FLOW CYTOMETRY/CELL SORTING and MASS CYTOMETRY CORE LABORATORY
CENTER FOR VACCINE DEVELOPMENT
UNIVERSITY OF MARYLAND - BALTIMORE
HEALTH SCIENCES FACILITY 1, Room 456
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RULES AND REGULATIONS (Revision March 10, 2015)

The primary goal of the flow cytometry/cell sorting laboratory is to ensure that University of Maryland investigators whose research projects require the use of a flow cytometer have access to such instrumentation.

The facility currently maintains two flow cytometers and a mass cytometer:

1) Beckman Coulter MoFlo Legacy flow cytometer/cell sorter equipped with three lasers (488 nm, 633 nm and 407 nm), FS and SS detectors, and 10 PMTs. This instrument has been installed and fully operational since September 2002.

2) Becton Dickenson LSR II SORP flow cytometer equipped with four lasers (488 nm, 552 nm, 641 nm and 407 nm), FS and SS detectors, and 15 PMTs . This instrument has been installed and fully operational since October 2007.

3) Fluidigm CyTOF mass cytometer able to detect up to 35 metal-labelled antigens. This instrument has been installed and fully operational since March 2013.

The flow cytometry laboratory personnel have the expertise and training to assist in the preparation of experimental protocols, as well as to appropriately and efficiently maintain and operate the equipment.

The following rules and regulations must be closely observed by ALL investigators to ensure the productivity and smooth operation of this valuable resource.

"User" is defined as the person generating the samples. "Principal Investigator (PI)" is defined as the person responsible for paying any facility charges. These may or may not be the same person.

A. NEW USERS

Before samples from a new user can be run on the flow cytometer, Principal Investigators (PI) who desire to have specimens run in the core facility are required to:

1. Schedule a meeting with the Core Director (Dr. Marcelo Sztein - 410-706-2345) or Supervisor (Regina Harley - 410-706-0095) to discuss whether the use of the flow cytometer/mass cytometer will be appropriate to accomplish the goals outlined by the investigator. At this time, the rationale for the use of flow cytometry/mass cytometry, the controls and staining techniques required for the experiments, the approximate number of samples to be run, the flow cytometer/mass cytometer requirements and time tables will be discussed in detail.

2. Receive and read the Rules and Regulations Form (Revision February 20, 2015).

3. Fill out and sign a Billing Authorization Form stating that the PI (and the PI's personnel) agrees to conform to

the rules and regulations governing the operation of the flow cytometry laboratory and is responsible for paying the fees incurred.

No samples will be run until we have received a signed copy of the Billing Authorization form.

B. SCHEDULING

1. The first experiment will be scheduled at the first meeting with Dr. Sztein and/or Regina Harley. Subsequent experiments can be scheduled with Regina directly by calling 410-706-0095, by stopping by the lab in HSF1 456, or by emailing cvdflowcore@medicine.umaryland.edu.

2. Every effort will be made to ensure that experiments are scheduled in a timely fashion. However, depending on the sample load, the quality and type of specimens and whether sorting is involved, scheduling times may vary.

3. Appointments to run live cells or sorting should be made at least a week in advance. In these circumstances, the PI should make every possible effort to conform strictly to the specified time, since failure to do so will result in conflict with other scheduled experiments.

4. **If you are going to be more than 15 minutes late (and it happens to everyone eventually) please let us know.** In this case, the experiments of the investigators that are late will either be rescheduled or run if the existing schedule permits doing so. In the latter situation, charges will begin at the scheduled time. If an experiment started on time demands additional time, the situation will be discussed with the investigators involved on a case-by-case basis.

5. For experiments involving the analysis of fixed cells (e.g. determination of surface markers with monoclonal antibodies), samples may be delivered to the flow laboratory before the scheduled time.

6. Cancellations should be made as soon as possible, preferably at least 48 hours in advance, to allow the reassignment of that time slot to another investigator.

C. SAMPLE ANALYSIS

1. Staining is the responsibility of the PI or the PI's personnel, unless otherwise arranged.

2. Samples must be provided as single cell suspensions free of cell debris and clumps, which can clog the instrument and will reduce the reliability of the data.

3. The number of cells required varies according to the analysis to be performed. These requirements, as well as whether viable cell preparations should be sterile or non-sterile, whether cells should be viable or fixed and the media in which they have to be resuspended will be discussed individually.

4. **All samples delivered to the flow cytometry laboratory must be accompanied by a Sample Log Sheet** stating the name of the PI, project title, date samples were generated, the name of the individual delivering the samples. The list of samples should have the following information: sample numbers, tube labels (if different than sample numbers), sample description (cell type and concentrations, experimental treatment, fluorochromes used). Sample Log Sheet templates are available in the Flow Cytometry Core Laboratory.

5. All samples will be run by the staff of the flow cytometry facility. Running of samples by the PI or user will not be permitted except under extraordinary circumstances. In that case, authorization will be provided only after a training period is completed and the Director of the facility personally evaluates their technical proficiency and understanding

of the principles of operation of the flow cytometer.

6. Assignment of Experiment # and Naming of Data Files

A. The facility will give each experiment run in the facility a unique 6-digit Experiment # as follows:
The first two digits are the user's initials.
The second two digits are the year.
The third two digits will be the number of the experiment run that year for that user.

B. Data files will be named with some combination of Experiment #, user initials, and date, depending on the instrument.

This information will be recorded on the Sample Log Sheet.

7. Biohazardous samples

The following samples <u>will not</u> be accepted for analysis under any circumstances:

1. Cells that have the potential to shed viruses (e.g., live cells obtained from AIDS patients)
2. Cells that are resuspended in solutions that may damage the instrument
3. Cells that have incorporated radioisotopes
4. Cells containing any pathogen that may be hazardous in any way to the personnel running the samples.

8. Log Book

A log book will be kept to record the name of the investigator, type of analysis, date and time spent in collecting samples and analyzing the data for each set of specimens. In some cases, for example for cell cycle analysis in which staining has to be performed immediately before analysis, the flow cytometry laboratory personnel are required to stain the specimens. In these situations, the time utilized to stain the specimens will also be recorded.

<u>D. DATA ANALYSIS AND RESULTS</u>

1. **Data analysis of the samples is the responsibility of the PI or the PI's personnel**, unless otherwise arranged. Data files will be provided to the user after completion of the experiment.

2. If core personnel are to do the analysis, results will be provided and discussed with the investigators as soon as data analysis is performed. The printouts of the results and, if needed, the data files will be provided. At this time the need for additional controls or changes to the experimental or staining protocols will be discussed.

<u>E. CHARGES</u>

1. As of February 1, 2015, all investigators will be charged as follows:
<u>Flow cytometry - Analysis</u>- $50.00/30 minutes, rounded up to the nearest 30 minutes.
<u>Flow cytometry - Sorting</u> - $50.00/30 minutes, rounded up to the nearest 30 minutes, plus a flat 2-hour set-up fee ($200.00).
<u>Mass Cytometry</u> - $85.00/30 minutes, rounded up to the nearest 30 minutes.

2. Rate increases might be necessary and may happen at any time. The decision to increase the hourly rate will be the joint responsibility of the Dean of the School of Medicine and the Director of the Flow Cytometry Core Laboratory.

3. The time used for both sample collection and data analysis will be included in the calculation of charges.

4. Only investigators without any source of funding who desire to procure preliminary results for a grant application will be exempt from the charge. In this situation, the PI should submit a signed statement indicating that they do not currently have any source of support and that it is their intention to use the results of the experiments to procure grant funds. Moreover, the PI should state that support for the performance of future experiments involving flow cytometry would be incorporated into budgets of grants submitted based upon the data obtained. These statements will be kept permanently in the files of the Flow Cytometry Laboratory.

F. COLLABORATIONS

Whether a particular project is categorized as service or collaboration will be decided in a case by case basis, by mutual agreement between the PI and the Director of the facility. This determination will be based largely on the extent of the involvement of flow cytometry personnel in the experimental design and discussion of the theoretical basis of each individual project.

G. PUBLICATIONS

1. All publications that include data generated by the flow cytometry laboratory should acknowledge that the samples were run in the Cellular Immunology and Flow Cytometry Section, Center for Vaccine Development, School of Medicine, University of Maryland at Baltimore.

2. **A copy of each publication (including abstracts) should be provided for the records of the flow cytometry laboratory.**

Marcelo B. Sztein, M.D.
Chief, Cellular Immunology
and Flow Cytometry Section, CVD

Regina Harley, M.S.
Supervisor , Flow Cytometry and
Mass Cytometry Core Laboratory

Original RULES AND REGULATIONS is on file in the Center for Vaccine Development Flow Cytometry Laboratory and is available for inspection upon request.