NEAR REAL-TIME QUANTITATION OF VIABLE MICROORGANISMS FOR PLANETARY PROTECTION AND CREW HEALTH. N.R. Wainwright, Charles River Laboratories, 1023 Wappoo Rd., Suite 43-B, Charleston, SC 29407, norm.wainwright@crl.com

Introduction: The need for rapid assessment of microbial bioburden is practically universal. This is as true for Planetary Protection and Crew Health as it is in the Biopharmaceutical and Food Sciences. For Planetary Protection, the knowledge of when minimum acceptable levels of contamination are exceeded is critical to keeping flight hardware in specification as manufacturing and assembly proceed. It is equally critical to mission science, especially when potential life detection science that may be impacted. For Crew Health, presence of potential pathogens are of primary concern. Whether detection methods are focused on preventative measures to keep environments clean, or on clinical diagnostic procedures that could help diagnose and treat infection, procedures and equipment to rapidly and simply provide information to the crew is paramount. We have developed a technology for the Biopharmaceutical field to detect and count viable microorganisms in a sample, without the need to culture, in about an hour. Sensitivity is as low as a single cell in a given volume. The robustness and portability of the system is amenable to both Planetary Protection and Crew Health requirements.

Background: Earlier work focusing on non-culture methods included measurement of ATP by the luciferin/luciferase system and bacterial endotoxin (LPS or lipopolysaccharide) by Limulus Amebocyte Lysate (LAL). Both of these methods have been approved as ancillary Planetary Protection testing methods [1]. From 2006 – 2009, we tested LAL on the International Space Station (ISS) as a technology demonstration (LOCAD-PTS). Much useful information and valuable experience was gained during that study in quantifying microbial contamination on surfaces [2,3,4]. However, we always realized that direct, selective measurement of <u>viable</u> organisms by non-culture methods was the ultimate objective.

New Technological Approach: To achieve the sensitivity required in the minimal time available, we designed a system with three major components, sample acquisition, fluorescent viability staining and laser scanning.

Figure 1. Full system shown with door open, revealing the rotary stage and optics module. A touch screen on top controls the unit. Approximate size: 1 cubic foot.

Sample acquisition. Samples should be suspended in an aqueous solution, either directly from a liquid or extracted from a surface or solid material. These can be loaded directly in a 10 µl capillary, or concentrated by deposition on a filter membrane, shown below.

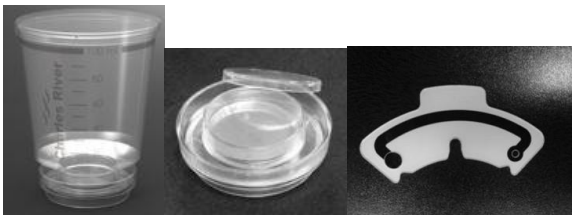

Figure 2. Left: 100 ml filter cup assembly; center: membrane with cup removed; right: 10µl capillary.

Fluorescent viability stain. Selective visualization of viable cells relies on a combination of dyes that accumulate in cells and a quencher that is only permeable to dead cells. A number of dye / quencher combinations are available that are directed to a number of targets, such as nucleic acids, redox reactions and esterase. In addition, fluorescent particles (0.8 µ) can be included as positive controls. Data from several examples will be presented.

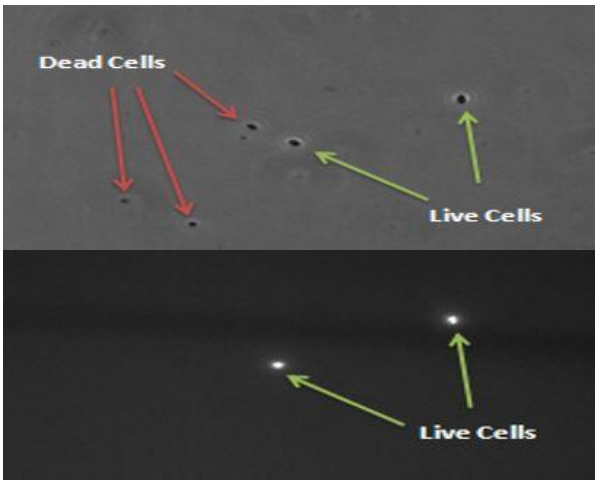

Figure 3. Upper panel: live and dead cells in phase contrast microscopy; lower panel: same field under fluorescence only.

Laser scanner. The scanner has a unique rotary mechanisms capable of scanning both membrane filters and capillaries. The system is comprised of a rotary stage on which the medium is held, a laser directing a collimated beam onto the medium to excite the fluorochrome stained cells, and an optic module housing three photomultipliers, each capturing a portion of the total emitted light spectrum.

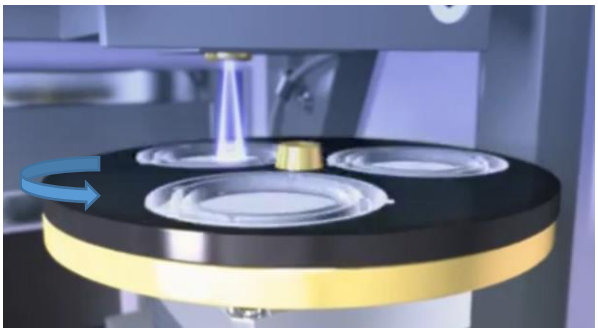

Figure 4. The rotary stage is shown with three membranes being scanned simultaneously.

Following a scan, software analyzes the number, position, color and intensity of fluorescence for each cell or control particle. Very strict criteria are set to eliminate false positives. Scan time is approximately 20 minutes for the three membranes (or four capillaries). Coupled to 15 – 30 minute stain time, the total assay time is less than an hour. As a validation of the process, the membrane filters may be placed on nutrient agar to grow and count colonies. A number of examples will be presented.

References: [1] NASA Technical Handbook. (2008) *Handbook for the Microbial Examination of space Hardware.* JASA-HDBK-6022. [2] Maule, J. et al. (2009) Rapid Culture-Independent Microbial Analysis aboard the International Space Station (ISS). *Astrobiology* 9 (8):759-775. [3] Morris, H.C., et al. (2010) Setting a Standard: The Limulus Amebocyte Lysate Assay and the Assessment of Microbial Contamination on Spacecraft Surfaces. *Astrobiology* 10(8): 845-852. [4] Morris, H.C., et al. (2012) Rapid Culture-Independent Microbial Analysis Aboard the International Space Station (ISS) Stage Two: Quantifying Three Microbial Biomarkers. *Astrobiology* 12(9): 830-840.