Flow Cytometry Core Facility-
IRBLleida
Performance Standards

Index

1. Objectives

1. To offer technical assistance and advice regarding new applications, experimental design, sample adquisition and data analysis to all research groups in need.
2. To design protocols of analysis.
3. Data processing and adquisition on demand.
4. Basic Training for Core users, and also theoretical and practical lessons in different Universitat de Lleida degrees.

2. Facilities and Equipment

The Flow Cytometry Facility Core is located in the Aux 4-1 Laboratory (4th floor) in the IRBLleida and Universitat de Lleida's Biomedicina 2 building beside Arnau de Vilanova University Hospital.

The Flow Cytometry facility is owner of an analytical FACS-Canto II Flow cytometer (Becton Dickinson) with 3 lasers and with 4-2-2 configuration, enabling up to 8 color panels (refer to technical specifications in the annexes). This flow cytometer is equipped with a computer with FacsDiva v. 6.1.3 analysis software installed. The facility also has aditional software packages for different applications such as Flowlogic (Inivai Technologies) for offline analysis, ModFit LT for cell cycle determinations, FCAP Array for CBA analysis (BD) and FlowCytomix Pro for FlowCytomix (eBiosciences) analysis.

The facility also includes a magnetic cell sorter **AutoMACS Pro Separator** (Miltenyi Biotec) which enables the serparationa and enrichment of different cell types using the MACS® technique. This instrument is located in a biosafety II cabinet.

Other equipment available are: 4ºC fridge, -20ºC freezer, vortex, micropipets, black and white printer, computer for data analysis post-acquisition.

3. Samples

The Flow cytometry Facitliy Core recieves samples from different origins (cell lines, mouse, rat primary cultures, whole blood, serum, human and animal plasma) potentially containing human pathogens, thus, it is of great relevance to maintain staff and user biosafety. For this reason, it is critical to know the sample origin and wether there are potentially infectious agents in order to maintain

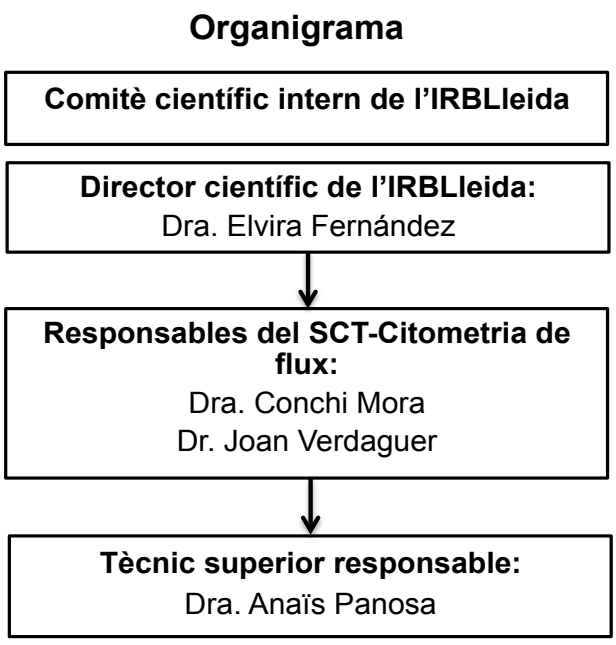
efective biosafety measures. To address these needs, the Flow Cytometer and Confocal Microscopy Core has developed a **Use Regulation** and a **Biosafety Questionnaire** that must be filled before starting any activity in the facility by the principal investigator (PI). In the same way, PI also must fill in the **Registration Questionnaire** in order to maintain the registration list updated.

4. Organization

The Flow Cytometry and Confocal Microscopy Core is an organized structure which depends directly on the IRBLleida Scientific Director.

4.1 Organization Chart and Staff

The daily workgroup in the Flow Cytometry Facility Core is composed of a Senior Technician with full time job and the Service Managers with part time dedication. Decision making are done in internal meetings between Service managers, the Scientific Director, and also the IRBLleida Internal Scientific.



The main duties of Flow Cytometry Facility managers are:

- To promote Flow Cytometry Facility activities among researchers.
- To ascertain that Flow Cytometry Facility use regulations are being followed.

- To call and assist in Flow Cytometry Facility Core internal meetings.

The main duties of the Senior technician are the following:

- To be aware of Core Use Regulation compliance.
- To always atend users in need.
- To apply Standard Operation Procedures for each instrument in the facility.
- To advice, assist and train users.
- To perform administrative and management tasks of the facility.
- To attend to Core internal meetings

4.2 Users

On the one hand, facility users are IRBLleida researchers: PhD students (with PI permission) and PIs. Also researchers from ICS Lleida, GSS and UdL are potential users of the core. All of them must fill in the **Registration Form** before starting their activities in the facility.

On the other hand, researchers from other Research Institutes or Private enterprises might be users if they request it puroposely.

5. Use Regulations

5.1 Ethical aspects

Ethical aspects, quality and viability of each research project must be verified by the Clinical Research Ethical Comitee (CEIC) following the current legislations:

- Llei 14/2007 d'investigació biomèdica (14/2007 Biomedical Research Law)
- European Council Agreement for human rights and dignity protection, considering biology and medicine applications, which become effective in Spain the 1st of January 2000.
- Additional protocol to the Human Rights and Biomedicine Convention, relative to biomedical research (2005).
- European Council Recommendation for research using biological materials of human origin from 15 march 2006.
- Guia de bona pràctica en la investigació en ciències de la salut. Institut Català de la Salut, 2003
- *Biosafety in Microbiological and Biomedical Laboratories*; US Department of Health and Human Services, 4th Edition.

5.2 Biosafety Aspects

In order to start using the Flow Cytometry Facility Core it is compulsory to fill in the Biosafety Questionnaire. This questionnaire must be filled and signed by the lab manager who is requesting the analysis, always before starting experiments or developing a project. Every questionnaire will be filed for granting that no information is subsequently modified.

5.3 Flow Cytometry Facility Users's Rights and Responsabilities

Before starting new experiments, user must follow these steps:

- To speak with the senior technician of the facility and explain the planed experiments in order for him to evaluate them and plan its execution.
- Every scientific project must be aproved by CEIC.
- To fill the **Registration For**m in order to be registered as a user of the facility.
- To read the **Use Regulation** document of the Flow Cytometry Facility Core and accept its conditions.
- To read and fill in the **Biosafety Questionnaire**.
- To attend a basic training course to learn how to use the flow cytometer and other instruments in the facility. This course will be held by Facility's staff and will be compulsory. Anyone in need to use instruments of the facility must have done the course and must be authorized by the staff when they consider the user is capable enough to use the instruments on their own.

5.4 Flow Cytometry Facility Staff's Rights and Responsabilities

- Facility staff is responsible for daily instrument maintenance and Use Regulation compliance
- Facility staff will consider each new user and will decide if he/she is skilled enough to perform experiments by him/herself.
- Facility staff reserve the right to not to perform any experiment if they consider that facility guidelines haven't been followed properly.

5.5 Rates

The Flow Cytometry Facility Core sets each year rates for the Flow Cytometer Analyser. These rates can change depending if it is an IRBLleida, ICS Lleida, GSS,

and UdL user or if it is an external user (companies, other research institutes, etc...). It may also vary depending if it is an assissted hour of use with the technician or it is an selfservice hour.
Rates are the following:

Preu/hora	Usuaris interns		Usuaris Externs
	Sense operador	Amb operador	Amb operador
Disseny experimental, consultoria	-	20€	35€
Adquisició de mostres FACS CantoII	12€	20€	35€
Anàlisi de dades	-	20€	35€

Internal users: IRBLleida, UdL, ICS Lleida and GSS members
External users: other institutions or companies
*Reductions in the rates may be applied depending on billing.
VAT not included

At the end of the week, Flow Cytometry and Confocal microscopy Core staff will count how many hours has the cytometer been used (using the **Use Form**) in order to calculate the amount of money each group will have to pay.
Each semester, a bill will be emitted and sent to PIs, which will correspond to services rendered to each group

6. Services and Applications

Applications already available in the **Flow Cytometry Core** are:

1. Immunophonotyping: Population analysis using from one to 8 different colors. Surface and intracellular antigen expression analysis.
2. Apoptosis Studies (TUNEL assays, annexin-V assays).
3. CBA (Becton Dickinson Cytometric bead array to calculate protein concentration) and FlowCytomix (from eBiosciences)
4. PhosFlow: Single cell cellular activiation level analysis of multiple proteins. It allows to combine surface and intracellular markers to study immunophenotype and intracellular signaling.

5. Oxidative stress studies (fluorescent probes for the detection of reactive oxygen species: DHE, Rho123, DCFDA, Mitosox, etc...).
6. Cell cycle analysis.
7. Proliferation assays using CFSE.

Facility staff is opened to develop new techniques and applications if the user requires them.

7. Service request

After being registered as a user in the Flow cytometry Facility Core and filled in all documents (User registration, Use Regulation, Biosafety questionnaire) each time the user wants to use the facility must do the following:

- To book in advance by using the booking application available in the IRBLleida intranet.
- It is not advisable to book the same day cause it may be no availability.
- Experiments must be prepared carefully, trying to adjust booked hours with real using time. In thay way we will avoid interferences with other people work plan.
- It is essential to arrive on time.
- Booking request cancellation must be done at least 24 h in advance in order to let other users book that time.
- Is user responsability to be sure that all results are copied in a data storage device. FACSDiva Database will be deleted from server every three months.
- Every incidence or error occured in an instrument during usage must be communicated immediately to the facility staff.

8. Annexes

Documents mentioned in the text:

- FACS-CantoII Flow cytometer Technical specifications
- Use Regulation of Flow Cytometry and Confocal Microscopy Core
- Biosafety Questionnaire
- Registration Form
- Use Form

FACS-Canto II Flow cytometer: 3 lasers: 4-2-2 combination (8 colors)

- 405 nm Violet Laser: 30 mW
- 488 nm Blue Laser: 20 mW
- 633 nm Red laser: 17 mW

Canto II 488 nm Blue Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	735	780/60	PE-Cy7
B	655	670 LP	PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, 7AAD
C	610	-	No PMT
D	556	585/42	PE, PI, EB
E	520	530/30	FITC, Alexa 488, GFP
F	none	488/10	SSC
G	-	-	-
H	-	-	-

Canto II 633 nm Red Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	735	789/60	APC-Cy7
B	685	-	No PMT
C	-	660/20	APC, Cy5

Canto II 405 nm Violet Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	502	510/50	AmCyan
B	-	450/50	Pacific Blue
C	-	-	-

Digital signal processing system allowing height, area and width pulse determinations. Compensation can be done offline and post-acquisition.

Analysis Software: BD FACSDiva v. 6.1.3

Cell cycle analysis Software: Modfit LT™ 3.0

FCAPArray Software: CBA analysis (BD)

FlowCytomixPro Software: FlowCytomix analysis (eBiosciences)

USE REGULATION OF FLOW CYTOMETRY AND CONFOCAL MICROSCOPY CORE

There are a few regulations user must follow in order to assure the good functioning of the facility.

- When a user decides to perform an experiment or a project it is compulsory to fill in the registration form (there is a booking sowftware in the intranet area in IRBLleida's website) and also the biosafety questionnaire.
- It is advisable to describe briefly the research project. With this information, the Core facility is able to write activity reports that will be used for funding and grant purposes.
- Bookings can be done by telephone or personally using the booking software in IRBLleida intranet.
- It is not recommended to book in the same day as it might be no availability.
- Experiments must be carefully prepared, trying to adjust booked hours with actual used hours. Behaving in such a way will avoid interfering in other user's work.
- It is essential to arrive on time. More than 15 minutes delay may involve losing the booked time and being billed for it. Booked time must be defined acording experiment complexity; if an experiment lasts more than the expected time and there is a user waiting for his turn, you must continue your experiment according to machine availability. On the other hand, if your experiment lasts less time than expected, total used time will be billed.
- In order to let the maximmum number of users to use the Flow cytometer, there will be a maximmum number of tubes for user and day depending on day's bookings. This maximmum number is 100 tubes/day.
- Booking request cancellation must be done at least 24h in advance, in order to let other users to book that time. If booking was not to be cancelled and investigator didn't appear on the schedulled time, booked hours will be billed.
- Is user's responsability to be sure to record his/her results in some data storage device. FACSDiva Database will be deleted from server every three months and anually for confocal microscopes computers. Users will

be asked to delete all experiments in advance. All not deleted data will be erased.

- Every incidence or error during use of any instrument of the facility must be alerted to facility staff promptly.
- Flow Cytometry Facility Core should be cited in the Acknowledgements of every published scientific paper that includes data obtained in experiments performed in the Facility. Citing the Facility reflects its work and is used to value the Flow Cytometry Core Facility and it is also a justification used in concurring in different scientific projects or public calls for funding in different administrations. An examle of citation could be:
    - We would like to acknowledge the IRBLleida Flow Cytometry Core Facility for the technical help in the flow cytometry experiments.

- **BIOSAFETY QUESTIONNAIRE**

Flow Cytometry and Confocal Microscopy Core Laboratories are multi-user facilities where many different samples from various sources that may contain known or unknown human pathogens are investigated. The safety of facility personnel and users is of ultimate concern. Information about the sample sources and potentially infectious agents is critical for effective biosafety mesures.
Consequently, this sample information form must be filled out completely and signed by the laboratory director who is requesting samples to be analysed before projects or experiments are started. The same biosafety questionnaire will be kept on file provided none of the information it contains has changed.

Laboratory Director (Principal Investigator)
Phone number
Fax
E-mail

Investigator (Experimentor)
Phone number
Fax
E-mail
Laboratory location

Project title, Funding entity and code (if any)

Project start date and end date:

Summary or description of project: Provide details related to cells that will be analyzed. Limit to one paragraph.

List type of sample and source (i.e., mouse spleen cells, human peripheral blood mononuclear cells, cells from an animal en-grafted with human cells, etc…); for cell lines, describe cell origin an biosafety level.

Does the sample contain any known infectious agent(s)? Yes ☐ No ☐

If yer, list infectious agents and name the biosafety level needed using the classification in *Biosafety in Microbiological and Biomedical Laboratories*; *US Department of Health and Human Services, 4th Edition*.

Has the infectious agent been inactivated or rendered non-infectious?

Yes ☐ No ☐

If yes, describe method inactivation. Provide proof of inactivation if applicable.

Were blood cell donors screened for bloodborne pathogens, i.e. HIV, HBV, HCV?

Yes ☐ No ☐

If yes, list test results, positive and negative.

Could the sample contain other known human pathogens? If yer, list agent(s).

Were the cells transformed using a virus such as EBV, HTLV-1, herpes samiri? If yes, list virus.

Yes ☐ No ☐

Have cells been tested for mycoplasma infection and/or viral infection (HIV, HBV, SIV, etc…)? Yes ☐ No ☐

If yer, give date of last test(s) and test(s) results. Tests must have been performed within one week prior to sample submission to the flow cytometry core laboratory.

Were the cells genetically engineered?

Yes ☐ No ☐

If yes, how were they genetically engineered? Was a gene therapy virus (adenovirus, retrovirus, lentivirus, herpesvirus, etc...) used to transfer genetic

information to the cells? If yes, describe method in detail, attach vector map and show packaging cell line.

Will the samples be fixed prior to submission to core? Describe the fixation protocol in detail, e.g. list concentration and exposure time.

I have read above questions carefully and certify the information provided to be correct.

Date:

Signature (Laboratory Director, Principal investigator)

Registration Form

Flow Cytometry and Confocal Microscopy Core

Name and last name:

Group/Department:

Research institution:

University:

Principal Investigator:

Contact Telephone number:

Briefly description of project:

Experimented user? Yes ☐ **No** ☐

Selfservice Yes ☐ **No** ☐

Would you need technical assistance?

Yes ☐

No ☐

Other comments or suggestions:

Full d'utilització Citòmetre FACS CantoII Servei de Citometria

IRBLleida

Dia	Hora	Duració	Grup (IP)	Usuari	Incidències