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 flow cytometer located in IQ Building, Rm 2521.

1.1 **BD Accuri C6 Capabilities**

The BD Accuri C6 is a compact flow cytometer that uses a lowpressure 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 of over 5 x 10⁶ 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:

- Tabbed views for collection, analysis, and statistics
- Digital signal processing and color compensation at any time
- Drag and drop plots
- Export of files in FCS 3.0 format

- Seamless data file importation into FCS Express
- Enhanced Analysis upgrade adds:
- Drag and drop of publication-quality images, event coloring, live gating
- Batch analysis of sample data

2.0 Reason for Issue

Maintain a document that describes the Standard Operating Procedures that allows for the standard safe and optimal use of the BD Accuri C6 flow cytometer within the Pharmacology and Toxicology Core Facilities.

3.0 **Process Description**

Allow Core Facility Users within the Pharmacology and Toxicology Department 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: 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: 3537.2521.001 applies to any User and / or Trainer of the BD Accuri C6.
- 3.3 **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 protocol, 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 Manager. A new account maybe 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 must fill out Appendix I Questionnaire prior to use of the instrumentation in the facility.
 - **d. All Users** must schedule equipment using the iLab Solutions portal.

- e. Approved Users must record all equipment use in the Equipment Usage Logbook post-use on the same day as the recorded use. The Logbook is located on the desk next to the BD Accuri C6 flow cytometer. Within the Logbook on the current log sheet, Users must record the following: Date, PI, Name, Lab Location, Phone Number, Program, Sample Type, Error Messages, as appropriate (Appendix II).
- f. Only covered samples may enter Room 2521. Samples must be brought to facility in a standard <u>spill control box/leak-proof</u> <u>secondary container</u> that will contain any multiple tube or plate spill. 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.
- **g.** Immediately after use, the BD Accuri C6 flow cytometer must be appropriately shut down (see Section 4.5).

.4 Equipment Safety Issues



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 HIV, HCV, HBV, and 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. Decontamination of BD Accuri C6 post-operation:

Following the Shutdown procedures (Section 3.6.10) will result in appropriate daily decontamination of the flow cytometer between uses.

c. 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).

- **d.** BSL-2 samples should be fixed, when applicable.
- e. Radioactively labeled samples are prohibited.
- f. Under normal operating conditions, the BD Accuri C6 cytometer <u>does not</u> create aerosols.

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 BMR (Biological Materials Registration) form must be submitted and approved by EH&S prior to scheduling analysis.

Spill control:

h.

Samples must be brought to facility in a standard spill control box that will contain any multiple tube or plate spill. 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 receptacle 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 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.

- **i.** Never place anything on top of the BD Accuri C6, including tube racks or Kim-wipes.
- **j.** Ensure that the BD Accuri C6 waste container is filled with enough bleach to result in 10% bleach solution following use.

Pour bleach waste down sink after minimum of 10 minutes contact time following shutdown and flush with additional water.

3.5 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.** <u>Signage:</u> Currents BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2521.

c. <u>Access</u>: 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.** <u>PPE Requirements:</u> 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.

epartment

vi.

- iii. Print Authorization to Invoice MSU Form to take to care facility. <u>https://www.hr.msu.edu/benefits/workers-</u> <u>comp/documents/InvoiceMSU.pdf</u>
- iv. Report to a Lansing Urgent Care facility for postexposure follow-up as soon as possible. <u>https://www.lansingurgentcare.com/</u>
 - v. Be prepared to provide information about the agent or cells involved in the accident. Additionally, route of exposure, dose/concentration, unusually 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 Frandor location is open 24 hours.

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 3.4. All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to his lab. Waste cans 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-wipes 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.

3.6 **Contact Information**

- **a. Matthew Bernard: Core Director,** Office, IQ Building, Rm 2315 (517)355-4076; (585)703-5008 (cell)
- **b.** Environmental Health & Safety: 355-1053
- c. Occupational Health (University Physician's Office): 353-8933
- **d. MSU Police:** 355-2221

3.7 **Quality Measures**

a. Daily: When in use, run ddH₂O on the system to ensure system is in proper working order before running samples. Event number should be <200 events within 2 minutes when instrument is run on FAST. If instrument fails this check, see Section 3.6.1 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 system check has been completed, the date, time and person who performed the validation, must be recorded in the Equipment Logbook (Appendix III).

4.0 Procedure: BD Accuri C6 Flow Cytometer Use

4.1 Startup

- a. Check fluid levels in all bottles, ensuring that the Waste is empty and the Sheath, Cleaner, and Decontamination bottles are full.
- b. Add appropriate amount of bleach to Waste container (~ 100 mL), which will result in $\sim 10\%$ final bleach concentration.
- c. Gently push the sample stage back, remove current tube of ddH₂O (discard), and place a tube containing at least 2 mL of fresh ddH₂O on the SIP (sample injection port).
- d. Turn on <u>in order</u>: computer, cytometer.
- e. Log into computer. Sign in under User Account Name and password, as appropriate.

- f. Click on the CFlow Plus desktop icon to start the software. This software runs the BD Accuri C6 flow cytometer.
- g. 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.
- h. Check the number of events:

If there are <200 events processed in 2 minutes, proceed with analysis

If there are between 200-500 events processed in 2 minutes, repeat with running instrument 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.

If there are >600 events processed in 2 minutes, perform any of the following procedures to resolve the issue:

Run Cleaning (or decontamination solution) through the instrument for 2 minutes on FAST.

Run "unclog" or "backflush" under instrument pull-down menu (make sure you use a waste tube for these procedures). Purge air from the system by running for 5 minutes on FAST, but pause and resume run approximately every 30 seconds.

Run the Cleaning Fluid Cycle (See Section 3.6.2).

i. Be sure to wipe the SIP with a Kim-wipe 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 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 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.

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.

- 1. 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 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 processed.
- p. Analyze the Validation Bead data according to the BD Accuri C6 Software User Guide (Section 3.7). CS&T bead data can be analyzed based on prior runs, focusing on Median Fluorescence Instensity and %CV.

q. Record Validation information in the Accuri Equipment Log (Appendix III).

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 ddH_2O 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 ddH_2O 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 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.

- iii. Place a tube with at least 500 μ L 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 μ L of fluid.
- iv. Select Instrument > Extended clean of flow cell.

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).

- v. Replace the tube of Extended Flow Cell Clean Solution with a tube of approximately 2 mL of ddH_2O .
- vi. 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.
- vii. Run ddH₂O for 10 minutes on fast speed.
- viii. Operate the cytometer as usual.

e. Replace the in-line sheath filter approximately every 2-3 months. If 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 Accuri Equipment log, as appropriate (Appendix III).

4.4 Acquisition

Please refer to the BD Accuri C6 Software User Guide (Section 3.7) for instructions for 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 the 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.

j. Shutdown the computer.

Empty waste container, containing $\sim 10\%$ bleach down sink while wearing appropriate PPE (ie, lab coat, safety glasses, gloves) and rinse with DI water.

1. 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 3.3e.

b. **Error Messages / System Issues** – All error messages and system issues must be relayed to the Equipment Champion and the Pharmacology & Toxicology Core Facility Manager. This information must be relayed on the same day as equipment use. Error messages/system issues must be recorded, refer to 3.3e. All error messages and system information must be relayed on the same day as equipment use. Error messages and system issues must be recorded, refer to 3.3e.

4.7 **Resource Index**

BD Accuri C6 flow cytometer and CFlow Plus software literature and resources for the follow 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 Ins trument Manual.pdf

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 Use r Guide.pdf

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: <u>Timothy Stewart@bd.com</u>

5 Competences, Authorization and Training

New Users must receive proper authorization from either the Equipment Champion and / or Pharmacology & Toxicology Core Facility Manager before equipment use. A new User may contact the Equipment Champion or Pharmacology & Toxicology Core Facility Manager 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 setup. All Users are individually responsible for current SOP familiarization. All New Users must refer to 3.3a during new BD Accuri C6 flow cytometer account creation.

6 SOP Performance and Equipment Review

The effectiveness of the SOP: 3537.2521.001 will be monitored by the Pharmacology & Toxicology Core Facility Manager, 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 Pharmacology & Toxicology Core Facility Manager or Equipment Champion. The Core Facility Lab Manager 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: 3537.2521.001 review will occur every two years.

7 Definitions

a. **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 stand for (xxx . xxx) equipment serial number . room number . SOP version number.

b. **Originator / Author** The individual representing the Pharmacology and Toxicology Core Facilities that created SOP: 3537.2521.001

c. **Stakeholder** Any individual that uses or performs the task of which is the subject of the SOP, including the Pharmacology and Toxicology Core Facilities Department.

d. **New User** An individual who has not completed the requirements of section 3.3.

e. **Approved User** An individual who uses the BD Accuri C6 flow cytometer and has fulfilled section 3.3. This title may only be given by the Equipment Champion and / or the Pharmacology and Toxicology Core Facility Manager.

f. **Champion** An individual who's direct expertise with the BD Accuri C6 instrum,ent has been recognized by the Pharmacology and Toxicology Core Facility Committee. This title may only be awarded by the Pharmacology and Toxicology Core Facility Committee.

Pharmacology & Toxicology Core Facilities

8 Approvals

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

This SOP was rev	iewed by:		
Dr. Jamie Willard	Jamie Willard	ume Wilzed d, on MSU, que FHS: sue adu, ce US 40.11 – 01207	
This SOP was rev	riewed by:		. 6
Ms. Erica Lange _	Turce La	nge ISUM	MI.
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Appendix I

MSU South Campus Flow Cytometry Questionnaire

The MSU South Campus Flow Cytometry core facility is now operating under BSL-2 laboratory conditions. This questionnaire serves to gather information important information that will help us render effective core facility services. Part I provides information about the Principal Investigator, each of the independently funded research projects, and the researchers associated with each project. Part II will identify the samples to be analyzed.

<u>Part I</u>

Principal Investigator: Department: College: Office Location (building/room): Office Phone: E-mail:

The following questions are designed to ID individual grants or projects.

Funding agency: Project Title: Grant # or project #: Account # to be charged for services rendered: Business Office Address:

Please ID the instrument samples will be analyzed on: Select an instrument

Identify researchers working on this project:

Part II – The Samples

List the type of samples (ie, animal, human, plant, bacteria) and sources (ie, spleen, bone marrow, cultured cells):

Has the research protocol used to generate these samples been reviewed by the appropriate Animal (IACUC, please provide AUF #) or Human use Committees (please provide IRB identification and/or EH&S BMR ID #)?

Biosafety level required:

Will the samples be fixed prior to flow cytometric analysis or sorting? \Box Yes	□No
If yes, describe the fixation protocol:	

Required for BSL-2 samples:

Were tissue/blood donors screened for the following pathogens: HIV, SIV, HepB, HepC, HepD, Herpesvirus simiae, HTLV-1, HTLV-2, LCMV, SARS, Mycobacteria tuberculosis, Mycobacterium bovis, Neisseria meningitides?

□Yes: (List pathogen and the test results) □No: Unknown

Does the sample contain any other known infectious agents, if so please describe?

Has the infectious agent been inactivated? If so, please describe the method:

What precautions does the facility need to employ to safely handle these samples?

Required for Genetically modified samples:

Identify the method of cell transformation. If a virus was used, please identify it:

Were the cells genetically engineered?

□Yes □No

If yes, how were they genetically altered?

What precautions should be taken with these cells?

BD Accuri C6 User Log (Appendix II)

Please record the following information after each use.

Date	Start Time	End Time	User	PI Name	Lab #	Phone #	Comments / Actions

Please contact Matthew Bernard at 517-355-4076 or <u>matthew.bernard@ora.msu.edu</u> with any issues.



Accuri Equipment Maintenance Log (Appendix III)

Please record the following information after each use.

Date	Time	Initials	Clean/Decon (2 Weeks)	Flow Cell Clean (Monthly)	Tubing/Filter Replace (2-3 Months)	Comments / Actions

Please contact Matthew Bernard at 517-355-4076 or <u>matthew.bernard@ora.msu.edu</u> with any issues.

