

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

AUTHORS: Andreia Santos
Rute Gonçalves
Mariana Fernandes

APPROVAL: Mariana Fernandes

Cell Analyzers

BD Accuri C6 Plus
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`

- 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 the training, you will be kept as an “in training” user on the Agendo booking system for the next few sessions and will be given full permission as soon as you are autonomous using the cytometers. If you are not an iMM user, you need to create an Agendo account (<https://imm.agendoscience.com/>).
- For the first few sessions, we strongly recommend working during facility hours (9am to 6pm on week days) so that we can be present to answer your questions as they arise and 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**.
- If you are the first user of the day, you must clean the machine before acquiring.

- If you will need help during acquisition you can ask for assistance while booking your slot on the Agendo booking system. Assistance requests for the analysers work as pre-reservations and require confirmation from the UCF according to staff availability. Therefore, make your reservations in advance to ensure that one of the staff members is available. If there are already two sortings booked, it may happen that nobody is available at that time.
- Do not book assistances during lunchtime. From 12:00 to 14:00 we will not give assistances.
- Users can book unlimited slots on the analysers, 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.
- For *BD LSRFortessas* and *Amnis ImageStreamX*: 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.

For *BD Accuri C6 Plus*: Weekly, UCF Staff always turn OFF the system at 6pm, if there is no one booked afterwards. Thus, **during weekdays from 9am to 6pm** you do not need to turn it OFF. However, **weekly after 6pm** or on **weekends**, 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 systems are 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 systems following the cleaning protocol. You must **record the cleaning steps** in FACSDiva (If you are using BD LSRFortessas) or in Accuri C6 Plus Software (If you are using BD Accuri C6).

- On *BD LSRFortessa*s: Always **refill the sheath tank** with PBS+Azide. If during your acquisition, **the waste container gets full**, a high pitch sound will go off. You should disconnect the black sensor (the noise will stop). As soon as possible, put the cytometer in **Standby**, and **disconnect the orange connector**. Close the container with the red cap that is on top of it and replace the container with a new one.

On *BD Accuri C6*: Always **check if the waste reservoir is full** (empty it if close) and if necessary, **refill the sheath tank with H₂O** at the end of your session.

On *Amnis ImageStreamX*: **Check the beads and the buffer containers** level and ensure the **waste tank is empty**.



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 Plus software

Each user is given an individual login and password for FACSDiva and Accuri C6 Plus software. This will enable us to track instrument usage per user and adjust billing charges. It is mandatory to **logout from FACSDiva and Accuri C6 Plus** 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 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 Protocols

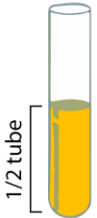
On weekdays, we clean the machines every morning at 9pm. However, on weekends and bank holidays, **if you are the first user of the day and the machine is OFF**, you must clean the machine before your acquisition.

For BD LSRFortessa 1 and 2:

Are you starting up? (First user of the day)


 **Please don't prime**

Turn on the Cytometer, the FFSS and the Computer
Login on Windows and FACSDiva
Follow the **startup cleaning procedure**:



CLEAN
RUN + HIGH
arm to the center
wait 10 min

➔




WATER+AZIDE
RUN + HIGH
arm to the center
wait 10 min

You are ready to run your samples!

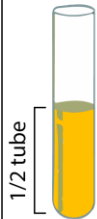
Thank you!

For BD LSRFortessa X20:

Are you starting up? (First user of the day)


 **Please don't prime**

Turn on the Cytometer and the Computer
Login on Windows and FACSDiva
Follow the **startup cleaning procedure**:



CLEAN
RUN + HIGH
arm to the center
wait 10 min

➔



WATER+AZIDE
RUN + HIGH
arm to the center
wait 10 min


You are ready to run your samples!

Thank you!

For BD Accuri C6 Plus:

Are you starting up? (First user of the day)

Turn on the Cytometer and the Computer
Login on Windows and Accuri C6 Plus software
Follow the startup **cleaning procedure**:



diH₂O
RUN + FAST
wait 15 min

You are ready to run your samples!

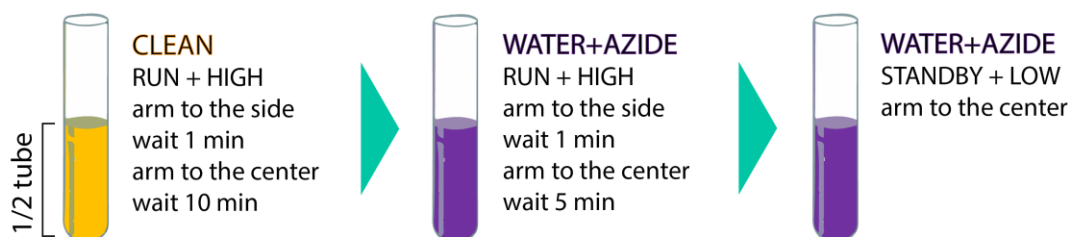
Thank you!

After your acquisitions, **you must always clean the system**, with the following protocols

For BD LSRFortessa and LSRFortessa X-20:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer
Prepare new **CLEAN** and **WATER+AZIDE** tubes
Start the **cleaning procedure** and **record the cleaning steps** in FACSDiva:



Log off from FACSDiva and Windows
Refill the sheat tank with PBS + Azide
Replace the waste reservoir, in case it gets full (sound bip)

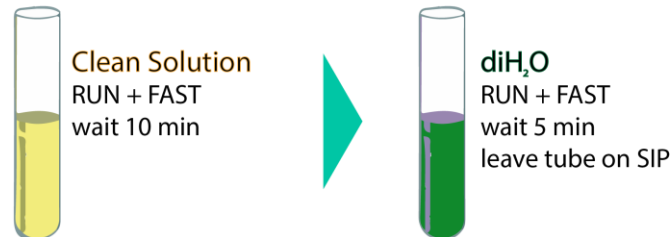


Thank you!

For BD Accuri C6 Plus:

Are you Done?

After acquiring your data, please **export it** to the server and **delete it** from the computer
Start the **cleaning procedure** and **record the cleaning steps** in Accuri C6 Plus:



Quit from Accuri C6 Plus and **Log off** from Windows
Refill the sheath tank with diH₂O
Empty the waste reservoir, in case it is halfway full

Thank you!

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

- **The sorters can only be operated by Flow Cytometry Facility Staff.**
- 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

- Book for yourself and not for others.
- Reservations for the cell sorters work as pre-reservations and require confirmation from the UCF according to staff availability. Therefore, make your reservations in advance to ensure that one of the staff members is available. If there is already a sorting and a training session booked, it may happen that nobody is available at that time.
- 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**.
- 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.

Important Informations and Communication with the Flow Cytometry staff

If you want to know **more about our Facility**;

If you want to use our cytometers and you want to know **How to Prepare your samples**;

If you want to know **What to expect when sorting**;

Come and visit our Wiki

imm.medicina.ulisboa.pt/facility/flowcytometry

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