**Title:  FACSAccuri Start-up, Shut-Down and Cleaning**

**Effective Date:**
**Approvals** (Signature and Date):

Author : Panagiota Stamou          Technical Authority (Signature)          Date: 24/05/2017

## 1.0     PURPOSE

To correctly Book, start-up, shut-down and maintain the FACSAccuri Flow Cytometer.

## 2.0     SCOPE

Correct start-up and shut-down knowledge is required by all users.
There is a cleaning procedure which is used after each run & a monthly cleaning procedure also.

## 3.0     RESPONSIBILITY

- Only registered researchers or students that have been trained by a member of facility staff, have signed the user licence agreement and instrument SOP may operate the instrumentation.  Registered and trained researchers and students are allowed to operate the flow platform instrument unsupervised only after approval by the Flow platform manager.
- To register with APC Flow Cytometry platform you need to send your details: Name, Laoratory, Contact details (email address) to Dr. Panagiota Stamou, E-mail: panagiota.stamou@ucc.ie, Phone: +35321490 1395
- All persons using the flow cytometer must follow the correct start-up and shut-down procedure as shown in this SOP.
- Exchanging sheath and waste canisters is part of operating a facility owned analyzer. Every user is expected to refresh sheath fluid if it is running empty and to exchange waste tanks either when they are 50% full or when they shut down the instrument as last user.
- Users should treat the analyzers with care when operating the system and loading samples. If you are in doubt of your operating procedures, please contact the staff and you will be shown proper use and procedure.
- Users are required to export and delete their experiment data form the Flow cytometer analyser equipment computer harddrive immediately after recording their experiments. Users can check the integrity of their data transfer before deleting the experiment form the database, but no more than a month must pass between recording and deletion.

- The facility takes no responsibility for the maintenance and physical integrity/storage of any user's data. Users are required to back up their data immediately after their analysis on their own groups secure server and to follow the above stated guidelines for data deletion and transfer at all times.
- Instrument can be booked on line via **iLab Operations Software** after user training.
- The instrument is user operated.
- There is a charging fee scheme for the machine as follows:

| SERVICE | EQUIPMENT | | APC Internal | UCC Departments Schools | Industry |
|---|---|---|---|---|---|
| FACS ANALYSIS (User Operated) | BD Accuri ANALYSER | Usage Charge per hour* | No Charge# | € 15 | € 30 |
| * FACS SHEATH, CS&T calibration beads and monthly maintenance consumables (FACS Clean, FACS Rinse) are included in the defined charge | | | | | |
| # APC users are required to provide their own stock of SHEATH FLUID | | | | | |

- Bookings of the instruments are not to be changed within a one working day period prior to the actual appointment. Booking durations are set within one working day period and can only be changed or modified in agreement with the Flow cytometry platform manager. Faliure to show up for a booked appointment without canelling (1 day earlier) 24 hours in advance will result in full charge for the duration of the booking.

## 4.0    REFERENCES AND APPLICABLE DOCUMENTS

- There is a laminated user's guide located next to the flow cytometer lab, Room 5.19. as well as a typed version attached to the front panel of the FACSAccuri.
- There is BD_FACSAccuri_instructions pdf file on the desktop folder of BD_FACSCalibur Computer

## 5.0    MATERIALS AND EQUIPMENT

Materials & Equipment provided by the APC Flow Cytometry Platform:
    FACSAccuri flow cytometer
    Decontamination Solution
    Cleaning Solution
    Distilled Water
Materials & Equipment provided by the registered users:
    BD Falcon flow cytometry tubes

    PPE (Lab Coat, gloves, eye protection)

| Instrument | Description/Availability |
|---|---|
| **BD FACS Accuri** FACS analyzer | Software: BD Accuri C6 Plus Software<br><br>Excitation lasers: 488 nm, 640 nm<br><br>Emission Detection<br>4 colors, Standard set installed:<br>• FL1 533/30 nm (eg, FITC/GFP)<br>• FL2 585/40 nm (eg, PE/PI)<br>• FL3 >670 nm (eg, PerCP, PerCP-Cy5.5, PE-Cy™7)<br>• FL4 675/25 nm (eg, APC)<br><br>Service: Instrument is cleaned thoroughly every month.<br><br>Specialty: This instrument can be used for up to 4 fluorescent parameters plus FSC, SSC.<br><br>Availability: Instrument can be booked on via **iLab Operations Software** after user training. The instrument is user operated. |

## 6.0 PROCEDURE

Daily FACSAccuri Start-up Procedure- Running Protocol

1. Open software and Switch on flow cytometer Wait for the indication the t the cytometer is ready. Follow the instructions to empty waste ontainer and refill buffers as required.
2. Place an empty flow tube on the SIP.
3. Click Backflush.
4. Replace Flow tube with a clean tube which contains 2 ml of deionized water (Do not use ordinary tap water) run for 2 minutes. If the number of events >20/sec proceed to cleaning protocol before starting the experiment.
5. Samples should be in running buffer containing the following ( 500ml PBS, 2.5gBSA, 1ml 0.5M EDTA)
6. Remove the tube of water and run samples at a medium flow rate to minimize clogging.
7. In the case of extended sampling times repeat steps 2-5 at a number of time intervals to ensure minimum build up of cells and clogging.

Daily FACSAccuri Shut-Down Procedure- Compalsory cleaning protocol

1. Open the file "Record Sheet for Cytometer Cleaning" on the desktop.
2. Place a tube of 2 ml of cleaning solution on the SIP.
3. Click on well A1 and set time limit for 6 min and set flow rate to fast.
4. Then Backflush.
5. Place a tube of 2 ml Sheath fluid on the SIP.
6. Click on well A2 and set time limit for 3 min and set flow rate to fast.
7. Then Backflush.
8. Check events per second. If events/sec> 20/sec then repeat steps 2-7 until this level is achieved. Allways follow a clean cycle with sheath fluid step.
9. In the case aof a severe clogging replace cleaning solution with decontamination solution and repeat steps 2-7.
10. Place a tube of 2 ml of deionized water on the SIP.
11. Click on A3 and set time limit for 2 min and set flow rate to fast.
12. Leave the tube on SIP and save the file with users name and date.
13. Switch off instrument.

## 7.0    HEALTH AND SAFETY CONSIDERATIONS

- Appropriate protective measures should be taken at all times (i.e. Gloves & lab coat). All used BD Falcon cytometry tubes and plates as well as gloves should be disposed of in an autoclave bag located in the main lab.

- All samples with biological safety category 2 or higher that have no approval from Health and Safety for live measurements must be fixed prior to entering the facility and running the analysis.

- All users must have prepared the samples for analysis at their laboratory bench outside the 5.19 room and all samples mut be carried at the 5.19 laboratory in tubes with lid in an appropriate container.

- All spillages and contaminated areas must be disinfected with 1.0% Virkon and be reported to the flow cytometry platform manager.

## 8.0      DOCUMENTATION REQUIREMENTS

- All users must make a booking and also write the booking via **iLab Operations Software** to use the machine. All users must ALSO document usage time in the LogBook (located within the flow cytometry room 5.19).

- Users must also document the performance of the equipment after each run taking care to record any problems. Please inform **Pepi Stamou** panagiota.stamou@ucc.ie of any problems.


Date:                   User Signature:


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