Fiscal Year:	FY 2011	**Task Last Updated:**	FY 10/05/2010
PI Name:	Tai, Yu-Chong Ph.D.		
Project Title:	In-flight Blood Analysis Technology for Astronaut Health Monitoring		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Smart Medical Systems and Technology Team		
Joint Agency Name:		**TechPort:**	Yes
Human Research Program Elements:	(1) **ExMC**:Exploration Medical Capabilities		
Human Research Program Risks:	(1) **Medical Conditions**:Risk of Adverse Health Outcomes and Decrements in Performance Due to Medical Conditions that occur in Mission, as well as Long Term Health Outcomes Due to Mission Exposures		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	91125	**Congressional District:**	29
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2007 NSBRI-RFA-07-01 Human Health in Space
Start Date:	10/01/2007	**End Date:**	09/30/2011
No. of Post Docs:	0	**No. of PhD Degrees:**	2
No. of PhD Candidates:	2	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Ho, Chih-Ming (University of California, Los Angeles) Kasdan, Harvey (IRIS International) Adams, Thomas (IRIS International)		
Grant/Contract No.:	NCC 9-58-TD01301		
Performance Goal No.:			
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Task Description:	The specific aims of the project include (a) 5-part WBC differential, (b) analysis of WBC subtypes (e.g., CD4+ T helper and natural killer cells), and (c) serum/plasma protein biomarker analysis (e.g., for infection, radiation and bone loss monitoring). This project is a continuation of a related project entitled "Handheld Body-Fluid Analysis System for Astronaut Health Monitoring", in which we explored electrical impedance sensing, fluorescence optical sensing, and flow separation of blood cells in microfluidic devices and portable platforms. We successfully demonstrated fluorescent sensing and counting for WBC count and 2-part differential with a portable prototype micro flowcytometer. For the current project, a major effort is proposed to extend the 2-part WBC differential to a 5-part WBC differential, add cell surface marker detection and analysis capability to the platform repertoire, and add plasma protein detection and analysis capability to the platform repertoire. Our approach to achieve the objectives is to extend the capability of the micro flowcytometer to enable a more comprehensive WBC differential, and allow detection of fluorescent labels attached to ligands used for cell surface marker and plasma protein detection. The second component necessary for extending the platform capability is the offline data analysis software. This software is being developed in Matlab to facilitate both quantitative assessment of fluorescence detection and cell and analyte recognition and quantitation. In the last funded year, we worked on searching for new staining method and optimizing the previously proposed Acridine Orange staining. We successfully developed a 4-part differential assay (i.e. Lymphocyte, Monocyte, Neutrophil and Eosinophil) with a cocktail staining of fluorescent dyes fluorescein isothiocyanate (FITC) and propidium iodide (PI). The differential assay is investigated in a correlation study with the commercial hematology analyzer, and further verified with the purified WBC types. For the Acridine Orange assay, the differential capability is also extended from 3-part (Lymphocyte, Monocyte and Neutrophil) into 4-part (Lymphocyte, Monocyte, Neutrophil and Eosinophil). The time and temperature dependence of the Acridine Orange staining are also investigated. We also worked on improving the proposed platform on its detection end. Two different approaches including a commercial mini-spectrometer and a 8-color fluorescence reader with an 8-channel PMT module have been implemented. Single cell fluorescence emission spectrum has been measured on the mini-spectrometer prototype. The information about the subtle differences of the spectrum can be used to optimize the wavelength range of the multi-color measurement for capturing these differences. Multicolor fluorescent beads have been successfully measured on the 8-color reader. Those two approaches are investigated in order to extract as much useful information as possible from the emitted signal. The additional spectral information should provide better discrimination between multiple fluorophores used simultaneously. It may also provide additional information about the intracellular environment in which Acridine Orange fluorescence occurs, leading to efficient WBC subtype discrimination. To analyze WBC subtypes, we initiated the development of synthesized peptides, which can be utilized to selectively target and bind to leukocytes. The binding peptides were custom synthesized with a fluorescein fluorophore attached to their n-terminus for binding quantification. A library of 72 potential peptide candidates has been tested using a modified protocol for leukocytes utilized in our studies. Several peptide binding assays has been performed to screen for potential peptides that will preferentially bind to leukocytes. Among the results, 4 peptides from our initial library exhibited a 2-3x higher binding strength to the B-cells compared to the other peptides, but showed minimal preference compared with the control group. We are in the process of running additional peptide binding assays using a new peptide library, against cultured cell lines (B- and T-cells). For the coming year, we plan to work on the prototype with the spectrum analysis capability and the one with the 8-color reader for developing the staining assay. Ability to discriminate among multiple color emissions will provide the capability to detect multiple ligands simultaneously, and may help in performing a 5-part WBC differential with a single stain such as Acridine Orange. Further, we will continue to work on developing the staining assay for the test platform, expanding the differential capability of the staining assays to 5-part (i.e. Lymphocyte, Monocyte, Neutrophil, Eosinophil and Basophil). A staining assay with a dye cocktail of PI, FITC and BO21 is currently being investigated. For the WBC subtype differential with synthetic peptides, we are in the process of running additional peptide binding assays using a new peptide library. These assays will be run using cultured cell lines (B- and T-cells) and additional leukocyte subtypes will also be tested when promising peptide candidates are identified.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The devices under development can be used for earth-based applications. The proposed device uses a cartridge in a hand held system. The cartridge will be cheap and disposable. The results will be available almost immediately to the patients without going through central lab facilities. The device can be used in an emergency room, ambulance and ultimately at home. As the senior population continuous to grow, this kind of device will find greater use in point-of-care applications. Part of the technology has been licensed to LeukoDx Inc. for the development of a point of care sepsis detection system initially targeted for the detection and monitoring of neonatal sepsis.
	For the second funding year, we proposed optimizing the blood staining and testing procedure for 5-part WBC differential, the hydrodynamic separator for WBC subtype separation, and plasma preparation for plasma biomarker detection. We are on the schedule. 1. Blood staining and testing procedure optimization: A 4-part WBC differential (Lymphocyte, Monocyte, Neutrophil and Eosinophil) assay using a staining cocktail of FTIC and PI has been developed. The differential capability has been investigated with a correlation study with a commercial hematology analyzer and further verified with purified individual WBC types. The differential capability of the previously proposed assay with metachromatic dye, Acridine Orange, was also expanded from 3-part differential (Lymphocyte, Monocyte and Neutrophil) to 4-part differential (Lymphocyte, Monocyte, Neutrophil and Eosinophil). 2. Purified WBC types preparation procedure for developing differential assays: A procedure of preparing purified WBC individual types (Lymphocyte, Monocyte, Neutrophil, Eosinophil or Basophil) has been developed. The purified WBC samples can be used to verify the differential capability of the proposed staining assays, and also provide new capability to identify new clusters in developing the 5-part differential assay. 3. Upgraded detection capability - spectrum analysis: One unit of the prototype has been upgraded from two-color detection to spectrum analysis with a commercial

Task Progress:	mini-spectrometer. The spectrum analysis capability can be used to identify the subtle differences of the fluorescence spectrum of different WBC types, and then the wavelength range of the multi-color measurement can be optimized to capture those differences. 4. Upgraded detection capability - 8-color fluorescence emission reader: Two units have been upgraded for 8-color fluorescence measurement reader using an 8-channel PMT module. One has been delivered to Caltech, and one remains in IRIS for additional tests and further development. Ability to discriminate among multiple color emissions will provide the capability to detect multiple ligands simultaneously, and may help in performing a 5-part WBC differential with a single stain such as Acridine Orange. 5. Synthetic peptides development for WBC type differential: A library of 72 potential peptide candidates has been tested using a modified protocol for leukocytes utilized in our studies. Several modifications were made to this protocol to minimize cellular damage and optimize the effectiveness of the binding assays. Purified human B-cells and peripheral blood mononuclear cells (PBMCs) were purchased for peptide binding assay studies. Our results demonstrate that peptides have potential to be used as targeting molecules to capture leukocytes. We are in the process of running additional peptide binding assays using a new peptide library, which would be run using cultured cell lines (B- and T-cells).
Bibliography Type:	Description: (Last Updated: 08/30/2018)
Articles in Peer-reviewed Journals	Lillehoj PB, Tsutsui H, Valamehr B, Wu H, Ho CM. "Continuous sorting of heterogeneous-sized embryoid bodies." Lab Chip. 2010 Jul 7;10(13):1678-82. http://dx.doi.org/10.1039/c000163e ; PMID: 20376391 , Jul-2010
Awards	Shi W. "IEEE Distinguished Student Humanitarian Prize, June 2010." Jun-2010
Papers from Meeting Proceedings	Shi W, Kasdan HL, Fridge A, Tai YC. "Four-part differential leukocyte count using uFlow cytometer." 23rd IEEE International Conference on Micro Electro Mechanical Systems (MEMS) 2010, Hong Kong, China, January 24-28, 2010. In: 2010 IEEE 23rd International Conference on Micro Electro Mechanical Systems (MEMS), p. 1019-1022, 2010. http://dx.doi.org/10.1109/MEMSYS.2010.5442382 , Jan-2010