Core Equipment ID:

Description: BD FACS Aria IIu

Room: IQ Building 2522

Champion: Matthew Bernard

1.0 Purpose

Standardize the process of control, maintenance and ownership, of the BD FACS Aria IIu instrument located in IQ Building Room 2522.

1.1 BD FACS Aria IIu SORP Capabilities

This BD FACS Aria IIu sorter is the original model FACSAria-I sorter that has been upgraded in the field with a FACSAria-II flow cell. It is equipped with 3 lasers: a blue 488nm (6 detectors), a red 633nm (3 detectors), and a violet 405nm (4 detectors). The optical configuration allows multi-parameter detection of up to 13 fluorescent parameters and 2 light scatter parameters. The FACS Aria IIu is capable of sorting through over 50 million cells per hour (dependent on cell type), with up to four populations simultaneously. The populations of interest can be sorted into 0.5 mL/1 mL Eppendorf tubes, 5 mL tubes, or 15 mL conical tubes. Populations can also be sorted directly into multi-well plates, including: 12-well, 24-well, 48-well, and 96-well.

1.2 FACSDiva v6.1 Software Capabilities

- Streamlines laboratory workflow in a multi-system environment by enabling users of BD platforms to use a single software application for acquisition and analysis.
- Provides easy-to-use instrument setup and quality control (QC) when used with BD FACSDiva™ CS&T research beads.
- Enables standardization across BD platforms for both inter- and intra-site experiments with use of application settings.
- Provides common feature sets that allow users to transition easily across BD platforms and from analysis to sorting applications. Provides flexible data management tools for users to export data for use with other third-party analysis software tools.

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 FACS Aria IIu instrument 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 FACS Aria IIu instrument. The process description details the standard use of the BD FACS Aria IIu instrument. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

3.1 SOP: P07900160.2522.001 for BD FACS Aria IIu instrument, authored by Matthew Bernard, created on 10/16/2017, issued on 12/14/2017.

3.2 SOP: P07900160.2522.001 applies to any User and / or Trainer of the BD FACS Aria IIu.

3.3 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the BD FACS Aria IIu instrument. All Users are responsible for the proper use, according to defined protocol, when using the BD FACS Aria IIu instrument

a. **New Users** need a FACS Diva 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 (**Appendix II**) post-use on the same day as the recorded use. The Logbook is located on the desk next to the BD FACS Aria IIu 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.

f. Only covered samples may enter Room 2522. Samples must be brought to facility in a standard **spill control box/leak-proof secondary container** 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 FACS Aria IIu flow cytometer must be appropriately shut down (see Section 4.10).

3.4 Equipment Safety Issues

a. Safety Issues – The Core Facility operates as a BSL-2 facility. 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 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.

Summary of Biosafety Practices for cell sorting in the Core Facility:

Type of cell/Procedure	Exempt BSL-1	BSL-1	BSL-2
Examples of cells	Wild-type cells from murine or other non-human/non-primate species that have **NOT** been exposed to any microbial agent (eg, viral, bacterial, fungal, protozoan, parasitic) and **have NOT been** genetically modified or Cells determine by EH&S to be recombinant NIH-exempt BSL-1	Cells from murine or other on-human/non-primate species that have not been exposed to any microbial agent, but have been genetically modified using non-viral methods (eg cells from transgenic animals or cells treated with nucleic acids). **Or** Cells determined by EH&S to be approved as non-recombinant BSL-1 or recombinant BSL-1	Cells of human or non-human primate origin or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (eg, viral, bacterial, fungal, protozoan, parasitic) and Have been approved by EH&S for BSL-2 containment and sorting.
EH&S BMR Form Update Requirement	Not Required	**Required**	**Required**
BBP Training	Not Required	Not Required	Initial and annual renewal required for work with human cells and other cells exposed to BBP.
Sort Sign-up	Required. Include name, phone #, and sample description.	Required. Include name, phone #, sample description, **and** a clear BSL-1 notation.	Required. Include name, phone #, sample description **and** a clear BSL-2 notation
Sample Transport	No Requirement	Leak-proof secondary container	Leak-proof secondary container
Room Restriction	None	None	Yes, door to 2522 must be closed during sort and the BSL-2 Biohazard sign posted on the outside.
PPE	Closed-toed shoes. When manipulating samples (ie, loading and unloading) gloves and lab coat are recommended	Closed-toed shoes. When manipulating samples (ie, loading and unloading) **gloves** and lab coat are recommended. Spills: Lab coat, nitrile gloves, goggles **required.**	Closed-toed shoes, lab coat, and gloves **required** at all times. When manipulating samples (ie, loading and unloading) face protection is also required. Spills: Lab coat, nitrile gloves, goggles **required.**
Waste	Contaminated materials must be decontaminated prior to disposal	Gloves and other waste must be disposed of as biohazardous.	Gloves and other waste must be disposed of as biohazardous.

b. **Aerosol Risk:** The BD FACS Aria IIu SORP designated for the acquisition and sorting of fixed or unfixed samples up to the BSL-2 level. High-speed cell sorters use higher system pressures and higher drop drive frequencies, which produce smaller droplets and satellite drops. During instrument failure (eg, partial blockage of the nozzle) the generation of secondary aerosols can occur (*Schmid I, Lambert T, Ambrozak D, Marti GE, Moss DM, Perfetto SP. International Society Cytology and Biosafety Standard for Sorting of unfixed Cells. Cytometry 2007; 71A:414-4371*). The potential exposure to escaped aerosols may be a health risk to sort operators. The BD FACS Aria IIu has been installed in a Baker BioProtect IV Biosafety Cabinet, specifically designed for this instrument, in order to mitigate exposure to aerosols when present, however aerosol containment must be properly assessed (Section 3.8).

c. **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 a sort.

d. **Decontamination of BD FACS Aria IIu post-operation:**
Following the Shutdown procedures (Section 4.10 will result in appropriate daily decontamination of the flow cytometer between uses.

e. **Decontamination of work surfaces:**
External surfaces in front of the BD FACS Aria IIu, inside the Biosafety cabinet and inside cabinet door can be cleaned with Envirocide (or equivalent) or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent). Wipe metal surfaces down with 70% ethanol after decontaminating to prevent corrosion.

f. **BSL-2 samples should be fixed, when applicable.**

g. **Radioactively labeled samples are prohibited.**

h. **Spill control:**

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.

Report spills to Core Facility staff.

i. Ensure that the BD FACS Aria IIu waste container is filled with enough bleach to result in 10% bleach solution following use. Pour bleach waste down sink after appropriate amount of time following shutdown and flush with additional water.

3.5 Laboratory Conditions

a. IQ 2522 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:** Currents BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2521. **A temporary must be posted on door during a BSL-2 sort to notify laboratory personnel and indicating only appropriate individuals are allowed to enter.**

c. **Access:** Access is limited to people with permission to run samples on the BD FACS Aria IIu, which has been booked through the iLabs web portal. Only individuals involved in training exercises, running samples on the cell sorter, or retrieving data should be in Rm 2522.

d. **PPE Requirements:** Standard PPE must be used at all times, which includes gloved hands, long-sleeve lab coat over full coverage shirt and pants, and full coverage shoes with intact soles. Face mask may also be worn.

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 EH&S website for instructions regarding what to do in the event of an exposure. The MSU Exposure Response Procedure is posted in Rm 2522.

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 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, 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.

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 2522 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. Dispose of PPE appropriately in the Core Facility. Remove disposable lab coat and place in biohazardous waste. Gloves should be discarded in the biohazardous waste container. Remove eye protection and wipe with Sani-Cloth Plus wipes.

j. Wash hands thoroughly before exiting the Core Facility.

k. 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 a *Performance Check* with CS&T beads (1 drop in 350 µL of water or appropriate diluent) in the CST Application to ensure system is in proper working order before running samples.

b. **Approximately every 6 months:** A CS&T Baseline should be run on the BD FACS Aria IIu flow cytometer. Run CS&T beads (3 drops in 500 µL of water or appropriate diluent) to establish a Baseline. Perform a Perfomance Check following Baseline. Once this has

been completed, the date, time and person who performed the validation, must be recorded in the Equipment Logbook (**Appendix III**).

c. **Approximately every year:** Have licensed professional certify Baker BioProtect IV biosafety cabinet.

The BioProect IV is a Class II Type A2 Standard biosafety cabinet designed specifically for the BD FACSAria product family. Baker has verified that it meets standards for both a Class II Type A2 biosafety cabinet and the National Sanitation Foundation Internal Standard 49. Baker performed microbiologic aerosol testing to confirm compliance with the NSF-49 protocols for containment and product protection with the BD FACSAria cell sorter running inside the cabinet. Biosafety cabinets protect operators and samples by controlling the airflow, HEPA filtering the air for contaminants, and directing exhaust air from the work area. The Baker Company BioProtect IV cabinet is designed to handle low to moderate risk biological agents, specified by NSF 49.

3.8 Assessment of Aerosol Containment

The following procedure is for the purpose of measuring the effectiveness of Aerosol Management on a droplet based, high pressure cell sorting flow cytometer as well as to ensure compliance to proper safety practices and procedures.

a. Aerosol Management: For proper aerosol containment, the following guidelines must be followed while sorting viable infectious material under high pressure. All sort operators must be trained by an equipment champion prior to any cell sorting:

i. The Baker BioProtect IV biosafety cabinet must be on and functioning according to the manufacturer guidelines. Using this system, the cabinet monitor should be set to **LOW** and the vacuum gauge should read between -0.15 and -0.25. If these values are outside of these ranges, the HEPA filter should be replaced and biosafety cabinet inspected.

ii. The waste tank must contain enough sodium hypochlorite (bleach) to provide a final concentration of 10% when filled (1L bleach to a final 10L waste collected).

iii. The Accudrop camera on the FACSAria IIu, which is focused on the sort stream, must be functioning normally. This camera system is used to monitor the sort stream and alerts the operator to potential sort stream disruption,

which can lead to increased aerosols. The FACSAria IIu is also equipped with a droplet breakoff monitoring technology, which is used during all sorting operations and can detect stream drifts due to possible clogs and automatically shuts down the stream.

b. Measurement of Containment:

i. After fluidics start-up and instrument warm-up (30 minutes) insert the 70 micron nozzle.

ii. Start stream, wait until stabilized, and turn on SweetSpot.

iii. Prepare Dragon Green 1 micron diameter microspheres (Bangs labs), or equivalent, for testing: Vortex vigorously and add 10 µL of beads into 1 mL of 1x PBS + 0.1% Tween-20.

iv. Set up vacuum system for cyclex-D filters

v. Set up template for beads, triggering on green fluorescence (FSC-A vs SSC-A, beads vs. other fluorescence detector, threshold on bead parameter)

vi. The Baker Bioprotect IV biosafety cabinet must be tested under simulated worst case failure mode. In this mode, the instrument is set with the stream hitting the waste aspirator to create excessive aerosols. Cover waste drawer in sort block with sample tubing (cut so that tubing can slide onto waste drawer) to simulate clog conditions, but do not move waste drawer in yet.

vii. Add 10+ paper towels to bottom of sort chamber (there will be a LOT of liquid)

viii. Don gloves, non-permeable back-close gown, sleeve protectors, N-95, goggles, and close inner facility door for testing, and put sign on the door to prevent anyone from entering.

ix. Run samples and make sure the event rate is approximately 50,000 events per second

xi. Run test and record results on Containment Test Record (Appendix IV):

1. Baker Biosafety Cabinet on **LOW**, cyclex-D filter vacuum at 20L/min.

- Sort block door closed, tube holder attached
- Filter on top of the sort collection chamber
- Waste drawer open, no tubing blocking (normal operation mode with no clog)
- Collection time: 5 minutes

2. Baker Biosafety Cabinet on **LOW**, cyclex-D filter vacuum at 20L/min.
    - Sort block door closed, tube holder attached
    - Filter on top of the sort collection chamber
    - Waste drawer open, tubing blocking
    - Collection time: 5 minutes
3. Baker Biosafety Cabinet on **LOW**, cyclex-D filter vacuum at 20L/min.
    - Sort block door closed, tube holder attached
    - Filter outside BSC at workstation
    - Waste drawer open, tubing blocking
    - Collection time: 5 minutes
4. Positive Control: Baker Biosafety Cabinet **OFF**, cyclex-D filter vacuum at 20L/min.
    - Filter to left of sort chamber, sort block door slightly open
    - tube holder attached
    - Waste drawer closed, tubing blocking
    - Collection time: 1 minute

xii. After testing is complete, unload beads, and run FACSClean, 10% bleach, or equivalent for 5 minutes at flow rate = 11.

xiii. While running, open waste drawer and turn BSC on **HIGH** for at least 2 minutes.

xiv. Decrease biosafety cabinet to **LOW**.

xv. When run is finished, run sterile water for 5 minutes at flow rate = 11.

xvi. Discard paper towels in bottom of sample chamber.

xvii. Prepare 4 microscope slides, one each per test: Add one drop of immersion oil to center of slide. Label slide with sample name or number/letter. Take apart cyclex-D filter and add coverslip to slide on top of immersion oil (make sure collection side faces down on oil). Repeat for each test. (You can add clear nail polish around coverslip if wanting to keep these slides for an extended period of time).

xviii. Analyze on an appropriate microscope with a FITC filter (make sure to schedule appointment on scope several days before testing).

c. Acceptable Tolerance:

Acceptable tolerances for the measurement of containment using the Dragon Green beads protocol are listed below:

Beads outside = Zero tolerance, no particles on entire slide. Any positive result must be investigated, resolved, and the instrument retested before proceeding with sorting potentially infectious samples.

Beads inside (positive control) = Greater than 100 per slide.

4.0 Procedure: BD FACS Aria IIu Use

4.1 Startup

a. Check fluid levels, ensuring that the Waste is empty and the Sheath tanks is full. Wear gloves when filling Sheath tank, as to avoid contamination of the tank/sheath.

b. Add appropriate amount of bleach to Waste container, which will result in ~10% final bleach concentration. When removing cap rest with filter cap up, do not invert, as clogging the filter in the cap could result in a pressurization issue.

c. Ensure that blue sheath-line (blue) is connected to sheath filter and air-line (clear) is connected to the sheath tank prior to startup.

d. Turn on <u>in order</u>: computer, cytometer (green button on left side of cytometer). Lasers must warm up for approximately 30 minutes prior to running qualification of instrument and/or samples.

e. Turn on Baker BioProtect IV Biosafety Cabinet (low setting). The BSC must be in operation for a minimum of 5 minutes prior to running any samples.

f. Log into computer. Sign in under User Account Name and password, as appropriate.

g. Click on the FACS Diva desktop icon to start the software. This software runs the BD FACS Aria IIu flow cytometer. Sign in using appropriate user name and password.

h. Sonicate desired nozzle (75, 85, or 100 µm) nozzle for at least 1 minute and dry using clean lens paper.

i. If instrument was shutdown following weekly ethanol shutdown procedure, run *Cytometer* > *Fluidics Startup*.

j. Following completion of Fluidics Startup and/or instrument warm-up, remove closed-loop nozzle and insert nozzle into the cytometer.

k. Start the stream. Ensure that stream in aligned with waste receptacle and not off-center, causing aerosols to be created. Adjust sort block alignment with appropriate tools, as appropriate.

4.2 Daily Instrument Qualification

a. Select *Cytometer>CST*. The cytometer disconnects from the BD FACSDiva interface and connects to the CS&T interface. Verify that the cytometer configuration under the System Summary is the appropriate configuration. The setup tab indicates the time of the last Performance check for your configuration.

b. Prepare CST beads. In a 12x75mm tube, add 0.35 mL sheath fluid and 1 drop CST gently vortexing. Label tube with "CST". Store the bead suspension at 2°C to 25°C in the dark until you are ready to use them (Note: beads are stable at 2°C to 25°C for no more than 20 minutes in direct light, and up to 8 hours if protected from light).

c. Select the correct bead Lot ID from the menu (bead lot is printed on each Bead vial). New bead lot files can be found on the BD Biosciences website and imported.

d. Install tube onto the cytometer loading port.

e. Under Setup Control, make sure that *Check Performance* is selected.

f. Click Run. The performance check takes approximately 5 minutes to complete.

g. Once the performance check is complete, click *View Report.*

h. Verify that the performance passed- In the Setup tab, the Cytometer Performance Results should have a green checkbox displayed and the word Passed next to it. If any parameters did not pass, refer to the cytometry supervisor or the BD Cytometer Setup and Tracking Application Guide for help troubleshooting.

i. Select *File>Exit* to close the CST window and connect back to the BD FACSDiva Interface. Click the Use CST Settings in the settings mismatch dialog box that appears. By selecting **Use CST Settings**, the laser delay, area scaling, and other cytometer settings will be updated to the latest optimized settings from the performance check.

4.3 Maintenance

a. At least weekly: Run *Fluidics Shutdown* if instrument will not be used within 48 hours. See section 4.10 for instructions for weekly shutdown.

b. Approximately every 6 months: Schedule preventative maintenance with Service Contract provider.

c. Approximately every 6 months: Run a new baseline with appropriate CS&T bead lot in the Cytometer Setup and Tracking application. App

d. Clean the Flow Cell approximately monthly.

i. Place a tube with approximately 3 mL of 20% bleach, 10% Contrad, or 100% Contrad (as appropriate) on the tube loader.

ii. Make sure stream is turned off and nozzles are removed.

iii. Place a lint-free swab wrapped in lens paper in the nozzle port.

iv. Select *Cytometer > Clean Flow Cell*. Run this 3x to ensure that flow cell is fully loaded with solution.

v. Allow to incubate for approximately 10-15 minutes.

vi. Perform *Clean Flow Cell* Function with ddH_2O 3x.

vii. Wipe out nozzle port with lint-free swabs, replace closed-loop nozzle.

viii. Run *Fluidics Startup*.

ix. Operate the cytometer as usual.

4.4 Preparing for a Sort

a. Autoclave sheath tank (remove probe) and water tank (remove probe) if running *Prepare for Aseptic Sort*.

b. Run Prepare for Aseptic Sort, as appropriate.

c. Set Drop Delay by running Accudrop beads (Section 4.7).

d. Adjust side-streams for sample collection tubes, as appropriate (Section 4.8).

e. Just prior to sorting sample, run 70% ethanol or FACSClean (10% bleach or equivalent) for at least 1 minute at 11. Backflush sterile sheath until sort stream appears normal.

4.5 Setting up an Experiment

a. Create a new Blank Experiment.

b. Rename the Experiment (right-click>Rename) using the date, lab name, a good descriptive term, as appropriate.

c. Create Applications settings the first time you run and experiment or new panel.

d. Application settings are associated with a cytometer configuration and include the parameters needed for the application, area scaling values, PMT voltages, and threshold values, but not compensation. Each time a performance check is run for a configuration, the application settings associated with that configuration are updated to the latest run. Using application settings provides an easy, consistent, and reproducible way to reuse cytometer settings for your commonly used applications.

e. Select Cytometer Settings in the Browser.

f. Delete all parameters you will not be using. In Parameters tab (In Instrument Window) click on small button to left of parameter name. Click delete button (use control key and highlight for multiple deletions). Repeat for each parameter you are not using.

g. Click the H and W checkbox to select Height and Width for FSC and SSC to enable doublet discrimination.

h. Right-click *Cytometer Settings* in the Browser, then select *Application Settings>Create Worksheet*. A second global sheet is added with the plots created according to your selections in the Parameters tab. You will use the gray boxes and crosshairs on this worksheet to guide your optimization.

i. Install the stained cells (labeled with all fluorochromes) tube onto the cytometer.

j. Optimize the FSC and SSC voltages to place the population of interest on scale.

k. Adjust area scaling factors first, as appropriate. In the Cytometer window select the *Lasers* tab. Create a worksheet with 4 dot plots: FSC-A vs. FSC-H, AF405-A vs. AF405-H, FITC-A vs. FITC-H, and APC-A vs. APC-H. Draw a diagonal line from the bottom left corner to top right of each plot. Load stained cells and acquire event data. Ensure that population is lined up on the diagonal. Adjust are scaling factor up or down, as appropriate to line up population with diagonal line that was created.

l. Verify that the positive populations are on scale. If a positive population is off scale, lower the PMT voltage for that parameter until the positive population is entirely on scale. Use the gray boxes as a guide when decreasing the PMT voltages. If the negative population is lowered below the gray box, you may decrease your ability to resolve dim populations from the negative population.

m. Stop acquisition and unload the tube. You do not need to record a data file.

n. To save, Right-click Cytometer Settings in the Browser, then select Application Settings>Save. Name the Application Settings appropriately and Click OK. The application settings are saved to the catalog. Application Settings do not include compensation settings. In a newly created experiment, ensure that the current CST settings are applied.

o. To use previously created Application Settings right-click the Cytometer Settings icon in the Browser and select Application Settings> Apply.

p. Select previously created Application Settings from the catalog.

q. Click *Overwrite* in the dialog that appears.

r. If a message appears about area scaling, click *Yes* to accept all changes to cytometer settings.

s. The parameter list and PMT voltages are updated to match the Application Settings that were previously created.

4.6 Compensation Setup

a. Ensure that the correct Application Settings are applied, or if not using, ensure that the correct parameters and PMT voltages are applied.

b. Select *Experiment > Compensation Setup > Create Compensation Controls*.

c. Click *OK* to close the Create Compensation Controls dialog. A compensation controls specimen is added to the experiment, along with an unstained control tube, and a stained control tube for each parameter. Worksheets containing the appropriate plots are added for each compensation tube.

d. Place the unstained control tube onto the loading port.

e. Set the current tube pointer to the unstained control tube in the Browser.

f. Click Load in the Dashboard.

g. Move the P1 gate to fully incorporate the singlet population.

h. Right-click the P1 gate and select Apply to all Compensation Tubes.

i. Click Record Data in the Dashboard to record the events from the unstained control tube.

j. Unload the unstained control tube.

k. Notice: Do not change the PMT voltages after the first compensation tube has been recorded. To calculate compensation, all tubes must be recorded with the same PMT voltage settings.

l. Click Next Tube in the Dashboard.

m. Acquire each compensation tube and record in this manner.

n. Verify that the snap-to interval gates encompass the positive populations.

o. Select *Experiment > Compensation Setup > Calculate Compensation*. If the calculation is successful a dialog appears. Appropriately name the compensation setup.

p. Click *Link & Save* to close the dialog box and save the compensation setup and link it to the experiment's cytometer settings.

4.7 Optimizing Drop Delay

a. Load a tube filled with a suspension of BD Accudrop beads (approximately 1 drop of beads in 1 mL PBS or H_2O).

b. Adjust the flow rate to achieve an event rate of ~2,500 events per second.

c. Turn on the voltage in the *Side Stream* window. Click *Sort* in the *Sort Layout* window.

d. Click Cancel at the Confirm dialog. There is no need to collect the beads. When the drawer is closed, the beads are sorted to waste.

e. Adjust the *micrometer dial* to obtain the brightest bead spot on the center stream.

f. Click the *Optical Filter* button in the Side Stream window. This control moves the emission filter that allows you to view the Accudrop beads in front of the lower camera. When the control is clicked, the image switches from a raw image to a processed (digitized) image. The two boxes indicate the region of the image where the left and center stream intensities are calculated during image processing. The numbers shown are percentages of the total intensity.

g. If the left side stream is not completely contained in the left region, adjust the voltage slider to place the stream in the center of the region.

h. Verify that the sort precision mode is set to ***Initial***.

i. Optimize the drop delay. Adjust the drop delay value in 1-drop increments (Ctrl-click arrow control) to achieve close to 100% intensity in the left side stream. Wait a few seconds after each click for a complete response to the delay change.

j. In the *Sort Layout* window, change the precision mode to ***Fine Tune***.

k. Optimize the drop delay. Adjust the drop delay value in 0.03-drop increments (click arrow control) until the left side stream intensity is greater than or equal to 90%. Wait a few seconds after each click for a complete response to the delay change.

l. As appropriate, verify *Auto Delay*.

m. Click the *Optical Filter* button to move the emission filter away from the camera.

n. Reset the window extension to its original setting (typically 2). Turn off the deflection plates.

4.8 Setting Up Side Streams

a. Place a collection tube(s) in the appropriate tube holder, and install the tube holder.

b. Turn on deflection plates: click on the small icon next to *Voltage* (green dot in the icon turns red).

c. Click on the icon next to *Test Sort*.

d. Click on the icon next to the *Waste Drawer* (you will audibly hear that the waste drawer moves back).

e. Open the sort block door (DO NOT TOUCH the two deflection plates!).

f. Adjust the side streams with the voltage slider controls, as appropriate for each stream.

g. Close the sort block door to verify image capture of streams in viewer window.

h. When the side streams are adjusted, click on *Waste Drawer* icon to close it.

i. Click on *Test Sort* and the *Voltage* icons to turn them off close sort block door.

j. Remove tubes from tube holder.

4.9 In the Event of a Clog

a. When an instrument clog occurs during sorting, ask any additional personnel or users to leave the room. Put on surgical mask or N95, as appropriate.

b. Ensure that the stream is off by clicking the *Stream* icon in the Breakoff window or hit emergency stop button on instrument and turn the Baker Bioprotect IV BSC to ***HIGH***.

c. Open the aspirator/waste drawer to allow aerosols to be removed.

d. Wait **5 minutes** for any potential aerosols to clear from the sort chamber before opening or touching the sort chamber.

e. Remove the sample tube from the cytometer loading port and the sample collection tubes and cap them. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, therefore, wipe with Sani-Cloth Plus wipe to

decontaminate. Use Envirocide (contact time 3 minutes) to decontaminate sort chamber, sample chamber, and immediate surrounding surface.

f. Reattach sample tube holder, close aspirator/waste drawer and restart stream to see if clog has cleared itself.

g. If clog has not cleared, turn off stream, and open aspirator drawer to allow aerosols to be evacuated. Wait **5 minutes** until opening the sort block.

h. Clean the flow cell three times by placing sterile diH_2O or Contrad (10-100%) in the cytometry loading port, then selecting *Cytometer > Cleaning Modes > Clean Flow Cell*, and click *OK*.

i. Close aspirator/waste drawer and restart stream to see if clog has cleared itself.

j. If clog has not cleared, turn off stream, and open aspirator drawer to allow aerosols to be evacuated. Wait **5 minutes** until opening the sort block.

k. If the instrument is still clogged, turn off the stream, remove the nozzle, and sonicate until the obstruction has been cleared. Turn on the stream and verify that it is normal. Repeat disinfection and/or cleaning the sort collection device and sort chamber, as appropriate.

l. Ensure that the deflection plate voltage is off and then clean and dry the plates.

m. Disinfect/Clean the sort collection device and sort chamber as necessary with 70% ethanol, 10% bleach, and/or Sani Cloth Plus wipes.

n. Turn on the stream and verify that it is normal.

o. Re-enable Sweet Spot and check Drop Delay with Accurdrop Beads.

k. Return the Bakes BioProtect IV biosafety cabinet to ***LOW***.

l. Filter the sample again through an appropriately sized cell strainer and resume sorting.

4.10 Shutdown/Decontamination

Daily (if instrument will be used within up to ~48 hours):

a. Run tube of FACSClean on 11 for 5-10 minutes.

b. Run tube of filtered milliQ water, or equivalent, on 11 for 5-10 minutes.

c. Turn off stream.

d. Remove nozzle and place in clean tube submerged in sterile filtered water, or equivalent.

e. Install closed-loop nozzle.

f. Select Cytometer>Cleaning Modes>Clean Flow Cell.

g. Install tube of filtered water (~3 mL) and click OK.

h. Following completion of flow cell clean, shutdown main power by depressing green button.

i. Exit Diva and shutdown computer.

j. Turn off Baker Bioprotect IV Biosafety Cabinet.

k. Fully depressurize sheath tank by pulling ring on pressure relief valve to prevent precipitates.

l. Spray down sample contact area with 70% ethanol until it evaporates. This includes: sort chamber, voltage plates, collection device, collection chamber, sample holder, and sample loading area.

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

n. Clean keyboard, mouse, and work surfaces in front of the BD FACS Aria IIu with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of surfaces.

Weekly or as needed (if instrument will not be used within ~48 hours):

a. Run tube of FACSClean or 10% bleach on 11 for 5-10 minutes.

b. Run tube of filtered milliQ water, or equivalent, on 11 for 5-10 minutes.

c. Turn off stream.

d. Refill ethanol (70%) sheath tank, if necessary.

e. Select *Cytometer>Fluidics Shutdown*

f. Remove nozzle and place in clean tube submerged in sterile filtered water, or equivalent. Select *Done.*

g. Install closed-loop nozzle and select Done.

h. Disconnect the air line and sheath line and connect to ethanol sheath tank (do not connect sheath filter to ethanol tank). Select *Done* to begin the cleaning process.

i. When prompted, install tube of filtered water (~3 mL) and click *Done*.

j. Click *Ok* when you see a message indicating system can be turned off.

k. Shutdown main power by depressing the green button on left side of instrument.

o. Exit Diva and shutdown computer.

p. Turn off Baker Bioprotect IV Biosafety Cabinet

l. Fully depressurize sheath tank by pulling ring on pressure relief valve to prevent precipitates.

m. Spray down sample contact area with 70% ethanol until it evaporates. This includes: sort chamber, voltage plates, collection device, collection chamber, sample holder, and sample loading area.

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

o. Clean keyboard, mouse, and work surfaces in front of the BD FACS Aria IIu with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of surfaces.

4.11 Records

a. **Records of Use** – All BD FACS Aria IIu 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/system issues must be recorded, refer to 3.3e.

4.12 Resource Index

BD FACS Aria IIu flow cytometer and BD FACSDiva 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 FACS Aria IIu flow cytometer in room 2522.

http://static.bdbiosciences.com/documents/BD_FACSAria_II_User_Guide.pdf?_ga=2.244315909.1278522869.1509555597-1487330724.1495554233

For detailed information about the functions, features, and use of BD FACSDiva v6.1 software or cytometer setup and tracking application, see the BD FACSDiva Software User Manual, available at:

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

http://static.bdbiosciences.com/documents/bd-cytometer-setup-tracking-application-guide.pdf?_ga=2.243661573.1278522869.1509555597-1487330724.1495554233

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

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 instrument 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 FACS Aria IIu instrument account creation.

6 SOP Performance and Equipment Review

The effectiveness of the SOP: P07900160.2522.001 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: P07900160.2522.001 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 FACS Aria IIu instrument. The SOP number stand for (xxx . 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: P07900160.2522.001

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

d. **Approved User** An individual who uses the BD FACSAria IIu instrument 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.

e. **Champion** An individual who's direct expertise with the BD FACSAria IIu instrument 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.

8 Appendix

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

This SOP was written/authorized by:

Dr. Matthew Bernard _____ Matthew Bernard 2018.01.10 15:28:38 -05'00'

This SOP was reviewed by:

Dr. Jamie Willard Jamie Willard Digitally signed by Jamie Willard DN: cn=Jamie Willard, o=MSU, ou=EHS, email=cherrymc@msu.edu, c=US Date: 2018.01.15 09:37:14 -05'00'

This SOP was reviewed by:

Ms. Erica Lange Erica Lange 15JAN18

Issue Date: 15 Jan 18 mPB

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.

Part I

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? ☐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 FACS Aria IIu User Log (Appendix II)

Please record the following information after each use.

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

Please contact Matthew Bernard at 517-355-4076 or matthew.bernard@ora.msu.edu for any Aria IIu issues.

PHARMACOLOGY & TOXICOLOGY CORE FACILITIES

BD FACS Aria IIu Equipment Maintenance Log (Appendix III)

Please record the following information after each use.

Date	Time	Initials	Startup Run?	CST Passed? (Daily)	Settings	Shutdown Performed?	Comments / Actions

Please contact Matthew Bernard at 517-355-4076 or matthew.bernard@ora.msu.edu for any Luminex issues.

SN:

PHARMACOLOGY & TOXICOLOGY CORE FACILITIES

Appendix IV

Containment Test Record

Sample 1: Normal Operational Mode

BSC:	**LOW**
Cyclex-D Filter:	**20 L/min**
Filter Location:	**On top of the sort collection chamber**
Collection Time:	**5 minutes**
Aspirator/Waste Door:	**Open**
Sort Door:	**Closed**
Tubing present:	**No**

Result_____

Sample 2:

BSC:	**LOW**
Cyclex-D Filter:	**20 L/min**
Filter Location:	**On top of the sort collection chamber**
Collection Time:	**5 minutes**
Aspirator/Waste Door:	**Open**
Sort Door:	**Closed**
Tubing present:	**Yes**

Result_____

Sample 3:

BSC:	**LOW**
Cyclex-D Filter:	**20 L/min**
Filter Location:	**Outside BSC, at workstation**
Collection Time:	**5 minutes**
Aspirator/Waste Door:	**Open**
Sort Door:	**Closed**
Tubing present:	**Yes**

Result_____

Sample 4: Positive Control

BSC:	**OFF**
Cyclex-D Filter:	**20 L/min**
Filter Location:	**On top of the sort collection chamber**
Collection Time:	**1 minute**
Aspirator/Waste Door:	**Closed**
Sort Door:	**Slightly open**
Tubing present:	**No**

Result_____

Date:

Operator: