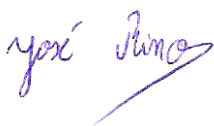


**INSTITUTO DE MEDICINA MOLECULAR JOÃO LOBO ANTUNES  
FLOW CYTOMETRY FACILITY  
USER GUIDELINES**

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## Cell Analyzers

BD Accuri C6  
BD LSRFortessa 1  
BD LSRFortessa 2  
BD LSRFortessa X-20  
Amnis ImageStreamX

### How to get training and permission to use the analyzers:

- All users, regardless of their previous expertise, must get training on a cell analyzer before using it. If you are an iMM user, you can contact us by phone, email or use the online booking system to request access and training in a cell analyzer. External users should contact us by email:

**[imm-flowcytometry@medicina.ulisboa.pt](mailto:imm-flowcytometry@medicina.ulisboa.pt)**

- A training session on a cell analyzer will be scheduled depending on the equipment availability and the planned date of your first experiment. Ideally, you should get trained on the same day or a few days before your first usage. The training duration will depend on your know-how and previous expertise. The training can be performed with a small group of users or include one-on-one tutorials on flow cytometry.
- After training, you will be given permission to use the iMM online booking system. If you are not an iMM user, we will create a user account for you and will ask you for affiliation information.
- For the first few sessions, we strongly recommend working during facility hours (9am to 6pm on week days) so that we can answer your questions as they arise. You should request assistance on the booking system and arrange for it with the Facility staff. We are available to help users within the facility schedule (9am to 6pm on week days), and will guide you through the acquisition protocol until you become an independent user.

### Rules for analyzer usage

- Book for yourself and not for others.
- You must have a valid reservation in the booking system before using an analyzer.
- The analyzers can be booked **up to 2 months in advance**.
- You must **include 15 min** in your booked time for the **cleaning protocol**.
- Users can book unlimited slots on the analyzers, but **can only cancel up to 3 hours before the slot starting time**.

- Last minute cancelations (within 3h before the starting booked time) must be requested by phone or email and may result in a penalization. The **penalty charge** corresponds to the running cost of the system, if the session is not booked by another user.
- Always record start and finishing times on the log sheet (cleaning time included). Also record if you detect any problems or if the user before you did not leave the cytometer in appropriate conditions.
- If you swapped a filter, you must replace it and leave the system with the default configuration.
- You must log out from the analyzer software and log off from Windows at the end of your session.
- If you are the **last user booked** or if the analyzer **will not be used for at least 3h**, you must **switch off** the system. If the system is not switched OFF at night, the last user booked will be charged the number of hours the system was left ON. The Facility staff will change your booking time to comprise the full time the system was ON.
- You must always export your data and delete it from the analyzer workstation regularly.
- Always clean the system following the cleaning protocol. If you are using a BD LSRFortessa or the BD LSRFortessa X-20, you must **record the cleaning steps** in FACSDiva.
- Always **check if the waste reservoir is full** (empty it if close or above to the limit line) and **refill the sheath tank** with PBS+Azide at the end of your session.



**Failure to comply with these rules will result in penalty charges and/or user banning**

### **Usage Log on BD LSRFortessa, BD LSRFortessa X-20 and BD Accuri C6 software**

Each user is given an individual login and password for FACSDiva and Accuri C6 software. This will enable us to track instrument usage per user and adjust billing charges. It is mandatory to **logout from FACSDiva and Accuri** software at the end of your session. We will use the software log records to calculate the monthly billing and you may incur in extra charges if do not log out. Users will be billed according to the actual starting time or booked starting time (whichever occurs first) and the actual ending time or booked ending time (whichever occurs last).

### **Data management on analyzers**

The Flow Cytometry staff is not responsible for data handling and storage

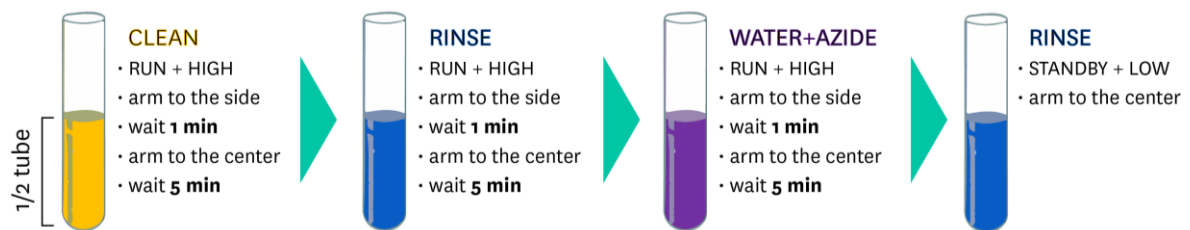
- Users **must always export** their data **and delete** it from the database. Do not book for data transfer or data analysis, otherwise you will be charged. If you only use the computer, check with an X the “Diva only” box in the log sheet. To analyze your data you can also use the PC located in room P2A32 or the MacFlow workstation (both are free of charge).
- Keeping data in the acquisition software may result in database problems which can only be solved by restoring the database with an empty one, **resulting in ALL DATA being deleted.**
- **Data transfer using USB connected devices is prohibited due to the high risk of virus or malware contamination.**

### Cleaning Protocol

You must always clean the machines following the cleaning protocol.

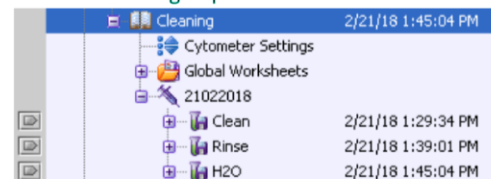
For BD LSRfortessa and LSRFortessa X-20:

- Prepare new **CLEAN**, **RINSE** and **WATER+AZIDE** tubes (don't fill them more than half volume)
- Start the **cleaning procedure** and **record the cleaning steps** in FACSDiva:



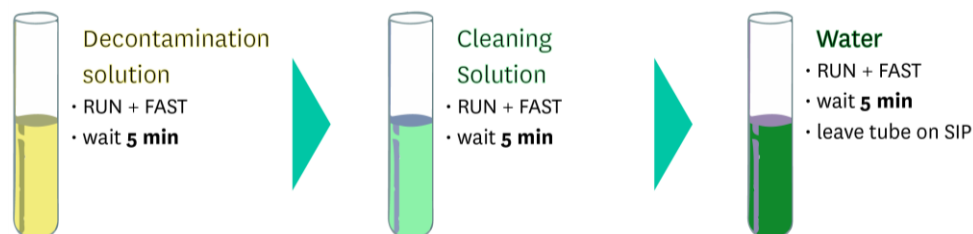
- Log off from FACSDiva and Windows
- Refill the sheath tank with PBS + Azide
- Empty the waste reservoir (if above the limit line)
- Follow the instructions on the desktop

record the cleaning steps



For BD Accuri C6:

- After acquiring your data, please **export it** to the server and **delete it** from the computer
- Start the **cleaning procedure**:



- Quit Accuri and **Log off** from Windows

We clean the machines every morning and perform weekly cleans, but this will not be enough if you don't clean after using the instruments. Make sure you do not leave the sheath empty and the waste full for the next user. Leave the workspace clean and ready for the next user. Remove anything that does not belong to the core facility, including used gloves, pipettes, tip boxes or FACS tubes.

In case of technical problems or necessary maintenance, resources will be blocked on the booking calendar by core facility staff as quickly as possible and users with conflicting reservations will be notified.

## Cell Sorters

BD FACSAria III  
BD FACSAria IIu

### Rules for sorter usage

- Users must contact the Flow Cytometry staff or use the online booking system to request access to the cell sorters booking. External users should contact us by email:

**[imm-flowcytometry@medicina.ulisboa.pt](mailto:imm-flowcytometry@medicina.ulisboa.pt)**

- Book for yourself and not for others.
- Users must have a valid reservation on the booking system for sorting. If you agree to swap you sort with a colleague, this must be updated in the booking system.
- The cell sorters can be booked **up to 2 months in advance**, between 10am and 6pm.
- If you need to book a sorter outside the regular working hours, please contact us beforehand to check for staff availability.
- When booking, **include time for new settings, purity checks and cleaning** (minimum 30 min).
- Please **contact us if you make a last-minute reservation on the booking system**.
- Always mention in the booking comments which nozzle (70 µm, 85 µm or 100 µm) and collection devices: tubes (1.5 mL, 5 mL FACS or 15 mL Falcon tubes) or plates (6-well, 24-well, 96-well or 384-well plate) you will be using.

- Sorter cancellations can be made **up to 7 days in advance**. Cancellations (within 6 days in advance) may result in a penalization. The **penalty charge** corresponds to the running cost of the system if the session is not booked by another user.
- **No shows** will be charged the running cost of the system for the duration of the booked slot.
- If you are late for a sort, let us know as soon as possible. If the sorter is already switched on when you notify us of the delay, you will be charged according to the booking start time.

### Sample Preparation

- If you are new to sorting, talk to us beforehand so we can help you plan your experiment.
- Come to the Flow Cytometry Facility fully prepared with
  - Controls (unstained sample and single color controls)
  - Samples (on ice if appropriate)
  - Collection devices (tubes or well-plates) with 1/3 of medium
  - Extra media for sample dilution
- Always **filter your cells** immediately before sorting. The mesh size should be half the size of the nozzle (for most users, 40 µm mesh BD Falcon cell strainers ref. 352340).
- For collection tubes, you should use polypropylene (Falcon 5 mL 12x75 mm ref. 35-2063; BD Falcon conical tubes 15 mL ref. 35-2096 or 1.5 mL polypropylene tubes)
- BSL-2 unfixed cells may only be sorted in the BD FACSAria III sorter with aerosol containment.
- The BD FACSAria IIu can only sort BSL-1 and fixed BSL-2 material.

### Communication with the Flow Cytometry staff

- Talk to us: before, during and after. We are here to help!
- Use the email [imm-flowcytometry@medicina.ulisboa.pt](mailto:imm-flowcytometry@medicina.ulisboa.pt) for cancellations, questions, suggestions, complaints, etc.