RAPID, POINT OF USE ASSAY TECHNOLOGY FOR CREW HEALTH AND MISSION SCIENCE: EXPERIENCE GAINED FROM TESTING ON ISS. N.R. Wainwright[1] A. Steele[2] and L.A. Monaco[3], [1] Charles River Laboratories, 1023 Wappoo Rd., Suite 43-B, Charleston, SC 29407, norm.wainwright@crl.com, [2]Geophysical Laboratory, Carnegie Institution of Washington, Washington DC 20015, [3] Jacobs ESTS Group, Huntsville, AL 35812.

Introduction: The endeavor to inhabit posts far from Earth necessitates the development of novel technologies to provide timely information for crew health, environmental monitoring and mission science. The lack of convenient support from ground resources and the limited flight mass that can be diverted to this purpose require innovative solutions to miniaturize equipment and maximize information collected.

With rapid advances in the disciplines of molecular biology, biochemistry, diagnostics, microfluidics and informatics, it has become possible to develop a single platform that could incorporate several technologies with enormous capabilities. Assay focus could be flexible, so simply changing a reagent cartridge module or software control could direct the technology to provide the information needed. We flew LOCAD (Lab on a Chip Application Development) to ISS as a very focused technology demonstration (2006 – 2011) for rapid, non-culture based microbiology testing needs [1]. We will review our experience in the context of how we might learn from those experiments in designing a more comprehensive, capable science assay platform to support future flight activities.

Figure 1. Flight instrument and disposables for LOCAD-PTS [1].

Positive Experiences: We chose a non-culture based test of microbial contamination for our technology demonstration for several reasons: 1) rapid results (minutes vs. days) 2) comparison to current culture methods, 3) reagents available and stable in dry form, 4) portable hardware is small and field tested (shown in figure 1), and most importantly, 5) simple fluidic cartridge is adaptable to many future applications. Our main goal was to show feasibility of the crew to operate the equipment and collect data in near real time. The primary assay was the well characterized Limulus Amebocyte Lysate (LAL), an FDA licensed reagent used in the Pharmaceutical industry to measure bacterial endotoxin to low (picogram) levels. Later experiments broadened the microbial targets to include lipoteichoic acid, beta glucan as well as endotoxin [2]. Side by side surfaces were swabbed for LAL testing and culture. Figure 2 shows crew member Suni Williams entering samples in the LOCAD-PTS (Portable Test System) using a surface sample acquisition tool.

Figure 2. Sample being loaded into LOCAD-PTS [1].

Given the limitations of the technology, good correlation was seen between the culture and LOCAD-PTS results. Figure 3 shows areas of the airlock surfaces tested expressed in EU (endotoxin units, 1 EU=~100pg). Note higher value shown in yellow on lower right (hatch handle). As one would assume, surfaces frequently touched by the crew showed higher values than those not usually handled.

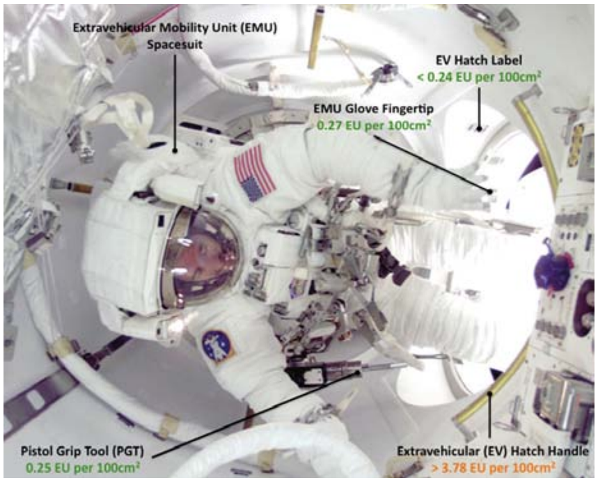

Figure 3. Airlock surfaces tested with LOCAD-PTS. Higher value (>3.78 EU/100cm^2) seen on hatch handle [1]

Problems to Overcome: Sample acquisition represented some of the greatest challenges. Since samples were suspended in an aqueous environment, providing a reliable source of clean water in our swab collection tool was a necessity. Evaporation losses of water packed with the swab created the challenge of dealing with an air bubble when dispensing the sample. We solved the problem in the short term by modifying the procedure used by the crew; however, a redesign of the water module was required to more permanently fix the issue. We initially thought bubbles in the fluidic line would be more of a problem, however, the diameter of the fluidic channels was ~1mm. Small enough to handle the 25 microliters sample volume, but not microfluidic dimensions.

Future Design: The technology demonstration of LOCAD-PTS can be considered a success, not in the sense it has definitively established a culture alternative. That will require much more validation, and frankly, addition of more technology. We did, however establish that point-of-use collection of data by crew to be quickly and reliably performed. Decisions that must be made on future flight missions to Mars and other destinations beyond low Earth orbit will surely benefit from expansion of this theme. Some generalizations we can draw from our experience that can be applied to future instrument development include:

- Sample acquisition must be reliable, simple and adaptable to multiple sample sources, i.e., liquid, air, surfaces.
- Miniaturization and portability is important.
- Multiple technologies should be integrated to provide breadth of information collected and alternative confirmation of positive assay results.
- Reagents should be kept to a minimum and be stable in a dry format for long (on the order of years) shelf life.
- User-friendly automation of assay protocols requires minimal crew expertise to achieve valid results.

Technologies such as enzymatic reactions, nucleic acid amplification, immunoassays, spectroscopy and others that operate in an aqueous environment can be handled by common means. In as much as the initial LOCAD-PTS instrument has been modified to take a range of chips to test for different substrates. Therefore samples collected from diverse formats will be testable with minimal adaptation of fluid flow within an instrument. Ultimately, questions that address biological and chemical issues, such as crew health, planetary protection, hazard determination and Astrobiology are addressable with a minimal set of flight hardware, enabling crew to function autonomously on long duration missions without intimate ground support.

References: [1] Maule, J., Wainwright, N.R., Steele, A., Monaco, L, Morris, H., Gunter, D., et al (2009) Rapid culture-Independent Microbial Analysis aboard the International Space Station (ISS). *Astrobiology*, 9 (8), 759. [2] Morris, H., et al. (2012) *Astrobiology*, (in preparation).