Core Equipment ID: P07900160

Description: BD FACSAria IIu

Room: IQ Building 2522

Champion: Matthew Bernard

1.0 Purpose

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

1.1 BD FACSAria IIu Special Order Research Products (SORP) Capabilities

This BD FACSAria 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 FACSAria 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

a. Streamlines laboratory workflow in a multi-system environment by enabling users of BD platforms to use a single software application for acquisition and analysis.

b. Provides easy-to-use instrument setup and quality control (QC) when used with BD FACSDiva™ Cytometer Set-up and Tracking (CS&T) research beads.

c. Enables standardization across BD platforms for both inter- and intra-site experiments with use of application settings.

d. 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 FACSAria IIu instrument within the MSU Flow Cytometry Core Facility.

3.0 Process Description

Allow Core Facility Users to properly and effectively use the BD FACSAria IIu instrument. The process description details the standard use of the BD FACSAria 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 FACSAria IIu instrument, authored by Matthew Bernard, created on 10/16/2017, issued on 12/14/2017.

3.2 SOP P07900160.2522.002 amendment, authored by Matthew Bernard, on 3/31/2020, issued on 4/10/2020.

3.3 SOP P07900160.2522.003 amendment, authored by Matthew Bernard, on 10/26/2020, issued on 10/27/2020. This amendment clarifies disinfection procedure following a BSL-2+ sort (Prepare for Aseptic Sort), updates procedure for cleaning prior to instrument maintenance by engineer, and updates shutdown procedure based on best practices.

3.4 SOP P07900160.2522.004 amendment, authored by Matthew Bernard, on 2/16/2024, issued on 11/01/2024. This amendment clarifies maintenance procedures and updates biosafety requirements. 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. Added Power Wash Procedure. Added detailed information on autoclaving Sheath tank.

3.5 SOP: P07900160.2522 applies to any User and / or Trainer of the BD FACSAria IIu.

3.6 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the BD FACSAria IIu instrument. All Users are responsible for the proper use, according to defined protocol, when using the BD FACSAria 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 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 (BBP) and Biosafety Principles (BSP), as required for respective research projects.**

c. **All Users receive EH&S/IBC approval of a Cell Sorting Addendum in the HURON Click system. Once approved, all users are required to complete a Biosafety Questionnaire for each approved cell type prior to 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 2522. 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 4.12d). 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 or BSL-2+ samples.

f. Immediately after use, the BD FACSAria IIu flow cytometer must be appropriately shut down (see Section 4.10).

3.7 Equipment Safety Issues

a. **Safety Issues** – The Core Facility operates 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 healthy 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.

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

Type of Cell/Procedure	**Exempt BSL-1**	**BSL-1**	**BSL-2**	**BSL-2+ (enhanced precautions)**
Examples of cells	**Wild-type cells from murine or other non-human/non-primate species that have NOT been exposed to any microbial agent (e.g., viral, bacterial, fungal, protozoan, or 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 (e.g., 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 (e.g., viral, bacterial, fungal, protozoan, or parasitic) and have been approved by EH&S for BSL-2 containment and sorting.**	**Cells of human or non-human primate origin or Cells that have been genetically modified using viral methods or Cells exposed to microbial agents (e.g., 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**	**Required**
BBP Training	**Not Required**	**Not Required**	**Initial and annual renewal required for work with human cells and other cells exposed to BBP.**	**Initial and annual renewal required for work with human cells and other cells exposed to BBP. N95 respirator fit test also required.**
Sort Sign-up	**Required. Complete sign-up in iLab calendar and provide a clear BSL-1 notation.**	**Required. Complete sign-up in iLab calendar, sample description, and**	**Required. Complete sign-up in iLab calendar, sample**	**Required. Complete sign-up in iLab calendar, sample description, and and**

		provide a clear BSL-1 notation	**description, and a clear BSL-2 notation.**	**provide a clear BSL-2+ notation.**
Sample Transport	**No Requirement**	**Leak-proof secondary container**	**Leak-proof secondary container**	**Leak-proof secondary container**

b. **Aerosol Risk:** The BD FACSAria 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 (e.g., 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 FACSAria IIu has been installed in a Baker BioProtect IV Biosafety Cabinet (BSC), specifically designed for this instrument, in order to mitigate exposure to aerosols when present, however aerosol containment must be properly assessed (Section 3.11).

c. **All samples exposed to or infected with bacterial or viral agents must be approved by EH&S/IBC on a case-by-case basis.** A related Cell Sorting Addedummust be submitted through the PI's HURON Click Biosafety Protocol and approved by the IBC/EH&S prior to scheduling a sort.

d. **Decontamination of BD FACSAria 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 FACSAria 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. **Radioactively labeled samples are prohibited.**

g. **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 4.12d). 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.

h. Ensure that the BD FACSAria IIu waste container is filled with enough bleach to result in 10% bleach solution following use. The waste solution needs to sit for at least 20 minutes before transferring it into an EHS approved 5 Gallon carboys.

3.8 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:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2522 **A temporary sign must be posted on door during a BSL-2 or 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 FACSAria 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. The room will be locked during a BSL-2+ sort.

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. Safety glasses and N95 respirator may also be worn. For sorting samples with a BSL-2+ designation, a front-closure gown, face shield and safety glasses and/or goggles, and N95 respirator are also required (PAPR may be worn in place of N95 and faceshield, following appropriate EH&S training).

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. **Negative Pressure:** Room 2522 is setup to maintain negative air pressure. For BSL-2+ sort, the room will be monitored for negative air pressure prior to sort initiation using the Aircuity system and/or smoke bottle.

g. **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, 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.

h. Sample handling and decontamination within IQ Rm 2522 is covered in Section 3.7. 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.**

i. **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 wipes are available for wiping **keyboards and personal electronic devices if cross-contamination accidentally occurs.**

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

k. Wash hands thoroughly before exiting the Core Facility.

l. **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.

3.9 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:** 355-1053

d. **Occupational Health (University Physician's Office):** 353-8933

e. **MSU Police:** 355-2221

3.10 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 the system is in proper working order before running samples. Ensure that the CS&T beads Lot# matches the CS&T Lot# used to establish the baseline.

b. **Approximately every 6 months:** A CS&T Baseline should be run on the BD FACSAria IIu flow cytometer. Run CS&T beads (3 drops in 500 µL of water or appropriate diluent) to establish a Baseline. Perform a Performance Check following Baseline. Once this has been completed, the date, time and person who performed the validation, must be recorded in the Equipment Maintenance Record

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

The BioProtect 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.

3.11 Assessment of Aerosol Containment

The following procedure is for the purpose of measuring the effectiveness of the Aerosol Management System (AMS) 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 AMS 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 Clean Flow cell with 10-20% bleach for 20 minutes, then perform a fluidics startup. After fluidics start-up and instrument warm-up (30 minutes) insert the 70 micron nozzle.

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

iii Prepare Dragon Green 1 micron diameter microspheres (Bangs labs, #FSDG004), 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 micro5, cyclex-D, or equivalent 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 the 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, N95 respirator, goggles, and close inner facility door for testing, and put a 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.

x Run test and record results on Containment Test Record (Appendix I):

1 Baker Biosafety Cabinet AMS on **LOW**, 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 AMS on **LOW**, 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 AMS on **LOW**, 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 AMS **OFF**, 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

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

xii While running, open the waste drawer and turn the AMS on **HIGH** for at least 2 minutes.

xiii Decrease the AMS to **LOW**.

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

xv Discard the paper towels in the bottom of sample chamber into a waste container.

xvi Prepare 4 microscope slides, one each per test: Add one drop of immersion oil to the center of the slide. Label slide with sample name or number/letter. Take apart micro5, cyclex-D, or equivalent filter and add coverslip to slide on top of immersion oil (make sure the 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).

xvii Analyze on an appropriate microscope with a appropriate filter (make sure to schedule an appointment on the microscope 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 the 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 FACSAria IIu Use

4.1 Startup

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

b. Add an appropriate amount of bleach to Waste tank, 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 the blue sheath-line (blue) is connected to the sheath filter and the air-line (clear) is connected to the sheath tank prior to startup.

d. Turn on external house air source on wall to pressurize sheath tank.

e. Turn on in order: 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.

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

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

h. Click on the FACS Diva desktop icon to start the software. This software runs the BD FACSAria IIu flow cytometer. Sign in using appropriate username and password.

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

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

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

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

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 CS&T beads. In a 12x75mm tube, add 1 drop of gently vortexed CS&T beads to 350 µL of water or appropriate diluent. Label tube with "CST", and make sure to gently vortex before use. 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 M**aintenance**

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

b. **Approximately every 6 months: Perform preventative maintenance.**

i Perform "Power Wash" of flow cell.

1 Make up in filtered milliQ water at 1.5% (45 uL in 3 mL)
2 Remove nozzle
3 Turn on stream with no nozzle
4 Load Citranox and set to 11 and run for 1 minute
5 Disconnect sheath from under sheath filter and run tube dry
6 Reconnect sheath line to filter
7 Run for 2-3 minutes
8 Shutdown stream and load a filtered water tube
9 Turn stream on and load water at 11 for 5 minutes
10 Clean nozzle port with swabs to dry out
11 Spray down sort chamber with 70% ethanol and let dry
12 Clean deflection plates with swabs
13 Insert nozzle, start stream and check QC

ii Replace all fluidics filters; o-rings on common connections.

iii Run Disk Cleanup and Disk Defrag on hard drive.

c. **As needed: Schedule repair visit with Service Contract provider.**

i Repairs may be necessary be periodically on the FACSAria IIu All surfaces must be decontaminated before the service engineer performs any maintenance on the instrument. The following surfaces should be wiped down with appropriate disinfectant at least 20 minutes prior to servicing:

ii All outer surfaces of the cytometer.

iii All table surfaces, keyboard, and mouse.

iv The inner and outer surfaces of the BSC near the front with which the service engineer might come in contact.

v Once this is done the BSC can be turned off.

vi Complete the equipment release form and attach to the exterior of the BSC.

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

e. **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 the 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 the 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) [or minimally clean with 70% ethanol and rinse with sheath] and water tank (remove probe) if running *Prepare for Aseptic Sort*.

i To autoclave sheath tank. Remove large o-ring and 2 plastic knobs from lid and probe from tank and place these parts inside the biosafety cabinet.

ii Cover tank with foil to cover probe port and lid opening. Also wrap lid and attachment in foil. Apply autoclave tape

iii Autoclave and let cool prior to reassembling.

iv Spray down probe with 70% ethanol prior to re-attaching. Wrap plumber's tape 4 times counter-clockwise around port where probe attaches and attach probe with wrench. Tighten until snug, being careful not to overtighten.

v Add Sheath and reassemble lid with o-ring and plastic knobs. Reattach sheath filter line and probe sensor to cart.

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 a sample, run 10% bleach or 70% ethanol for at least 3 minutes, followed by sterile water for at least 1 minute at a flow rate = 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, and a good descriptive term, as appropriate.

c. Create Applications settings the first time you run an 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 the Parameters tab (In Instrument Window) click on the small button to left of the parameter name. Click the delete button (use the 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 area scaling factor up or down, as appropriate to line up the population with the 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).

o. 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 the streams in the viewer window.

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

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

j. Remove tubes from the 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 a surgical mask or N95 respirator, as appropriate.

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

decontaminate. Use Envirocide (contact time 3 minutes) to decontaminate the 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 the clog has not cleared, turn off the stream, and open the 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 the aspirator/waste drawer and restart the stream to see if clog has cleared itself.

j. If clog has not cleared, turn off the stream, and open the 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 in sterile water or Coulter Clenz in a capped 12x75mm tube until the obstruction has been cleared (if Coulter Clenz is used, sonicate again in sterile water before re-inserting). Re-insert nozzle and turn on the stream and verify that it is normal. Repeat disinfection and/or cleaning the sort collection device and sort chamber, as appropriate. Discard tubes and any liquid into their respective biohazardous waste containers.

l. **It may be necessary to clean the sort block following a clog:** 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. Once cleaning is complete, change and discard gloves into a biohazard waste container prior to closing the sort block, close the sort block door, turn on the stream, and verify that the stream is normal.

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

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

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

4.10 Shutdown/Decontamination

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

i Run a tube of sterile filtered 10% bleach at a flow rate = 11 for 5-10 minutes.

ii Run a tube of filtered milliQ water, or equivalent, at a flow rate = 11 for 5-10 minutes.

iii Turn off stream. Allow stream to turn off and aspirate fully to waste.

iv Remove nozzle and place in a clean, dry tube. The nozzle may be sonicated in Coulter Clenz followed by sterile water, as appropriate.

v Install the closed-loop nozzle.

vi Open the sort block and rinse waste drawer with 10% bleach or 70% Ethanol (squirt bottle). Ensure that enough time has passed after sorting a sample/clog has cleared and that appropriate PPE is being worn before opening the sort block. Close the sort block once liquid has been fully aspirated.

vii Select Cytometer>Cleaning Modes>Clean Flow Cell.

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

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

x Exit Diva and shutdown computer.

xi Turn off the house air supply to the cart.

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

xiii Spray down the 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.

xiv Disconnect and empty the waste tank, containing ~10% bleach and empty **into large carboy provided by EH&S, as no liquids containing fluors or beads can be disposed of down the drain**. Wear appropriate PPE (e.g., lab coat, safety glasses, and gloves).

xv Reconnect the waste tank.

xvi Clean the keyboard, mouse, and work surfaces in front of the SONY LE-MA900 with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent). Subsequently, wipe metal surfaces with 70% ethanol to avoid corrosion of the surfaces.

xvii Turn off the Baker Bioprotect IV Biosafety Cabinet, as appropriate.

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

i Run a tube of filtered 10% bleach at a flow rate = 11 for 5-10 minutes.

ii Run a tube of filtered milliQ water, or equivalent, at a flow rate = 11 for 5-10 minutes.

iii Turn off stream.

iv Allow stream to turn off and aspirate fully to waste.

v Remove the nozzle and place in a clean, dry tube. The nozzle may be sonicated in Coulter Clenz followed by sterile water, as appropriate.

vi Install the closed-loop nozzle.

vii Open the sort block and rinse waste drawer with 10% bleach or 70% Ethanol (squirt bottle). Ensure that enough time has passed after sorting a sample/clog has cleared and that appropriate PPE is being worn before opening the sort block. Close the sort block once liquid has been fully aspirated.

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

ix Disconnect and empty the waste tank, containing ~10% bleach and empty **into large carboy provided by EH&S, as no liquids containing fluors or beads can be disposed of down the drain**. Wear appropriate PPE (e.g., lab coat, safety glasses, and gloves)..

x Reconnect the waste tank.

xi Select *Cytometer>Fluidics Shutdown.* Select *Done* and *Done* if nozzle has been removed and closed-loop nozzle has been installed.

xii Disconnect the air line and depressurize the sheath tank. Disconnect the sheath line and connect to the ethanol sheath tank (do not connect the sheath filter to the ethanol tank). Connect the air line to the ethanol tank last (this avoids spray from the sheath or ethanol filter). Select *Done* to begin the cleaning process.

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

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

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

xvi Exit Diva and shutdown the computer.

xvii Turn off the house air supply to the cart.

xviii Fully depressurize the sheath and ethanol tank by pulling the ring on the pressure relief valve to prevent precipitates.

xix Spray down the 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.

xx Empty the waste container, containing ethanol into the appropriately labeled hazardous waste container while wearing appropriate PPE.

xxi Clean the keyboard, mouse, and work surfaces in front of the BD FACSAria 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 the surfaces.

xxii Turn off the Baker Bioprotect IV Biosafety Cabinet, as appropriate.

4.11 Procedures for Sorting BSL-2+ with Enhanced Precautions

a. Perform assessment to ensure that Room 2522 is setup to maintain negative air pressure. For BSL-2+ sort, the room will be monitored for negative air pressure prior to sort initiation using the Aircuity system and/or smoke bottle.

b. Following startup, empty the waste tank. Add enough fresh bleach to the waste tank to provide a final concentration of approximately 15% when filled.

c. Place a sign on the outside of the door indicating what agent is in use, potential hazards posed by the infectious agent, and what protection should be worn when working with the infectious agent. The door should remain locked at all times.

d. Put on PPE: This includes a back-closure gown, gloves, face shield, goggles/safety glasses, and N95 respirator for BSL2+ sorts.

e. Prepare the proper disinfectant (e.g., 20% bleach) and wipes in case of a sample spill. Tubes will be vortexed inside the BSC with caps on.

f. Check and clean the sort block, sort collection chamber, and gaskets with 70% Ethanol prior to sorting. Pay specific attention to any salt deposits that can interfere with the proper function of the components involved in sorting. Clean these off with sterile water.

g. **After sorting is completed**, collection tubes should be capped and wiped on the outside with appropriate decontaminant and placed in a biohazard transport container. Gloves should be changed and discarded into a biohazardous waste container.

h. Run 10% bleach for 20 minutes in the sample injection port (SIP) to decontaminate the sample tubing.

i. Run sterile water for 10 minutes to clear the bleach from the sample tubing.

j. **Turn off the stream and run AMS on high for at least 5 minutes with the waste drawer open.**

k. **Nozzle Cleaning:** In the BSC, remove the nozzle and place it in a sterile capped 12x75mm tube with 1 ml of Coulter Clenz. Snap the cap tightly and place in the sonicator for 1 min.

l. Remove the tube and aspirate the liquid using a disposable transfer pipet. Add 1 ml of sterile water. Change gloves. Snap the cap shut and place in the sonicator for 1 min.

m. Aspirate the liquid again. Remove the nozzle from the tube and dry with a kimwipe. Place the nozzle back into the nozzle holder underneath the cover. Change gloves.

n. Insert the closed-loop nozzle and run **Prepare for Aseptic Sort** (ensure sufficient levels of fluids/buffers are present in appropriate fluid tanks (fresh sterile water,

70% ethanol or 10% bleach) to ensure that flow cell is properly disinfected (this is especially important if the Aria was clogged during the BSL-2+ sort).

o. Open the sort block and rinse waste drawer with 10% bleach (squirt bottle). Close the sort block once liquid has been fully aspirated.

p. After decontamination of the system's fluidics is complete, ensure the waste tank is mixed. If a Weekly Fluidics Shutdown will not be run, allow the waste tank to sit overnight before transferring it into an EHS approved 5 Gallon carboy.

q. If running a Fluidics Shutdown, disconnect and empty waste container, containing ~15% bleach down sink while wearing appropriate PPE and rinse with DI water (ensure at least 20-minute contact time).

r. Reconnect the waste tank, if appropriate.

s. Run a Daily (4.10a) or Weekly (4.10b) Shutdown, as appropriate.

t. Turn off the instrument and decontaminate the sort block and collection chambers with proper disinfectant (70% ethanol, 10% bleach, Envirocide, and/or Sani Cloth Plus wipes) by spraying and wiping off. Spray and wipe off the collection tube holder and sample tube holder and keep them in the BSC on a paper towel.

u. Wipe all surfaces down with disinfectant, including the outside of the cytometer and the table surface.

v. Disinfect and clean the surfaces around the cytometer, especially near the sort chamber, with proper decontaminant and treat all work surfaces with appropriate disinfectant.

w. If a Fluidics Shutdown was not run, empty waste container, containing ~15% bleach into an EHS approved 5 Gallon carboy.

x. If a Fluidics Shutdown was run, empty the waste container, containing ~35% ethanol into the hazardous waste container while wearing appropriate PPE.

y. With a clean pair of gloves, clean the keyboard, mouse, and work surfaces in front of the BD FACSAria 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 the surfaces.

z. Before leaving the room, place all disposable PPE in the biohazardous waste container and wash hands thoroughly. **Leave the BSC turned on overnight.**

Records

Error Messages / System Issues – All error messages and system issues must be relayed to the Equipment Champion and the Core Staff and appropriately recorded in the Equipment Maintenance Log on the same day as equipment use.

4.12 Resource Index

a. BD FACSAria IIu User Guide:
BD FACSAria IIu flow cytometer and BD FACSDiva 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 FACSAria IIu flow cytometer in room 2522.

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

b. BD FACSDiva v6.1 User Guide:
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.bu.edu/flow-cytometry/files/2010/10/BDFACSDivaSoftwareReferenceManual.pdf

http://static.bdbiosciences.com/documents/bd-cytometer-setup-tracking-application-guide.pdf

c. BD Technical Support:
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 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 Section 3.6a during new BD FACSAria IIu instrument account creation.

6.0 SOP Performance and Equipment Review

The effectiveness of the SOP: P07900160.2522.003 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 Staff 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 iLab. Updated SOPs will be published and Approved Users will be notified. SOP: P07900160.2522.003 reviews will occur every two years.

7.0 Definitions

7.1 **SOP:** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance, and ownership of the BD FACSAria IIu instrument. The SOP number stands for (xxx . xxx . xxx) equipment serial number . room number . SOP version number.

7.2 **Originator / Author:** The individual representing the MSU Flow Cytometry Core Facility that created SOP: P07900160.2522.

7.3 **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.

7.4 **New User:** An individual who has not completed the requirements in section 3.4.

7.5 **Approved User:** An individual who uses the BD FACSAria IIu instrument and has fulfilled the requirements in Section 3.6. This title may only be given by the Equipment Champion and / or the Core Facility Staff.

7.6 **Champion:** An individual whose direct expertise with the BD FACSAria IIu 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.

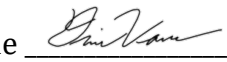
8.0 Approval

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  11/13/2024

Issue Date: November 13th, 2024

Appendix I

Containment Test Record

Sample 1: Normal Operational Mode

AMS:	**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:

AMS:	**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:

AMS:	**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

AMS:	**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:	**Yes**

Result_____

Date:

Operator: