



Guidelines

Laboratory of Flow Cytometry and Cell Sorting



GUIDELINES FOR THE USE OF THE LABORATORY OF FLOW CYTOMETRY AND CELL SORTING FACILITY

The Laboratory of Flow Cytometry and Cell Sorting was established in 1986, with funding from the Research Centers in Minority Institutions (RCMI) Program, to provide a state-of-the-art facility for qualitative and quantitative cellular analysis and automated cell sorting. The objectives of the Laboratory of Flow Cytometry and Cell Sorting are: to provide access to flow cytometry technology to investigators, students and technical personnel; to train interested users in the utilization of flow cytometry instrumentation, and its multiple applications in biological research; to provide limited technical support in flow cytometry methodology to interested users of the facility, and to encourage the development of new methodology and applications in the area of flow cytometry. Some of the applications used in flow cytometry are: immunophenotyping, cell cycle analysis, quantitation of protein surface expression on cells, respiratory burst activity, analysis of mitochondrial membrane potential, cell isolation and cloning by sorting and DNA analysis among others. Technical expertise is available through affiliated faculty, technical personnel and graduate students. The RCMI Program oversees that users of the facility comply with the rules and guidelines. Potential users of the facility are required to formally request access to the instrumentation and other services available.

Description of the facility

A. Physical location:

Department of Microbiology
3rd Floor A-382
(787) 758-2525 Ext. 7033

B. Administrative Staff

Emma Fernández-Repollet, Ph.D.
Scientific Director
(787) 758-2525 Ext. 1620, 1621
e-mail address: efernandez@rcm.upr.edu

Diana Ramos
Technical Specialist
Advanced Flow Cytometry Facility
(787) 758-2525 Ext. 5121,1303
e-mail address: dramos@rcm.upr.edu

Hours for technical assistance 8:00am- 4:00pm

I. Instrumentation

1. FACSort Cell Sorting Flow Cytometer System- Becton Dickinson; classifies and separates cells based on various parameters. The configuration of this instrumentation is depicted in the illustration included in **Appendix A**. It has acquisition software such as: Cell Quest, SimulSet and FACSComp.

2. Facstation analytical computer (Attached to the FACSort) - This system reads, analyzes and prints data acquired using the FACSort instrumentation. The system includes:

- a. Power Macintosh G3
- b. 17- inch Multisync Trinitron Color Display Monitor
- c. Laser printer- HP DeskJet 1200 C/PS
- d. Software:
 - Cell Quest- for acquisition and analysis
 - DNA Application Disk- for acquisition
 - FACSComp- to perform instrument QC
 - Attractors- to perform batch analysis
 - SimulSet- clinical acquisition and analysis
 - ModFit LT- for DNA analysis

3. Off-Line Analysis Station- Computer system used exclusively to analyze and print data files (**Appendix B**). The station includes:

- a. Power Macintosh G4
- b. Xerox DocuPrint N2125 Laser
- c. 17- inch MultiSync FE 750
- d. Software:
 - Adobe PS Read Me Files
 - Acrobat Reader
 - Cell Quest
 - FACSComp
 - FACSCConvert
 - ModFit LT 3.0

4. Refrigerator / freezer- to store samples and reagents on a temporary basis.

5. Flow Cytometry Related Literature
Cytometry- Journal (1994-Current)
User's Guide - Immunocytometry Systems- Becton Dickinson

II. Eligibility

A. All investigators that justify the use of the laboratory with a research project will be eligible for the use of the facility. To facilitate this process, the Laboratory of Flow Cytometry and Cell Sorting requires that users provide evidence of training in the use of the flow cytometer (i.e., attendance to annual workshop, which is organized and sponsored by the RCMI Program; formal training with flow cytometer manufacturer; or previous experience in a flow cytometry laboratory).

B. Priorities for the use of the facility are as follow:

- 1st Priority: RCMI Active Investigators / Technicians.
- 2nd Priority: Investigators of Medical Sciences Campus.
- 3rd Priority: Graduated students of Medical Sciences Campus and Technicians.
- 4th Priority: Investigators of other campuses of the University of Puerto Rico or private institutions/laboratories.

Note: Users that are going to acquire data have priority over those performing data analysis. Data analysis has been facilitated by the addition of an independent data analysis station.

C. Registration of users

Faculty and/or students interested in using the flow cytometry facility are required to fill out a request form (**Appendix C**) to use the instrumentation. A password is assigned after the user is authorized to have access to the laboratory.

D. Special requirements:

- 1- Users should provide **all reagents** for calibration and analysis.
- 2- Users should provide **printer supplies**.
- 3- Users should provide **data storage materials**. (zip drives, floppy disk)

III. Rules and Regulations

A. The following rules and regulations must be followed by all users of the Laboratory of Flow Cytometry and Cell Sorting:

- 1- Reserve the use of the equipment at least 24 hours before in the Weekly Schedule form placed in the facility (**Appendix D**). Use of the flow cytometer is limited to 4 hours period per laboratory, per day, unless special arrangements are authorized. Users must notify any cancellation at least 24 hours in advance.

- 2- Sign the Log Book placed in the facility on the date of the use of any of the instruments of the facility.
- 3- Transport all samples to be run in the flow cytometer in racks and properly labeled.
4. Follow Universal Precautions (**Appendix E**) at all times.
- 5- Wear gloves, laboratory coats and mask for all procedures involving handling of human material.
- 6- Follow appropriate procedures regarding handling of biohazardous materials and proper disposal of biohazard wastes.
- 7- Wash all laboratory surfaces with 10% bleach before and after laboratory procedures.

B. Tips on the proper use of the Flow Cytometer

- 1- Make sure that the instrument is working properly, i.e. set up and calibrate for your particular application.
- 2- If problems arise during analysis, fill out a Problem Report Form (**Appendix F**) and deliver the form to the Technical Specialist working in Lab A-331 (Ext 1303). **Do not use the instrument. Do not attempt to fix the instrument.**
- 3- Only the Scientific Director and/or the Technical Specialist are authorized to call the manufacturer to request maintenance and/or repair services.
- 4- After each session, clean and shut down the instrument as described in the instruction manual found on the shelf of the facility.
- 5- Always leave a tube with no more than 1 ml of distilled water inserted in the suction area.
- 6- Leave the working area clean.
- 7- Each person is responsible to dispose of their own biohazard materials.

Note: Do not use computer for other purpose than Flow Cytometry acquisition and analysis, use of the computer for other purposes voids the service contract. Any user that violates this rule will then have to pay for the cost of repair.

IV. Recommended References

- 1- User's Guide- Flow Cytometry Training Manual- Becton Dickinson
- 2- Cytometry- Journal (1994-2001)
- 3- Purdue Cytometry CD ROM, Volumes 1- 6*
- 4- Biological Safety Guidelines (**Appendix E**)
- 5- Current Protocols in Cytometry*

* These references are available at Advanced Flow Cytometry Facility, Ext. 1303

V. Fee for Services

A nominal charge of \$5.00/hr will be implemented, effective October 1, 2002, for flow cytometry data acquisition and/or analysis at the Flow Cytometry Facility (Appendix G). These funds will be used to cover expenses related to the daily maintenance of the instrument. Technical support is available on a limited bases, for an additional cost. **Technical support does not include sample preparation it only include data acquisition and analysis. Request for services requiring technical support should be made a week prior to the date of service.**

VI. RCMI Acknowledgment

*All investigators using the **Flow Cytometry and Cell Sorting Facility** for their investigations should acknowledge this support by including the following sentence in their publications:*

“THIS INVESTIGATION WAS SUPPORTED, IN PART, BY A RESEARCH CENTERS IN MINORITY INSTITUTIONS AWARD, G12RR-03051, FROM THE NATIONAL CENTER FOR RESEARCH RESOURCES, NATIONAL INSTITUTES OF HEALTH.”

Your cooperation in this respect is vital to the success of the program and the support of this facility.

Copies of publications acknowledging support to the RCMI Program should be sent to our office to include the reference in our progress report:

RCMI PROGRAM
OFFICE 621-A
6th FLOOR
UPR MEDICAL SCIENCE CAMPUS
Tel: (787) 763-9401
Fax: (787) 758-5206
E-mail: efernandez@rcm.upr.edu

VII. Approval of Guidelines:

Approved by:



Emma Fernández-Repollet, Ph.D.
Director, RCMI Program

Date: June 6, 2002

APPENDIX

APPENDIX A



APPENDIX B



APPENDIX C

**REQUEST FOR USE OF SHARED
INSTRUMENTATION**

Instrument: _____ Location: _____

Name: _____ Degree: _____

Institution: _____ Department: _____

Lab.#: _____ Extension: _____

PI: _____ Role in project: _____

Duration of project: _____ Funding: _____

Project title (attach a copy of abstract):

Training Information:

A. By whom you were trained? _____

B. Number of hours trained? _____

C. The training consisted of:

D. What will this instrument be use for?

E. How many hours per week the instrument will be used? _____

Approved by: _____ Date: _____

APPENDIX D
FACSort Weekly Schedule
July 7 – July 13

	Mon 7	Tue 8	Wed 9	Thu 10	Fri 11	Sat 12	Sun 13
7:00 am							
8:00							
9:00							
10:00							
11:00							
12:00 pm							
1:00							
2:00							
3:00							
4:00							
5:00							

APPENDIX E

FLOW CYTOMETRY FACILITY

University of Puerto Rico

Telephone: (787) 758-2525 ext. 1620

BIOSAFETY POLICY

Universal Precautions: is an approach to infection control. According to the concept of universal precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other blood borne pathogens. (OSHA Regulations)

Scope: Biohazards originate from biologically active material, which may possess hazardous properties to humans or other animals, or plants. All unfixed human materials are considered biohazardous. Additionally, the use of a fixative does not necessarily eliminate biohazardous properties. All researchers and their staff must know the potential biohazards associated with their work. Precautions commensurate with the maximum-level hazard should always be taken.

Biosafety Levels: The Flow Cytometry laboratory is suitable as a Biosafety Level 1 facility. This is the lowest, or least controlled, possible rating. Biosafety Level 1 is defined for use with agents that present no known or, at most, minimal potential hazard to laboratory personnel and the environment. Work is normally conducted on the bench top and no special containment equipment is required.

Precautions: Procedures appropriate for Biosafety Level 1 are: (1) Laboratory doors must be closed while experiments are in progress; (2) Work surfaces must be decontaminated on a regular basis, and following any spills; (3) All contaminated liquid or solid waste must be decontaminated prior to disposal; (4) Mouth pipetting is prohibited; (5) Eating, drinking, and storage of food are not permitted; (6) Persons must wash their hands after handling viable materials and before leaving the laboratory; (7) All procedures must be carefully performed to minimize the creation of aerosols; (8) The wearing of coats or gowns is recommended.

Wastes disposal: The user is responsible to handle and take out from the facility all the biohazards materials.

In accordance with the above guidelines, we require all users of the Flow Cytometry Facility to apply appropriate procedures for the safe handling of their particular specimens and wastes. Generally, this means that all users will dispose of their own wastes. Bench top "Biohazard" bags, suitable for autoclaving, are provided for users' convenience.

Instruments lines that are in contact with biologically active materials must be decontaminated by each user. This includes the sample-foed and waste tubing, and the waste reservoir. The waste reservoir must be emptied and decontaminated following each use. Forty milliliters of pure sodium hypochlorite solution (Clorox) placed in the waste reservoir each time it is emptied suffices to decontaminate most materials that accumulate there. Users are also responsible for flushing the sample feed and waste tubing with appropriate decontamination agents (usually 30% Clorox in water, for 2-5 minutes)

All users of the Flow Cytometry Facility will be held accountable for the procedure listed above. Violation of these procedures will result, at minimum, in revocation of further laboratory access. If you have questions concerning these biosafety regulations, please ask a staff member to review them with you. We appreciate your cooperation in allowing us to operate a biologically safe laboratory.

APPENDIX F

Flow Cytometry Unit

Problem Report Form

Date: _____
Time: _____
Operator: _____

Instrument: _____

Description of Problem: _____

Send to : _____
Rcvd/date: _____

Action Taken: _____

Appendix G

To: Flow Cytometry Standards Corp.
P. O. Box 194344
San Juan PR 00919

Invoice#:

Billing Date:

Evaluation of flow cytometry quantitative measurements using Quantum Simply cellular microbeads; including flow cytometry analysis, assessment of reagent stability, accuracy and reproducibility of results as well as intra and inter assay variation.

TOTAL:

Diana Ramos
Technical Coordinator
RCMI Flow Cytometry Facility

Make check payable to: University of Puerto Rico

Send Payment to: RCMI Program
Office 621-A
UPR-Medical Sciences Campus
P.O. Box 365067
Río Piedras, Puerto Rico 00936-5067

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Last updated: 5/2007