

Core Equipment ID: 3537

Description: BD Accuri C6

Room: IQ Building, Rm 2521

Champion: Matthew Bernard

1.0 Purpose

Standardize the process of control, maintenance, and ownership of the BD Accuri C6 instrument located in IQ Building Room 2521.

1.1 BD Accuri C6 Capabilities

The BD Accuri C6 is a compact flow cytometer that uses a low-pressure pumping system to drive the fluidics allowing for the derivation of sample volume and calculation of absolute counts or sample concentration per microliter. The flow cytometer is capable of running up to 10,000 events per second at sample concentrations $> 5 \times 10^6$ cells/mL. It is equipped with 2 lasers: a blue 488nm (3 detectors + FSC/SSC; FL1 [530±15 nm], FL2 [585±20 nm], FL3 [>670 nm]) and a red 640nm (1 detector; FL-4 [675±12.5 nm]). The optical configuration allows multi-parameter detection of up to 4 fluorescent parameters and 2 light scatter parameters. Optical filters allow for detection of commonly used fluorescent markers, including, but not limited to:

FL1- FITC, AF488, GFP, CFSE, YFP

FL2- PE, PI

FL3- PerCP, PerCP-Cy5.5, PE-Cy7, RFP, mCherry

FL4- APC, AF647, APC-Cy7, APC-H7

1.2 CFlow Plus v1.0.227.04 Software Capabilities

BD Accuri C6 Software (CFlow Plus) controls the BD Accuri C6 flow cytometer system in order to acquire data, generate statistics, and analyze results.

BD Accuri C6 Software provides the following features:

- a. Tabbed views for collection, analysis, and statistics
- b. Digital signal processing and color compensation at any time
- c. Drag and drop plots
- d. Export of files in FCS 3.0 format
- e. Seamless data file importation into FCS Express
- f. Enhanced Analysis upgrade adds:
 - i Drag and drop of publication-quality images, event coloring, live gating
 - ii Batch analysis of sample data

2.0 Reason for Issue

Maintain a document that describes the Standard Operating Procedures that allow for the standard safe and optimal use of the BD Accuri C6 flow cytometer within the MSU Flow Cytometry Core Facility.

3.0 Process Description

Allow Core Facility Users to properly and effectively use the BD Accuri C6 flow cytometer. The process description details the standard use of the BD Accuri C6 flow cytometer. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

- 3.1 SOP: 3537.2521.001 for BD Accuri C6 flow cytometer, authored by Matthew Bernard, created on 10/04/2017, issued on 12/14/2017.
- 3.2 SOP 3537.2521.002 amendment, authored by Matthew Bernard, on 3/31/2020, issued on 4/10/2020.
- 3.3 SOP 3537.2521.003 amendment, authored by Matthew Bernard, on 2/16/2024, issued on 11/01/2024. Appendices I, II, and III have been removed, as Biosafety Questionnaire, User Log, and Equipment Maintenance log are no longer needed due to the online Biosafety Questionnaire, the iLab system scheduling, and the electronic [Equipment Maintenance Log](#), respectively. Hazardous waste disposal procedure updated.
- 3.4 SOP: 3537.2521 applies to any User and / or Trainer of the BD Accuri C6.
- 3.5 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the BD Accuri C6 flow cytometer. All Users are responsible for the proper use, according to defined protocols, when using the BD Accuri C6 flow cytometer
 - a. **New Users** need a Windows user account created for equipment access, before initial use. New accounts are authorized and created by the Equipment Champion and / or the Core Facility Staff. A new account may be created after training and equipment approval has occurred.
 - b. **All Users are expected to have completed EHS training programs Bloodborne Pathogens and Biosafety Principles, as required for respective research projects.**
 - c. **All Users are required to complete a Biosafety Questionnaire for each cell type to be analyzed prior to use of the scheduling use of instrumentation in the facility.**
 - d. **All Users** must schedule equipment using the iLab Solutions portal.
 - e. Only covered samples may enter Room 2521. Samples must be brought to the facility in a standard **spill control box/leak-proof secondary container** that will contain any multiple tube or plate spill, per EHS standards (see Section 4.7d). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm. Spill control boxes must be labeled with Biohazard identification for BSL-2 samples.

- f. Immediately after use, the BD Accuri C6 flow cytometer must be appropriately shut down (see Section 4.5).

3.6 Equipment Safety Issues

- a. **Safety Issues** – The Core Facility operates at up to BSL-2 plus. Biosafety level and limitations for this facility are restricted to WHO and NIH risk groups defined as:

Risk Group 1 – Agents that are not associated with disease in health adult humans (no or low individual or community risk).

Risk Group 2 – Agents that are associated with disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).

Examples of risk groups 1 and 2 which may be analyzed include: 1) Plasma or serum from non-primate animals; 2) cell supernatants from cell lines of ATCC origin and those tested negative for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV); 3) primary human serum or plasma if tested for HIV, HBV, HCV, and EBV; 4) Supernatants from primary human cells if tested for HIV, HBV, and HCV; 5) Supernatants from genetically modified cell lines using third generation lentivirus systems.

Research involving BSL-3 or BSL-4 requirements are not supported, which includes WHO and NIH risk groups 3 and 4.

- b. **BSL-2 samples are required to be fixed.** Please consult Core Staff for discussion of exemptions.
- c. **Decontamination of BD Accuri C6 post-operation:**
Following the Shutdown procedures (Section 4.5) will result in appropriate daily decontamination of the flow cytometer between uses.
- d. **Decontamination of work surfaces:**
External surfaces in front of the BD Accuri C6 can be cleaned with Envirocide (or equivalent) or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent).
- e. **Radioactively labeled samples are prohibited.**
- f. **Under normal operating conditions, the BD Accuri C6 cytometer does not create aerosols.**
- g. **All samples exposed to or infected with bacterial or viral agents must be approved by EH&S on a case-by-case basis. A related HURON Click must be submitted and approved by EH&S prior to scheduling analysis.**
- h. **Spill control:**
Samples must be brought to the facility in a standard spill control box that will contain any multiple tube or plate spill (see Section 4.7d). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm.

Report spills to the Core Facility staff.

In the event of a spill for BSL-2 samples, the spill should 1st be covered with absorbent paper towel, which will then be saturated with 10% bleach and allowed to soak a minimum of 10 minutes. The wet towel should be placed in a biohazard waste container after contact. The spill area will then be covered with 10% bleach, allowed to soak briefly, and then wiped up with an absorbent towel. After cleaning the spill, dispose of the absorbent material and gloves into a biohazard waste container. Squeeze bottles of 10% bleach are made fresh daily for spill control.

Report spills to Core Facility staff.

- i. Never place anything on top of the BD Accuri C6, including tube racks or kimwipes.

3.7 Laboratory Conditions

- a. IQ 2521 is a BSL-2 research lab with negative air pressure air flow. The lab door must be closed at all times. The room contains a sink for hand washing, germicidal soap, emergency eye wash station, and spill control kit/equipment.
- b. **Signage:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2521.
- c. **Access:** Access is limited to people with permission to run samples on the BD Accuri C6, which has been booked through the iLabs web portal. Only individuals involved in training exercises, running samples on the Luminex or other instrumentation in the room, or retrieving data should be in Rm 2521.
- d. **PPE Requirements:** Standard PPE must be used at all times, which includes gloved hands, long-sleeve lab coat over full and coverage shirt and pants, and full coverage shoes with intact soles.
- e. All samples will be handled with BSL-2 precautions, including proper handling, storage, transportation, disposal, and decontamination according to the MSU Biosafety Manual and BBP Exposure Control Plan.
- f. **Exposure Control Plan:** Please refer to the Exposure Control Plan available on the MSU EHS website for instructions regarding what to do in the event of an exposure. The MSU Exposure Response Procedure is posted in Rm 2521.
 - i. **Eye/Mucous Membrane Exposure:** Flush immediately at nearest eyewash station for 15 minutes.
Wounds/Needlesticks: Wash the area immediately, use warm water and sudsing soap to scrub the area for 15 minutes.
 - ii. Notify your supervisor immediately if he/she is available.
 - iii. Print Authorization to Invoice MSU Form to take to the care facility.
<https://www.hr.msu.edu/benefits/workers-comp/documents/InvoiceMSU.pdf>
 - iv. Report to a Lansing Urgent Care facility for post-exposure follow-up as soon as possible.
<https://www.lansingurgentcare.com/>

- v Be prepared to provide information about the agent or cells involved in the accident. Additionally, route of exposure, dose/concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

Note: Any required follow up visits must also take place at Lansing Urgent Care. The location in Frandor is open 24 hours.
- vi Follow up by completing the Report of Claimed Occupational Injury or Illness Form with your supervisor within 24 hours.
- g. Sample handling and decontamination within IQ Rm 2521 is covered in Section 3.6. All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to their lab. Waste containers are available for non-hazardous waste. A biological waste container for waste generated during a biohazard cleanup is available in the lab. **No needles are permitted in the Core Facility.**
- h. **Eating, drinking, or use of personal care products are prohibited in the facility.** Use of personal electronics will not be allowed if that use interferes with proper operation of the instrumentation in the facility. Those operating flow instrumentation in the facility must remove gloves and wash their hands before using any personal electronic device. Sani-Cloth Plus germicidal disposable wipes are available for wiping **keyboards and personal electronic devices if cross-contamination accidentally occurs.**
- i. **Medical:** Users of the facility should have all current vaccinations, including those for HepB. Anyone who may be immune-compromised should visit Occupational Health before working in the facility.

3.8 Contact Information

- a. **Matthew Bernard: Core Director**, Office, IQ Building, Rm 2315 (517)355-4076
- b. **Daniel Vocelle: Assistant Director**, Office, BPS Building, Rm 4198 (517)-355-1536
- c. **Environmental Health & Safety:** 517-355-1053
- d. **Occupational Health (University Physician's Office):** 517-353-8933
- e. **MSU Police:** 517-355-2221

3.9 Quality Measures

- a. Daily: When in use, run ddH₂O on the system to ensure the system is in proper working order before running samples. The event number should be <200 events over 2 minutes when the instrument is run on FAST. If the instrument fails this check, see Section 4.1g for subsequent recommended procedures.
- b. Approximately Monthly: A validation should be performed on the BD Accuri C6 flow cytometer. See Section 4.2 to perform these monthly quality assurance measures. Once a system check has been completed, the date, time and person who performed the validation, must be recorded in the [Equipment Maintenance Log](#).

4.0 Procedure: BD Accuri C6 Flow Cytometer Use

4.1 Startup

- a. Check fluid levels in all bottles, ensuring that the Waste tank is empty and the Sheath, Cleaner, and Decontamination bottles are full.
- b. Gently push the sample stage back, remove the current tube of ddH₂O (discard), and place a tube containing at least 2 mL of fresh ddH₂O on the SIP (sample injection port).
- c. Turn on in order: computer then cytometer.
- d. Log into the computer. Sign in under User Account Name and password, as appropriate.
- e. Click on the CFlow Plus desktop icon to start the software. This software runs the BD Accuri C6 flow cytometer.
- f. When the BD Accuri C6 Traffic Light turns green and BD Accuri C6 Software displays the message C6 is connected and ready, run ddH₂O for at least 2 minutes on FAST before processing samples.
- g. Check the number of events:
 - i *If there are <200 events processed within 2 minutes, proceed with analysis*
 - ii *If there are between 200-500 events processed in 2 minutes, repeat running ddH₂O on the instrument for 5 minutes on FAST until <100 events/min are observed. It may also help to run a few consecutive runs of 30 seconds on FAST.*
 - iii *If there are >600 events processed in 2 minutes, perform any of the following procedures to resolve the issue:*
 - 1 Run Cleaning (or decontamination solution) through the instrument for 2 minutes on FAST.
 - 2 Run “unclog” or “backflush” under the instrument pull-down menu (make sure you use a waste tube for these procedures). Purge air from the system by running ddH₂O for 5 minutes on FAST, but pause and resume run approximately every 30 seconds.
 - 3 Run the Cleaning Fluid Cycle (See Section 4.3c).
- h. Be sure to wipe the SIP with a kimwipe in between samples.

4.2 Validation

- a. Run 6-peak and 8-peak beads (Spherotech), CS&T, or equivalent approximately monthly to check validation.
- b. Place a tube with 2 mL of filtered, deionized water on the SIP.

- c. Enable the Run with the Limits radio button in the Instrument Control Panel. Enable the Time check box next to the Min and Sec fields in the Instrument Control Panel and type in a run time of 15 minutes.
- d. Set to run on FAST and click on the RUN button to rinse the SIP.
- e. Once the run is finished, click on the Delete Sample Data button to delete data collected during the rinse.
- f. Run 8-Peak Validation Beads:
Disable the Time check box next to the Min and Sec fields, enable the Events check box and enter 50,000 into the Events field.
- g. Select Ungated Sample from the associated drop-down list.
- h. Vortex a sample tube containing suspended 8-peak validation beads, prepared according to the package instructions. Place the tube on the SIP.
- i. Select the SLOW radio button in the Fluidics section of the Control Panel and click the RUN button to start acquisition.

NOTE: The R1 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- j. Name the sample by typing a name in the text box just above the Sample Grid. Include the date in the sample name to differentiate it from samples collected on other dates.
- k. Run 6-Peak Validation Beads:
Vortex a tube of suspended 6-peak validation beads, prepared according to the package instructions. Place the tube on the SIP.
- l. Select an Empty Well and verify that the check box by Events is still enabled and set at 50,000 and that Ungated Sample is still selected from the drop-down list.
- m. Click on the RUN button.

NOTE: The R2 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- n. Alternatively, run CS&T beads.
- o. Name the sample with a name that includes the date it was processed.
- p. Analyze the Validation Bead data according to the BD Accuri C6 Software User Guide (Section 4.7a). CS&T bead data can be analyzed based on prior runs, focusing on Median Fluorescence Intensity and %CV.
- q. Record Validation information in the [Equipment Maintenance Log](#).

4.3 Maintenance

- a. **Run Decontamination Fluid cycle at least every 2 weeks.** The BD Accuri C6 automatically runs the decontamination fluid cycle when the system is shut down

normally. The decontamination fluid cycle lasts about 15 minutes. To manually run the decontamination fluid cycle:

- i Place a tube with approximately 2mL of ddH₂O on the SIP.
- ii Select Instrument > Run decontamination fluid cycle.
- b. **Prepare fresh Cleaning Solution approximately every 2 weeks.**
- c. **Run Cleaning Fluid Cycle at least every 2 weeks.** The cleaning fluid cycle pulls cleaner fluid from the cleaner tank and runs it through the fluidic lines of the fluidics system. After filling the system with cleaner fluid, the cleaning fluid cycle purges the cytometer with fresh sheath fluid and performs a backflush. This cycle takes about five minutes.
 - i Place a tube with approximately 2mL of ddH₂O on the SIP.
 - ii Select Instrument > Run cleaning fluid cycle.
- d. **Run an Extended Flow Cell Clean approximately monthly.** During extended flow cell cleaning, the flow cell fills completely with a cleaning solution from the sample tube on the SIP. This cycle automatically shuts down the cytometer with cleaning reagent in the flow cell, allowing the flow cell to soak.
 - i Place a tube with at least 500 µL of Extended Flow Cell Clean Solution (PN 653159) on the SIP. Never run the Extended Clean of Flow Cell cycle without a tube containing at least 500 µL of fluid.
 - ii Select Instrument > Extended clean of flow cell.
 - iii After the cytometer is automatically shut down, allow the cytometer to rest for at least 30 minutes (up to overnight for a more thorough cleaning).
 - iv Replace the tube of Extended Flow Cell Clean Solution with a tube of approximately 2 mL of ddH₂O.
 - v Restart the cytometer. The cytometer performs a longer fluidics startup cycle and the Software displays the message "Extra startup time needed due to cleaning or improper shutdown". This longer cycle purges cleaning reagent from the flow cell.
 - vi Run ddH₂O for 10 minutes on FAST.
 - vii Operate the cytometer as usual.
- e. **Replace the in-line sheath filter approximately every 2-3 months.** If the cytometer is used daily, consider changing monthly. See BD Accuri C6 User's Manual for instructions.
- f. **Replace the peristaltic pump tubing approximately every 2-3 months.** See BD Accuri C6 User's Manual for instructions. Tubing comes in contact with biological samples and therefore should be considered hazardous. Wear gloves and appropriate PPE during this procedure.

- g. Record Maintenance in the [Equipment Maintenance Log](#).

4.4 Acquisition

Please refer to the BD Accuri C6 Software User Guide (Section 4.7a) for instructions on operating the CFlow Plus software and analyzing samples.

4.5 Shutdown

- a. Place a tube with approximately 2 mL of decontamination solution on the SIP.
- b. Select an empty data well in the Collect Tab of BD Accuri C6 CFlow Plus software.
- c. Set the time limit for two minutes and the fluidics speed to FAST.
- d. Click on the RUN button.
- e. Once the run is finished, remove the tube of decontamination solution from the SIP.
- f. Place a tube with approximately 2 mL of ddH₂O on the SIP and set a time limit for five minutes and fluidics to FAST.
- g. Click on the RUN button.
- h. When the run is finished, leave the tube on the SIP.
- i. Press the power button.
The shutdown cycle runs for approximately 15 minutes, then the cytometer automatically powers off. **Do not hold the power button down for more than 3 seconds**, as this will result in a hard shutdown, where the fluidics cycle fails to run.
- j. Shutdown the computer.
- k. Empty the waste tank into larger carboy where an appropriate amount of bleach can be added and pickup scheduled with EH&S. Ensure that appropriate PPE (e.g., lab coat, safety glasses, gloves) is utilized.
- l. Clean keyboard, mouse, and work surfaces in front of the BD Accuri C6 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent).

4.6 Records

- a. **Records of Use** – All BD Accuri C6 system use must be recorded. Refer to 3.4e.
- b. **Error Messages / System Issues** – All error messages and system issues must be relayed to the Equipment Champion and the Core Facility Staff and appropriately recorded, refer to 3.4e, on the same day as equipment use.

4.7 Resource Index

- a. BD Accuri C6 User Guide:
BD Accuri C6 flow cytometer and CFlow Plus software literature and resources for the following items can be found at the links below. Printed versions of these resources can also be found with the BD Accuri C6 flow cytometer in room 2521.

https://www.bdbiosciences.com/documents/BD_Accuri_C6Flow_Cyto_Instrument_Manual.pdf

- b. BD CFlow Plus v1.0.227.04 User Guide:
For detailed information about the functions, features, and use of BD CFlow Plus v1.0.227.04 software, see the BD CFlow Plus v1.0.227.04 Software User Manual, available at:

https://www.bdbiosciences.com/documents/BD_Accuri_C6_Software_User_Guide.pdf

- c. BD Biosciences Technical Support is available to users in the U.S. and Canada by calling 1-877-232-8995.

BD Biosciences Company Representative:

Timothy Stewart
Research Instrument Sales Specialist
2350 Qume Drive, San Jose, CA 95131-1807 USA
Cell: 724.494.9787 Tel: 800.451.4557 ext: 1017
E-mail: Timothy_Stewart@bd.com

- d. Transport of Biological Materials:
For detailed information about the transport of biological materials, see the EHS recommended procedures available at:

<https://ehs.msu.edu/lab-clinic/shipping/bio-transport-local-vehic.html>

5.0 Competencies, Authorization and Training

New Users must receive proper authorization from either the Equipment Champion and / or Core Facility Staff before equipment use. A new User may contact the Equipment Champion or Core Facility Staff to schedule training. Training includes SOP and flow cytometer familiarization and any additional required or specialized training. Once training is complete authorization may be issued and a system account and password may be set up. All Users are individually responsible for current SOP familiarization. All New Users must refer to 3.4a during new BD Accuri C6 flow cytometer account creation.

6.0 SOP Performance and Equipment Review

The effectiveness of the SOP: 3537.2521 will be monitored by the Core Facility Staff, Equipment Champion and All Users. Any procedural or qualitative deviations will be reflected within an updated SOP. Any Approved User should aptly report any procedural or qualitative issues and / or errors to the Core Facility Director/Manager or Equipment Champion. The Core Facility Staff and Equipment Champion's name and contact information can be found on the Pharmacology and Toxicology Core Laboratory in iLabs. Updated SOPs will be published and Approved Users will be notified. SOP: 3537.2521 reviews will occur every two years.

7.0 Definitions

- 8.0 **SOP:** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance, and ownership of the BD Accuri C6 flow cytometer. The SOP number stands for (xxx . xxx . xxx) equipment serial number. room number. SOP version number.

- 8.1 **Originator / Author:** The individual representing the MSU Flow Cytometry Core Facility that created SOP: 3537.2521
- 8.2 **Stakeholder:** Any individual that uses or performs the task of which is the subject of the SOP, including the MSU Flow Cytometry Core Facility Department.
- 8.3 **New User:** An individual who has not completed the requirements of section 3.5
- 8.4 **Approved User:** An individual who uses the BD Accuri C6 flow cytometer and has fulfilled section 3.5. This title may only be given by the Equipment Champion and / or the Core Facility Staff.
- 8.5 **Champion:** An individual whose direct expertise with the BD Accuri C6 instrument has been recognized by the MSU Flow Cytometry Core Facility Staff. This title may only be awarded by the MSU Flow Cytometry Core Facility Staff.


9.0 Approvals

The below signatures and dates are required for full SOP approval and implementation.

This SOP was written/authorized by:

Dr. Matthew Bernard  01Nov2024

This SOP was reviewed by:

Dr. Daniel Vocelle  13Nov2024

Issue Date: November 13th, 2024