2 Medical and Scientific Evaluations aboard the KC-135

2.1 Microgravity-Compatible Flow Cytometer

FLIGHT DATES:
June 29, 2004
August 10 – 11, 2004
September 2 and 9, 2004

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GOAL:
To validate a prototype spaceflight-compatible flow cytometer and an associated microgravity-compatible cell staining device for medical support during long-duration space missions. Five KC-135 evaluations of the developed hardware were performed in FY04.

OBJECTIVES:
A spaceflight-compatible flow cytometer would be useful for the diagnosis of astronaut illness during long duration spaceflight and for conducting in-flight research to evaluate the effects of microgravity on human physiology. Until recently, the primary limitations preventing the development of a spaceflight compatible flow cytometer have been largely mechanical. Standard commercially available flow cytometers are large, complex instruments that use high-energy lasers and require significant training to operate. Standard flow cytometers function by suspending the particles to be analyzed inside a ‘sheath’ fluid for analysis. This requires the presence of several liters of sheath fluid for operation, and generates a corresponding amount of

liquid hazardous waste. The particles are then passed through a flow cell which uses the fluid mechanical property of 'hydrodynamic focusing' to place the cells in single-file (laminar flow) as they pass through a laser beam for scanning and evaluation. Many spaceflight experiments have demonstrated that fluid physics is dramatically altered in microgravity (MSF [Manned Space Flight] Fluid Physics Data Sheet-August 1997) and previous studies have shown that sheath-fluid based hydrodynamic focusing may also be altered during microgravity (Crucian et al, 2000). For these reasons it is likely that any spaceflight compatible design for a flow cytometer would abandon the sheath fluid requirement. The elimination of sheath fluid would remove both the problems of weight associated with large volumes of liquids as well as the large volume of liquid waste generated. It would also create the need for a method to create laminar particle flow distinct from the standard sheath-fluid based method.

The spaceflight prototype instrument is based on a recently developed commercial flow cytometer possessing a novel flow cell design that creates single-particle laser scanning and evaluation without the need for sheath-fluid based hydrodynamic focusing. This instrument also possesses a number of design advances that make it conditionally microgravity compatible: it is highly miniaturized and lightweight, uses a low energy diode laser, has a small number of moving parts, does not use sheath fluid and does not generate significant liquid waste. Although possessing certain limitations, the commercial cytometer functions operationally like a standard bench top laboratory flow cytometer, aspirating liquid particle samples and generating histogram or dot-plot data in standard 'FCS' file format. In its current configuration however, the cytometer is limited to three parameter/two-color capability (two color PMTs + forward scatter), does not allow compensation between colors, does not allow linear analysis and is operated by rather inflexible software with limited capabilities. This is due to the fact that the cytometer has been designed and marketed as an instrument specific to a few particular assays, not as a multi-purpose cytometer.

The NASA-JSC Center Directors Discretionary Fund has funded the Cell & Molecular Research Laboratory to: (1) construct a prototype flight instrument based on the framework of the commercial cytometer; (2) perform ground-based and microgravity validation of the instrument; (3) design and validate a set of medical assays compatible with the prototype instrument; (4) design and validate a microgravity compatible cell staining device for sample processing that can interface with the instrument. In FY04 the initial stages of instrument design and validation were successfully completed, as well as the development of the cell staining unit and medical assays. The FY04 KC-135 evaluations that took place were as follows:

Flight #1	6-29-2004	Validation of fluidics/function, standard sample acquisition
Flight #2	8-10-2004	Std. Data acquisition, evaluation of precision sample delivery pipettes
Flight #3	8-11-2004	Initial evaluation of new cell staining unit and associated instrument port
Flight #4	9-2-2004	Complete blood-to-data system evaluation part 1
Flight #5	9-7-2004	Complete blood-to-data system evaluation part 2

METHODS AND MATERIALS

Blood donors. Whole blood samples were obtained from adult donors into ACD anticoagulant vacutainers. This includes the KC-135 flight experiment and all developmental work performed

in preparation for the experiment. The subjects had been screened by the NASA-JSC Human Test Subject Facility for most major infectious diseases and were found to be in good health. Institutional Review Board (NASA-JSC) approval was obtained for this study and written informed consent was obtained from all subjects.

Cell staining. A complete 2-color antibody panel was formulated to resolve most major leukocyte subsets yet remain within the limitations of the instrument. The cell populations resolved included: leukocyte subsets; T cells; B cells; NK cells; T cell subsets and activated T cells. Cell surface markers were stained prior to flight. For the bead-based cytometry samples either fluorescent calibration microspheres or linearity fluorescent microspheres were used.

Flow cytometry analysis. For ground-based analysis, a Beckman-Coulter XL flow cytometer was configured as a reference cytometer for 3 parameter/2 color analysis so that it would mimic the function of the cytometer. Analysis was performed using the XL as a reference cytometer, ground-based control data was generated using the prototype flight cytometer. For data collection during microgravity the sampling apparatus of the cytometer was altered for ease of operation during parabolic flight. To ensure data collection occurred during microgravity only, samples were mixed and affixed to the sampling probe and the instrument was primed prior to the initiation of the microgravity phase. Priming took place during the 2g phase between parabolas. Data collection was initiated as the parabola was initiated and ended as the aircraft exited the parabola.

RESULTS:

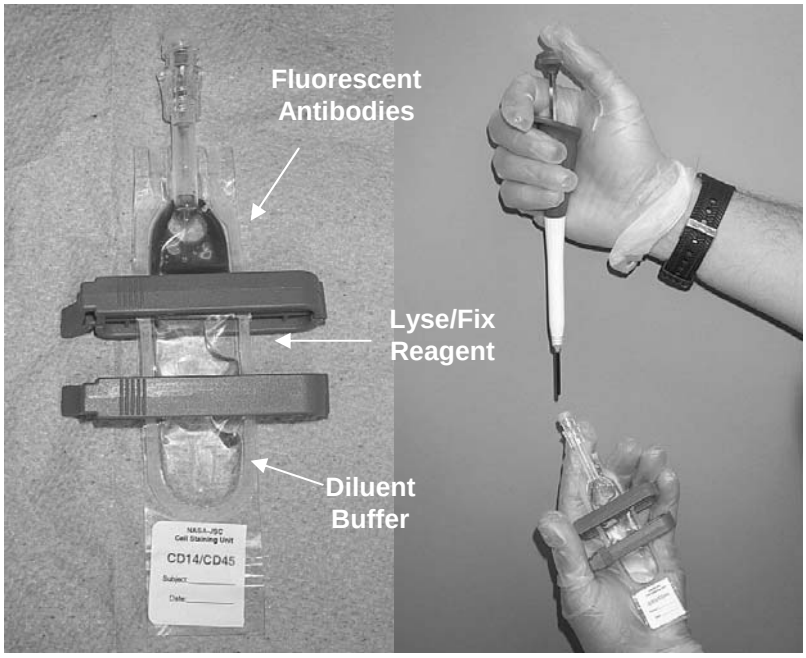
In FY04 the basic configuration of the prototype flight cytometer was prepared, the associated cell-staining unit (interfaces with instrument) was developed and validated, the medical assay panel was developed and validated and microgravity evaluation of all hardware was successfully performed.

Development of prototype instrument: The internal components of a novel commercial cytometer were used as a basis for development of the basic prototype flight cytometer. The basic prototype flight cytometer consists of the unit with the minimal alterations required for microgravity evaluation and further development. (An advanced prototype flight cytometer will be completed in year two of this project that will be completely re-engineered for optimum compatibility with on-board flight operations) The areas of the commercial cytometer that were deemed not microgravity compatible were modified. These included: (1) replacing the waste collection system with an inflatable Teflon bag fixed to a one-way check valve and (2) modifying the sample uptake port with 10cm of silicone peak tubing and an injection port. The newly developed injection port was designed to be compatible low-flow fluidics and compatible with the injection port of the cell staining unit.

Development of cell staining unit/sample processing procedure: A novel cell staining unit was developed by the CMR Laboratory in 1996, and evaluated onboard the Shuttle on STS-71. This unit, the Whole Blood Staining Device (WBSD), consists of tubing separated into chambers with clips with an injection port at one end. By filling the chambers with staining reagents, a blood sample may be injected and processed during microgravity. The WBSD was initially considered and evaluated with the prototype flight cytometer. It was found, however that the hard tygon tubing unit body created a negative pressure that hindered the instrument from withdrawing sample. This effect was found to dramatically alter the capacity of the instrument to provide

absolute cell counts. A novel second generation unit was designed that used a sterile low-adherence collapsible Teflon bag, a Baxter Interlink Injection Site and Weland clips for chamber separation. This unit, (the WBSD2) interfaced perfectly with the instrument, provided reliable absolute counts and was found to be robust and reliable.

To generate absolute counts it is necessary to deliver a precise volume of blood sample into the staining device. For this purpose a 100ul positive displacement pipette was used. Several types of blood collection tubes were evaluated, and a 4.0 ml EDTA Starsted monovette was found to have several distinct advantages: (1) they are plastic; (2) the top screws into place and is not held by vacuum; (3) they are without vacuum during storage, the user creates the vacuum at the time of sample collection; (4) they make excellent storage vessels if there is an unexpected delay in processing; (5) the port is thinner than a vacutainer, meaning that the plastic pipette tip may pass through and sharp needles are not required.



Development of medical assay panel: A panel of clinical laboratory hematology and immunology assays, important in infection and immunity, was developed to be compatible with the limitations of the Prototype Flight Cytometer. This assay panel was achievable within the two-color cytometry limit of the instrument and would provide critical medical information currently not available on-orbit. The developed and evaluated assays were as follows: white blood cell count, leukocyte differential, lymphocyte subsets, T cell subsets, activated T cell levels and DNA content. The two-color antibody matrix developed for these assays was as follows:

PE	PC5	
CD14	CD45	WBC Differential: granulocytes/lymphocytes/monocytes.
CD3	CD45	T cell percentage
CD19	CD45	B cell percentage
CD16	CD45	NK cell percentage
CD3	CD4	Helper inducer T cell subsets
CD3	CD8	Cytotoxic/suppressor t cell subsets
CD45RO	CD4	T cell subsets; memory/naïve T cell subsets
CD45R0	CD8	T cell subsets; memory/naïve T cell subsets
CD69	CD3	Activated T cell subsets.

Microgravity evaluation of instrument and associated hardware: The instrument, all associated hardware, and the panel of assays were all evaluated real-time in microgravity conditions onboard the KC-135 aircraft. The sample delivery/processing hardware was found to function well during microgravity conditions, in a manner comparable to ground operation. The operators noted that care needed to be exercised to prevent air bubbles in the liquid samples from skewing the volume of sample primed in the pipette for delivery. However, the clear-bore design of the precision 100ul positive-displacement pipette allowed for easy inspection of the sample. The WBSD2 units functioned extremely well during microgravity and the interface with the instrument was found to be simple and reliable.

The Prototype Flight Cytometer bolted to the floor of the KC-135 aircraft for microgravity evaluation (left) and evaluation of the precision sample delivery and cell staining hardware

The cytometer itself was found to function well in microgravity condition. Due to the sample priming delay caused by the temporary addition of the exterior peak tubing to which the WBSD sample port was attached, the operators had to begin priming the samples during the 2g phase of flight; however data were only collected during the microgravity phase. All peripheral blood leukocyte subset data collected during microgravity were found to be essentially identical to ground data and reference cytometer control data.

DISCUSSION:

The flow cytometer is an extremely versatile laboratory instrument with a broad-spectrum of uses in both clinical medicine and basic science research. It is therefore highly desirable to develop a spaceflight compatible cytometer for use on the International Space Station. While currently limited in ability, the cytometer represents an attractive design option for the design of a spaceflight compatible flow cytometer. It has the potential to be made completely microgravity compatible and serve as a prototype spaceflight instrument with relatively minimal alterations in design specifications.

The cytometer's level of miniaturization, use of a low energy diode laser, and elimination of the sheath fluid requirement all uniquely meet the existing prerequisites for use during spaceflight. It is likely that the current limitations of this instrument could be overcome by modifying the software, adding additional lasers, color PMTs, side scatter, color compensation ability and further miniaturization. The sample delivery apparatus of the cytometer is not microgravity compatible and would require significant modifications. These limitations notwithstanding, the cytometer may be well suited to be the prototype from which a spaceflight compatible flow cytometer is designed.

The successful microgravity evaluation of the cytometer and the need to collect real-time experimental data onboard ISS for both research and clinical diagnosis purposes warrant the continued development of a spaceflight prototype flow cytometer. The versatility of the flow cytometer for general research (biological, microbial, environmental and physiological studies) and diagnostic medicine would be a major asset to the ISS/lunar/mars programs.

CONCLUSION:

A basic evaluation version of a prototype spaceflight-compatible flow cytometer was successfully created and evaluated. An associated sample processing system that interfaces with the instrument was also successfully developed.

REFERENCES:

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PHOTOGRAPHS:

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JSC2004E35188
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JSC2004E35293
JSC2004E35652 to JSC2004E35673
JSC2004E40306 to JSC2004E40320
JSC2004E40641 to JSC2004E40659

VIDEO:

- Zero-g June 29 – July 2, 2004, Reference Master: 718394
- Zero-g August 9 – 13, 2004, Reference Master: 718620
- Zero-g August 30 – September 9, 2004, Reference Master: 718586

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

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