

RVC Flow Cytometry Core Facility Rules and Guidelines

The RVC Flow Cytometry Core Facility is a resource for numerous researchers and clinicians.

As with all laboratories, eating and drinking is not allowed in the flow cytometry laboratory.

If you have questions or requests, please email hharris@rvc.ac.uk.

LSRFortessa™ X-20

- To gain access to the self-service LSR Fortessa™ X-20 in the RVC Flow Cytometry Core Facility
 1. Complete an [application](#) and forward it to hharris@rvc.ac.uk
 2. Complete training to a competent standard - minimum one assisted training session and one solo attempt and up to two attempts at each. All training session details are recorded in Flow Cytometry Training Register that monitors user experience level.
 3. Once training is completed, you will gain access the online SharePoint booking system. For any technical support required during your slot, please email hharris@rvc.ac.uk.
- Availability is scheduled on a first-booked-first-serve basis
- The self-service instruments are available during normal working hours i.e 08:00 to 18:00, unless they have been booked for servicing and repairs. Users found to gain access out of normal working hours or on colleagues' ID cards will have their access rights suspended until this situation is corrected and their PI will be notified
- Users are responsible for performing QCs on the instruments on a daily basis. CS&T must be performed by the first user each week. Thereafter 8 peak beads must be recorded at for tractability, recorded by each user as evidence of the equipment performance on day of use. Make a note in the appropriate equipment logbook.
- Do not book more than 4 hours at a time. Please discuss exceptional booking needs exceeding this limit in advance with the RVC Flow Cytometry Core Facility Manager by email hharris@rvc.ac.uk
- Users who do not arrive within 30 minutes of their start time forfeit the booking. Anyone that makes use of the cytometers after this 30-minute grace period can do so without being asked to leave
- If you are unable to attend your booking you **must** delete the booking from the calendar no later than 1 hours before it is scheduled to begin. After this deadline, the scheduled time can be billed in full
- The booking system will be reviewed regularly to ensure smooth transition from one user to another throughout a day, with no delays.
- Only samples of Biosafety Level of 2 or below can be analysed or sorted in the RVC Flow Cytometry Core Facility. Agents above Biological Safety Level 2 must be fixed to inactivate the infectious agent and inform the RVC Flow Cytometry Core Facility Manger (hharris@rvc.ac.uk) before running these sample on the cytometers. Failure to do so will result in immediate termination of all access rights
- Bring all samples to the laboratory in a secondary containment with securely fitted lid
- Due to limited capacity the RVC Flow Cytometry Core Facility cannot refrigerate user samples for longer than the stay in the laboratory
- The flow cytometry core does not provide standard consumables such as FACS tubes, antibodies, 96 well plates etc., these are to be brought by the user
- Check if you are the last booked user of the day. On any instrument the last user is responsible for shutdown. If you have booked the equipment as last user but no longer

require use, communicate that clearly with the previous user or attend to complete the shutdown only.

- Save your data using a relevant naming procedure so that anyone at any time can understand what the experiment was for. This is best performed by appropriately naming your experiment in DIVA as you start.
- Data is stored on the equipment computer during acquisition. At the end of your booking, immediately transfer this data to a networked drive to a location that your PI has advised.
- Data on the hard disk will be removed during regular database maintenance without notification, ensure you have exported your data on the day of use.
- Internet browsing and the use of USB sticks or external hard drives for data transfer on the instrument workstations is not allowed to reduce the risk of virus infections.
- Users are responsible to pay the agreed fees for all services. If payment is outstanding for more than 45 days, the user account will be suspended until payment is received in full.
- Users must inform the RVC Flow Cytometry Core Facility in writing to hharris@rvc.ac.uk two months before any grant code is due to expire and provide a new valid funding source.
- Users must notify the RVC Flow Cytometry Core Facility if their lab affiliation changes. This ensures correct invoicing.

Nikon Ti2 Inverted Fluorescence Microscope

- To gain access to the self-service Nikon microscope in the RVC Flow Cytometry Core Facility
 1. Complete an [application](#) and forward it to hharris@rvc.ac.uk
 2. Complete training to competent standard - minimum one assisted training session and one solo attempt.
 3. Once training is completed, you will gain access the Outlook Microscope Booking Calendar.
- For any technical support required during your slot, please email hharris@rvc.ac.uk.
- Availability is scheduled on a first-booked-first-served basis
- Self-service instruments are available during normal working hours i.e 08:00 to 18:00, unless they have been booked for servicing and repairs. Users found to gain access out of normal working hours or on colleagues' ID cards will have their access rights suspended until this situation is corrected and their PI will be notified
- Do not book more than 4 hours at a time. Please discuss exceptional booking needs exceeding this limit in advance with the RVC Flow Cytometry Core Facility Manager by email hharris@rvc.ac.uk, e.g. for time-lapse incubation imaging
- Users who do not arrive within 30 minutes of their start time forfeit the booking. Anyone that makes use of the cytometers after this 30-minute grace period and may not be asked to leave
- If you are unable to attend your booking you **must** delete the booking from the calendar no later than 1 hours before it is scheduled to begin. After this deadline, the scheduled time can be billed in full
- The booking system will be reviewed regularly to ensure smooth transition from one user to another throughout a day, with no delays.
- Only samples of Biosafety Level of 2 or below can be imaged in the RVC Flow Cytometry Core Facility. Agents above Biological Safety Level 2 must be fixed to inactivate the infectious agent. Failure to do so will result in immediate termination of all access rights
- Bring all samples to the laboratory in a secondary containment with securely fitted lid
- At the end of your session remove all samples and belongings from the microscope area and ensure it is clean and tidy
- Replace all sample holding brackets to their storage location at the end of every session
- Due to limited capacity the RVC Flow Cytometry Core Facility cannot refrigerate user samples for longer than the stay in the laboratory
- Data can be stored on the equipment computer during acquisition. At the end of your booking, immediately transfer this data to a networked drive to a location that your PI has advised.

- Internet browsing and the use of USB sticks or external hard drives for data transfer on the instrument workstations is not allowed to reduce the risk of virus infections.
- Save your data using a relevant naming procedure so that anyone at any time can understand what the experiment was for
- Data on the hard disk will be removed during regular database maintenance without notification, ensure you have exported your data on the day of use
- Users are responsible to pay the agreed fees for all services. If payment is outstanding for more than 45 days, the user account will be suspended until payment is received in full
- Users must inform the RVC Flow Cytometry Core Facility in writing to hharris@rvc.ac.uk two months before any grant code is due to expire and provide a new valid funding source
- Users must notify the RVC Flow Cytometry Core Facility if their lab affiliation changes. This ensures correct invoicing.
- If a problem occurs with any of the instruments, please seek assistance first from the RVC Flow Cytometry Core Facility Manager by phone using the speed dial option in the laboratory telephones. Alternatively send an email to hharris@rvc.ac.uk to notify them of the problem and record the issue in the appropriate Microscope Logbook to notify other users

FACS AriaFusion™

- Setup of cell sorters is a service provided by trained facility operators only.
- To gain access to the AriaFusion™ sorter:
 1. Complete an [application](#) and forward it to hharris@rvc.ac.uk
 2. Before any first time run of an operator-run service please schedule a consultation session with hharris@rvc.ac.uk to discuss details of any potential optimisation steps
 3. To book time on facility operated platforms, please email hharris@rvc.ac.uk to arrange a mutually acceptable time. You will receive an outlook appointment as confirmation. The SharePoint booking calendar will be controlled by Facility trained operators only.
- The Fusion instrument is available during normal working hours i.e 09:00 to 17:00, unless they have been booked for servicing and repairs.
- Access to the Fusion sorter for any self-service is restricted to users that have a certificate of participation in Advanced BD FACSAria™ Operator Course
- Users will be notified immediately via email of any equipment break downs that affects their booking. A service contract is held for all instruments and will be repaired as soon as possible. No charges will apply if an unexpected break down affects an allotted sort booking.
- At least 48hours before a scheduled sort, if you need to cancel email to hharris@rvc.ac.uk otherwise you will be charged, unless the facility can fill the vacancy with another sort. Users will be informed if we could fill the opening.
- The Fusion sorter uses sterile FACSFlow (1X PBS with sodium azide preservative) as sheath fluid. If beneficial to their cell type users can request a different sheath fluid. The user will supply a minimum of 10L of alternative sterile sheath fluid at least 2 days before their sort. To change the sheath fluid an additional fee of £100 will incur to cover the cleaning and refilling preparation of the sheath tank.
- Users that wish to process clinical samples that have unpredictable schedules are urged to discuss their needs with the RVC Flow Cytometry Core Facility Manager hharris@rvc.ac.uk before their project begins to arrange the availability of trained facility staff.
- Due to limited capacity the RVC Flow Cytometry Core Facility cannot refrigerate user samples for longer than the stay in the laboratory
- Users are responsible to pay the agreed fees for all services. If payment is outstanding for more than 45 days, the user account will be suspended until payment is received in full.
- Users must inform the RVC Flow Cytometry Core Facility in writing to hharris@rvc.ac.uk two months before any grant code is due to expire and provide a new valid funding source.
- Users must notify the RVC Flow Cytometry Core Facility if their lab affiliation changes. This ensures correct invoicing.

Standard Maintenance Rules for All Flow Cytometry Users

- FACS tubes containing cleaning fluids must be at the beginning and end of each experiment, users **must** record this information in the appropriate FACS logbook associated with each equipment.
- If users do not follow cleaning procedures regularly they may have their access rights suspended for a period of time. The core reserves the right to charge £50 for cleaning an instrument if it is found dirty for the next person.
- During regular business hours (Monday to Friday 09:00 to 17:00) users must check for any other bookings and if other users are booked after you set instruments on standby after your recorded cleaning steps, leaving the waste connected. Make a note in the appropriate logbook shutdown comments that the machine is on standby. Otherwise complete the shutdown fully.
- Users that fail to shut down the instruments when they are the last user will be billed the hourly rate of the equipment between their log-off until 09:00 on the following workday. Their PI will be notified.
- Do not allow FACS tubes to run dry. This introduces air bubbles into the system which can be time consuming and difficult to clear out.
- Ensure the bench space around the cytometers is clean and clear of any spills. Remove any discard tubes, tips, etc. back to their origin lab or into the biohazard waste containers. Non-biohazard waste is collected in regular waste bins.
- If a problem occurs with any of the instruments, please seek assistance first from the RVC Flow Cytometry Core Facility Manager by phone using the speed dial option in the laboratory telephones. Alternatively send an email to hharris@rvc.ac.uk to notify them of the problem and record the issue in the appropriate FACS Logbook to notify other users.