth to a given celestial body and backward contamination is preventing contamination of eventual sample return missions from Mars back to Earth. PP verifies that NASA Planetary Protection requirements are met in part via the NASA Standard Assay in order to support the launch approval process and to comply with international law. The NASA Standard Assay verifies these bioburden requirements by quantifying any aerobic, heterotrophic, and heat-shock resistant isolates.

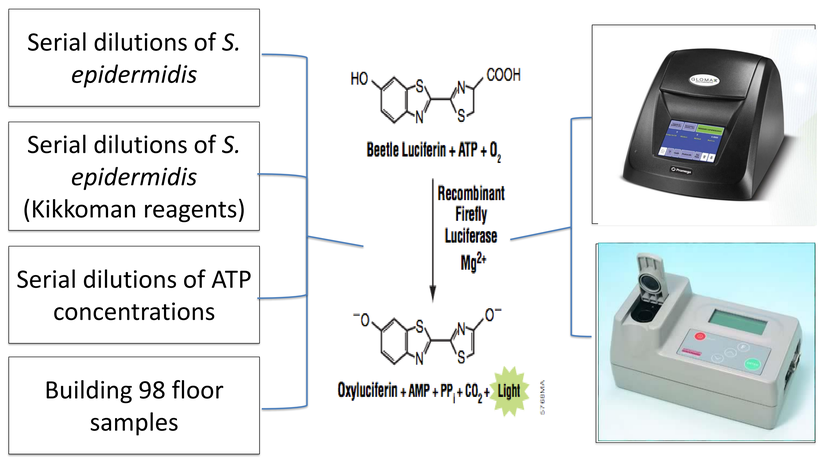
ATP analysis is also used to prescreen surfaces for biological cleanliness. The more cells present on a surface, the higher the ATP (Adenosine Triphosphate) levels. To test for levels of ATP the NASA handbook recommends the Kikkoman HSPlus kit, which uses the firefly protein/enzyme combination of luciferin and luciferase to produce light proportional to the levels of ATP present. Kikkoman, a Japanese based company, manufactures the reagents for this analysis and ordering these reagents is a long lead item (8 weeks)! To potentially circumvent the long lead time a US commercially available device and reagent set (Promega) was compared to the currently employed Kikkoman system.

Goal

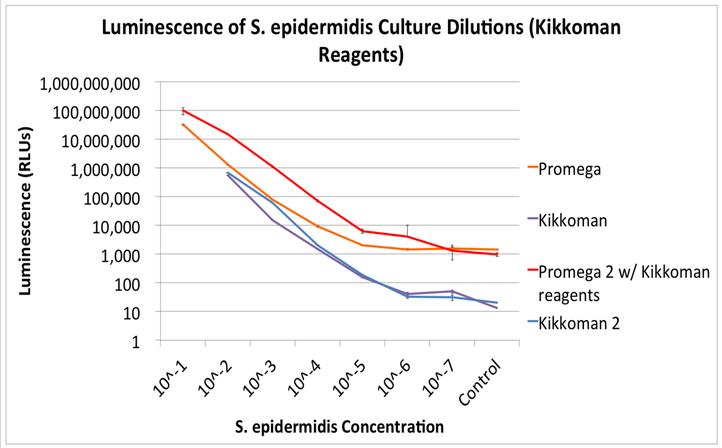
- Technically evaluate two commercially available rapid microbiological cleanliness assay systems (Promega vs. Kikkoman)
- Test assay systems in four different conditions
  1. Serial dilutions of *Staphylococcus epidermidis*
  2. Serial dilutions of ATP
  3. Environmental sample analysis
  4. Serial dilutions of *S.epidermidis* using only Kikkoman reagents



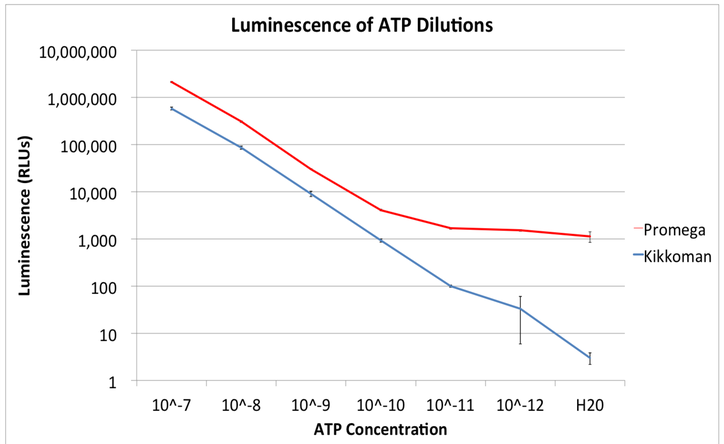
Experimental Design



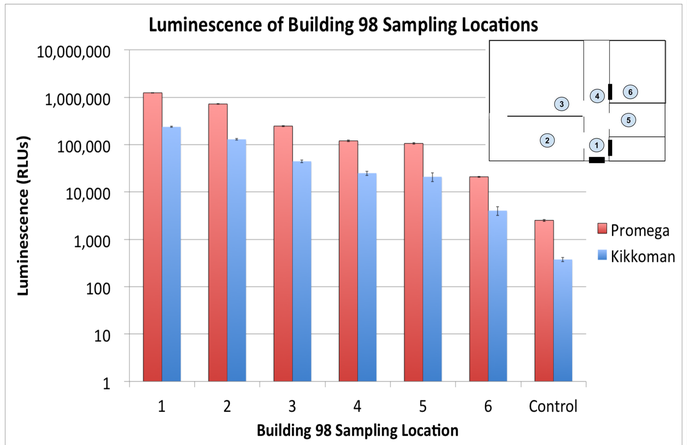
Results and Discussion



Two dilution series of *24 hour S. epidermidis cultures* were tested on both assays. The Kikkoman results were very similar, and both were unable to read the high levels of the 10^-1 dilutions. The two Promega results differed significantly because each trial used either the Kikkoman specific reagents or the Promega specific reagents.



ATP dilutions were tested on both assays. Both assays showed similar slopes, however, the Promega device had a higher background for water, making the differences between water, 10^-12, and the 10^-11 indistinguishable. **The Kikkoman system had distinguishable results at lower concentrations**, which is required for low biomass Planetary Protections samples.



Samples taken from the Building 98 lab were tested on both assays. The Promega device consistently had RLU levels approximately 5 times higher than the Kikkoman device. However, both assays showed accurate ATP levels at all the sample locations. Sample location 1 was located in front of the door to the lab and sample 6 was in the clean room.

Conclusions

Technically due to the background levels of standard spacecraft processing, detection limits within the 1000 RLU or less levels are desirable. Given these two options the Kikkoman device is more technically sensitive at lower detection limits thus making it more feasible to resolve out environmental biomass levels compared to the Promega Device.

This material is based upon work supported by the Chevron Corporation, Howard Hughes Medical Institute, the National Marine Sanctuary Foundation, National Science Foundation, and S.D. Bechtel, Jr. Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funders.
The STAR program is administered by the Cal Poly Center for Excellence in STEM Education (CESAME) on behalf of the California State University.